Doctoraatsproefschrift nr. 993 aan de faculteit Bio-ingenieurswetenschappen van de K.U.Leuven

Evaluation of pigs' welfare and meat quality in relation to housing, transport and slaughterhouse procedures

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Proefschrift voorgedragen tot het behalen van de graad van Doctor in de Bio-ingenieurswetenschappen

November 2011

ISBN 978-90-8826-214-2
D/2011/11.109/47

Voorwoord

Graag zou ik iedereen willen bedanken die mij geholpen heeft bij het tot stand komen van dit eindwerk:

- Prof. R. Geers voor de adviezen, ondersteuning en geboden mogelijkheden;
- De Europese Commisie, Belpork vzw, VLAM vzw (Vlaams Centrum voor Agro- en Visserijmarketing), FOD (Federale Overheidsdienst), Vlaamse Overheid, KHK (Katholieke Hogeschool Kempen) en K.U.Leuven voor de financiële steun;
- Prof. J. Buyse, Prof. R. De Schrijver en Prof. M. Hendrickx als assessoren van dit werk;
- Dr. J. Claes, Dr. S. De Bie, Prof. J. Buyse, Prof. R. De Schrijver, Prof. B. Goddeeris en Prof. M. Hendrickx voor de inspanningen als juryleden;
- De varkenshouder voor de bereidwillige samenwerking tijdens de studie van bijtproblemen bij varkens;
- Dhr. M Vandebroeck en het personeel de slachthuizen Abattoir Marquet S.A., Comeco N.V., Covameat N.V., De Lokery N.V., Detry S.A., De Coster, Tielt-De Brauwer, Liège, Goossens N.V., Noordvlees Van Gool N.V., Bens N.V., Porc Meat Zele N.V., Geel N.V., Genk N.V., Sint-Truiden, Torhout en Westvlees N.V. voor de medewerking;
- An Ceustermans en Wendy Wuyts voor hun hulp tijdens de hammenproef;
- Dhr. R. Deliever van de Dienst Onderwijsondersteuning voor de assistentie bij het analyseren van het eiwitgehalte in de hammen;
- Prof. G. Verbeke voor de statistische raadgevingen;
- Het personeel van het Zoötechnisch Centrum voor de assistentie tijdens de experimenten;
- (oud-) collega’s van het Labo voor Kwaliteitszorg in de Dierproductie voor de ondersteuning en de leuke werksfeer;
- De studenten voor de hulp tijdens de praktische proeven;
- Liesbet Permentier en Sanne Van Beirendonck voor het nalezen van dit werk;
- Liesbet Permentier voor de geapprecieerde hulp tijdens de lay-out;
- Vrienden en familie voor de interesse en steun;
- Vriendin Natalie en dochter Marie voor hun onvoorwaardelijke steun en geduld.

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Summary

In Belgium, fresh pig meat is one of the most exported food products. The total export in 2010 was 702,576 tons, which is 68% of the total Belgian pig meat production and is 2 % more than the previous year. These results confirm the rising trend of the past five years. Accordingly, it is very important to deliver good quality of meat and financial losses need to be avoided. Stress and consequently impaired animal welfare might be an important cause of financial loss by affecting pigs' behaviour and meat quality. The aim of this research is to study the effect of (1) stressors such as ventilation and environmental enrichment on pigs' biting behaviour and (2) pre-slaughter stress on meat quality of fresh and processed meat.

Tail and ear biting are severe problems in modern pig production, have a multi-factorial origin and might result in a reduction of animal welfare and productive performance. This abnormal behaviour is investigated in the first part of the study (Chapter 2 and 3). In Chapter 2, the effect of genetics and ventilation on pigs' biting behaviour was investigated. All pigs originated from one of two Piétrain boars, originating from two different lines. Genetics and ventilation were found to have a significant impact on the biting behaviour and the appearance of wounds. More precisely, pigs descendant of a boar, which had a better conformation and higher lean tissue content than pigs originating from the other boar, were more vulnerable for inappropriate ventilation, i.e. more pigs showed biting behaviour and more wounds were seen. In Chapter 3, the effect of a sequence of toys (weekly change) versus a single toy (chain) on pigs' biting behaviour during the complete fattening period are reported. Toy contact and biting pen mate behaviour were observed on the day of introduction of the toy and five days later. The continuous sequence of seven enrichment objects reduced biting pen mate behaviour and the number of wounds compared to the providing only a single toy. The study also confirmed that not every object was feasible as an enrichment object for growing pigs. Generally, the most toy contact was observed together with the highest biting pen mate behaviour and could be induced by the competition for popular toys. However, biting pen mate behaviour was still the highest in pens with only a chain as enrichment object. Furthermore, habituation still occurred since toy contact behaviour was lowest at observation day five or decreased when the same toys were provided for the second or third time. The ideal sequence should maintain toy contact behaviour without competition in order to avoid biting pen mate behaviour and reduced animal welfare. No effect on growth and feed conversion was seen. In consensus with

Chapter 2, biting pen mate behaviour decreased over age since the activity of pigs decreased over age.

PSE meat is still a common meat quality defect in Belgium and pre-slaughter stress is the determining factor being multifactorial in its nature. The effect of several pre-slaughter parameters concerning transport, unloading, lairage, pig handling, stunning and season on fresh meat quality based on pH measurements 30 min after slaughter are reported in Chapter 4. Ten pre-slaughter parameters had a significant effect on meat pH after separate introduction of the variables as a fixed effect in the model. Simultaneous analysis of these variables in the global model revealed that the pH was influenced by four main risk factors, namely the mean noise level produced during unloading, the percentage of panting pigs, the use of an electric prod and season. Meat quality in terms of the percentage of potentially PSE carcasses was better in summer than spring or autumn and could be explained by a lower observed pre-stunning stress in summer.

The processing of meat with PSE or DFD characteristics has determinable effects on the quality of the end product. As a result, measurements in the slaughter line that can predict the quality of the processed meat product would be very interesting and is studied in Chapter 5. Meat quality measurements (pH, electrical conductivity, colour and/or water holding capacity) were carried out 30 min, 24 and/or 35 h after slaughter in three different muscles: *M. gracilis*, *M. semimembranosus* and *M. longissimus dorsi*. From these measurements, a tendency towards a higher proportion of PSE meat during summer was found compared to winter. Moreover a higher protein, a higher dry matter content, a lower water/protein ratio and a lower slicing yield were found for the cooked hams suggesting a higher PSE prevalence in the summer. These results, in combination with the results of Chapter 4, showed that pre-slaughter stress, temperature fluctuations and weather conditions might be more important factors than season concerning the occurrence of PSE meat. Temperature fluctuations and weather conditions were not measured and need to be considered in further research. Chapter 5 also showed that multiple meat quality measurements can provide more information about the meat quality but are not easy to perform on an industrial scale. From this study, it can be concluded that the ultimate pH measurements (after 24 h and 35 h) in the three examined muscles, and certainly the ultimate pH in the *M. gracilis*, are good measurements to predict colour 24-35 h post-mortem and the meat quality of hams after cooking.

The results of this thesis showed that good rearing practices for pigs also involves appropriate ventilation and the provision of enrichment objects that can maintain novelty. If the proposed pre-slaughter stressors are taken into account, i.e. minimal pre-slaughter stress, meat quality can be improved. However, further research is needed to associate good rearing practices for pigs with their final meat quality. This can practically be true when pre-slaughter stress is very low.

Samenvatting

Vers varkensvlees is één van de meest geëxporteerde voedingsmiddelen uit België. De totale export bedroeg 702.576 ton in 2010, wat 68% van de totale productie is. Bovendien is de export met 2 % gestegen ten opzichte van het voorgaande jaar. Dit bevestigt de stijgende trend van de laatste vijf jaar. Het is bijgevolg héél belangrijk om kwaliteitsvlees te leveren en financiële verliezen te vermijden. Stress en bijgevolg verminderd dierenwelzijn kan een belangrijke oorzaak zijn van financiële verliezen, omdat stress het gedrag en de vleeskwaliteit kan beïnvloeden. Het doel van dit onderzoek is effecten te onderzoeken van (1) omgevingsfactoren zoals ventilatie en omgevingsverrijking op het bijtgedrag van varkens en van (2) stress vóór het slachten op de vleeskwaliteit van vers en verwerkt varkensvlees.

Staart- en oorbijten zijn ernstige problemen in de moderne varkenshouderij, hebben een multifactoriële oorsprong en kunnen resulteren in een verminderd dierenwelzijn en prestatievermogen. Dit abnormale gedrag werd in het eerste gedeelte van deze studie bestudeerd (hoofdstuk 2 en 3). In hoofdstuk 2 werd het effect van genetica en ventilatie onderzocht. De biggen waren afkomstig van twee Piétrainberen, maar van verschillende genetische lijnen. Genetica en ventilatie hadden een significant effect op het bijtgedrag en het voorkomen van wonden. De biggen van de ene beer hadden een betere conformatie en mager vleesaandeel dan de biggen van de andere beer en waren meer gevoelig voor slechte ventilatie. Dit was te merken aan het hoger bijtgedrag en het meer voorkomen van wonden. In hoofdstuk 3 werd het effect van een sequentie van speeltjes (wekelijks vervangen) ten opzichte van één enkel speeltje (ketting) beschreven naar speel- en bijtgedrag. De ganse afmestperiode werd bekeken. Speel- en bijtgedrag werden geobserveerd op de dag van introductie van het speeltje en vijf dagen later. De continue sequentie van de zeven speeltjes verlaagde het bijtgedrag en het aantal wonden ten opzichte van de aanwezigheid van één enkel speeltje. Deze studie toonde ook aan dat niet elk speeltje geschikt was als omgevingsverrijkend materiaal voor varkens. In het algemeen werd gevonden dat het meeste speelgedrag samenhangt met het hoogste bijtgedrag. Dit gedrag kan het gevolg zijn van competitie voor de populaire speeltjes. Ondanks dit competitiegedrag was het bijtgedrag in de hokken met enkel een ketting als speeltje nog steeds het hoogste. Ook werd er nog steeds gewenning geobserveerd aangezien het speelgedrag het laagste was op observatiedag vijf of daalde wanneer dezelfde speeltjes voor een tweede of derde maal werden aangeboden. De ideale sequentie zou het speelgedrag moeten blijven stimuleren zonder competitie om zo het bijtgedrag te vermijden en het dierenwelzijn te verbeteren.

Geen effect op groei en voederconversie werd waargenomen. In overeenstemming met hoofdstuk 2 werd een daling van het bijtgedrag en de activiteit van varkens met de tijd waargenomen.

PSE vlees is nog steeds een veel voorkomende afwijking op het gebied van vleeskwaliteit in België. Stress voor het slachten is hierbij de bepalende factor en is multifactorieel van oorsprong. Het effect van verschillende stressparameters net vóór het slachten op de vleeskwaliteit, in het bijzonder voor transport, lossen, rusten, omgang met varkens, verdoving en seizoen, werd bestudeerd in hoofdstuk 4. De vleeskwaliteit werd bepaald aan de hand van pH-metingen 30 minuten na het slachten. Tien parameters hadden een significant effect op de pH na enkelvoudige inbreng van elke variabele in het model als fixed effect. Na inbreng van alle variabelen tegelijk in het globaal model werd gezien dat de pH door voornamelijk vier risicofactoren beïnvloed werd. Deze factoren waren het gemiddelde geluid dat geproduceerd werd tijdens lossen, het percentage "pompende" varkens, het gebruik van de elektrische prikkelaar en het seizoen. Op basis van het potentiële percentage PSE vlees werd gezien dat de vleeskwaliteit in de zomer beter was dan in de winter. In de zomer was er minder stress net voor het verdoven, dit kan een mogelijke verklaring zijn.

Het verwerken van vlees met PSE of DFD eigenschappen heeft negatieve effecten op de kwaliteit van het eindproduct. Bijgevolg zou het heel interessant zijn om de vleeskwaliteit van het eindproduct al te kunnen voorspellen in de slachtlijn. Dit werd bestudeerd in hoofdstuk 5. De vleeskwaliteitsmetingen (pH, conductiviteit, kleur en/of waterverlies) werden 30 minuten, 24 en/of 35 uur na slachten uitgevoerd in drie verschillende spieren: *M. gracilis*, *M. semimembranosus* en *M. longissimus dorsi*. Deze metingen gaven een tendens van meer PSE vlees in de zomer dan in de winter weer. Er werd eveneens een hoger eiwitgehalte, hoger droge stofgehalte, hoger snijverlies en lagere water/eiwit verhouding gevonden voor de gekookte hammen, wat tevens een hogere PSE prevalentie in de zomer suggereert. Deze resultaten, samen met de resultaten uit hoofdstuk 4, tonen aan dat stress voor het slachten, temperatuurfluctuaties en weersomstandigheden belangrijkere factoren kunnen zijn dan seizoen inzake het voorkomen van PSE vlees. Temperatuurfluctuaties en weersomstandigheden werden niet gemeten en moeten dus zeker meegenomen worden tijdens verder onderzoek. Het onderzoek toonde ook aan dat verschillende vleeskwaliteitsmetingen meer informatie geven over de vleeskwaliteit, maar niet altijd makkelijk uit te voeren zijn op industriële schaal. Uit deze studie kan besloten worden dat de ultieme pH-metingen (na 24 en 35 uur) in de drie onderzochte spieren, en voornamelijk in de *M. gracilis*, goede metingen zijn om de kleur 24-35 uur na het slachten te voorspellen, samen met de vleeskwaliteit van hammen na het koken.

De resultaten van deze thesis tonen aan dat een goede opkweek van varkens ook een aangepaste ventilatie en de voorziening van geschikte omgevingsverrijking inhoudt. Wanneer de vooropgestelde stressfactoren voor het slachten in rekening worden gebracht (minimale stress vóór het slachten), kan de vleeskwaliteit verbeteren. Meer onderzoek is echter nodig om een goede opkweek van varkens te kunnen koppelen aan de uiteindelijke vleeskwaliteit. In het bijzonder wanneer de stress vóór het slachten héél laag is, zodat een langetermijneffect al dan niet kan uitgesloten worden.

List of abbreviations

a*	red-greeness
ACTH	adrenocortictropic hormone
ANOVA	analysis of variance
ANS	autonomic nervous system
ATP	adenosine triphosphate
b*	yellow-blueness
CRF	corticotrophin releasing factor
CRH	corticotrophin releasing hormone
DFD	Dark Firm Dry
e.g.	exempli gratia
HPA	hypothalamic-pituitary-adrenal axis
i.e.	id est
Jap	Japanese colour scale
L*	lightness
LD	Musculus longissimus dorsi
LSS	low stress system
MG	Musculus gracilis
MS	Musculus semimembranosus
P	probability
pH_i	initial pH
pH_u	ultimate pH (24 h post-mortem)
pH_{u35}	pH 35 h post-mortem
PQM	pork quality meter
PSE	Pale Soft Exudative
Q3	third quartile
r	correlation coefficient
RFN	Red Firm Nonexudative
RSE	Red Soft Exudative
s.a.	sine anno
SAS	statistical analysis system
SD	standard deviation
SE	standard error
SEM	standard error of the means
WHC	water holding capacity

Chapter 1: General introduction

1.1 Definition of the problem

Pork is the most consumed meat in the world with 102.953 million tonnes in 2010 and is still a growing industry (Pig International, 2010; USDA-FAS, 2010). In the past, meat industry has always striven for quantity and less for quality. Today, consumers are becoming more discriminating and the demand for high quality meat is growing. However, there is still a large heterogeneity in meat quality which results in financial losses. Many factors exert an influence on meat quality and solutions to improve quality are mostly ambiguous. Animal stress has an important effect on meat quality. Consequently, meat quality is associated with another dimension of quality, i.e. animal welfare.
In this introduction several sources of stress are reported and their consequences on animal welfare and meat quality are explained.

1.2 Stress and animal welfare: a double unity?

1.2.1 Definitions

Stress is an inferred internal state and has no clear definition because stress has no defined etiology or prognosis. One of the most accepted definitions of stress is 'a biological response to an event (stressor) that the individual perceives as a threat to its homeostasis' (Moberg, 2000). Another often cited definition of stress was proposed by Fraser et al. (1975): 'An animal is said to be in a state of stress if it is required to make abnormal or extreme adjustments in its physiology or behaviour in order to cope with aspects of its environment and management'. Successful coping depends highly on the controllability and predictability of the stressor (Ursin & Olf, 1993).

If pigs fail to cope or fail to adapt successfully, stress responses may be not merely ineffective but actively delirious (National Research Council, 2008) and consequently affecting animal welfare. Broom (1986) defined the welfare of an animal as: 'The welfare of an individual is its state as regards its attempts to cope with its environment'. This vision implies that when an animal is confronted with environmental challenges it reacts with behavioural and physiological feedback mechanisms to maintain constant internal characteristics of the body (homeostasis). Consequently, behavioural and physiological measurements can give more information about the welfare of an animal (Broom, 1986).

Additionally, a good animal welfare implies "five freedoms" (Farm Animal Welfare Council, 1979) and is still the basis of present EU legislation on animal welfare (Korte et al., 2007). These freedoms are: 1) freedom from thirst and hunger, 2) freedom from discomfort, 3) freedom from pain, injury and disease, 4) freedom to express normal behaviour, and 5) freedom from fear and distress. In this thesis, behaviour measurements such as biting behaviour or biophysical measurements such as pH, PQM … were monitored to describe animal welfare and are consequently connected with the five freedoms. No single measurement can cover all the dimensions of welfare (Botreau et al., 2007).

In the last decennia, society has been confronted with epidemics such as avian influenza, foot and mouth disease and mad cow disease. As a result, ideas rapidly changed on how to keep animals (non anthropocentric thinking) and a new definition/EU legislation for animal welfare is needed. The Five Freedoms reflect a more ethical view than a scientific-based approach and are no longer helpful (Korte et al., 2007). Moreover, the latest concept in physiology, allostasis, has been neglected in the animal welfare definition. Homeostasis and allostasis are endogenous systems responsible for maintaining the internal stability of an organism. Homeostasis assumes that the controlled physiological variables are kept at their set point ('stability through constancy'). In detail, homeostasis describes mechanisms (Figure 1.1) that hold constant a controlled variable by sensing its deviation from a setpoint and feed back to correct the error (Sterling, 2004). In contrast, allostasis considers an unusual physiological parameter not as a failure to defend a set point, but rather as a response to some prediction ('stability through change') (Sterling & Eyer 1988; Sterling, 2004). Allostasis describes mechanisms (Figure 1.1) that change the controlled variable by predicting what level will be needed and then overriding local feedback to meet the anticipated demand (Sterling, 2004).

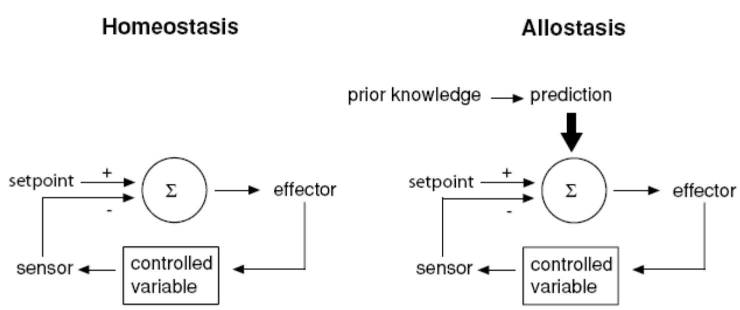

Figure 1.1. Homeostatic and allostatic model of regulation (Source: Sterling, 2004).

Allostatic load refers to the cumulative cost of allostasis to the body. Body systems are in a state of flux and stress pushes these systems beyond normal bounds of the fluctuations (Schreck, 2010). Furthermore, allostasis has the potential to replace the homeostasis as the core model of physiological regulations (Sterling, 2004). As a result, a new concept of animal welfare is defined by Korte et al. (2007):

- Stability through change and capacity to change are crucial to good health and good animal welfare. “Health” in this concept has the same meaning as defined in the World Health Organization's (WHO) constitution as: ”a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity”.
- Good animal welfare is characterized by a broad predictive physiological and behavioral capacity to anticipate environmental challenges.
- Good animal welfare is guaranteed when the regulatory range of allostatic mechanisms matches the environmental demands.
- A low allostatic load is a key for good health and good animal welfare.
- In organisms, structural design should match functional demand.
- Interpreting behavior and physiology in terms of animal perceptions and not exclusively in terms of human values.

Stressors can be categorized as those that evoke one of the two distinct responses by the organism: those that cause emergency-types of responses (type one) and those that cause coping-types of responses (type two) (McEwen & Wingfield, 2003). Stressors of type one can be associated with more acute stressors (Schreck, 2010). Acute stress is the state of an organism that appears with a sudden decrease in predictability and/or possibility to influence relevant changes in the environment. Common acute stressors are e.g.: noise, light and crowding... Stressors of type two can be associated with more chronic stressors (Schreck, 2010). Chronic stress is the state of an organism that appears when relevant changes in the environment can not be predicted or influenced during a long period (at least several days) of time. Common chronic stressors are e.g.: loneliness, inadequate climate (humidity, ventilation…) …

1.2.2 Physiological response to stress

The primary systems employed by vertebrates to cope with stress are the sympatho-adrenal medullary axis, with the secretion of catecholamines (epinephrine and norepinephrine) and the hypothalamic-pituitary-adrenal (HPA) axis, with the secretion of corticosteroids (Chrousos & Gold, 1992; Frankhauser, 1986). Both systems are anatomically and functionally intertwined (Ehrhart-Bornstein et al., 1998). Adrenal cortical secretion of steroids is regulated both by the sympatho-adrenal and hypothalamic-pituitary systems (Ulrich-Lai & England, 2005). The central components of these systems are located in the hypothalamus and the brain stem.

a) Sympatho-adrenal medullary axis

The sympathetic-adrenal response takes the message from the brain to the adrenal medulla via the sympathetic nervous system, which secretes epinephrine (adrenalin) and norepinephrine (noradrenalin) into the bloodstream. This flight-or-fight response is also called the 'Emergency Reaction' (Cannon, 1929) and involves:

- Increased blood pressure and heart rate;
- Increased blood flow to muscles and brain, while decreasing flow to digestive tract and internal organs through the constriction of certain blood vessels;
- Release of more glucose into the blood by stimulation of the liver, giving the muscles and brain the energy they need;
- Pupil dilatation;
- More sweating;
- Increased breathing rate.

Initiation of stress activates the autonomic nervous system (ANS) and is controlled by the hypothalamus. Two branches of the ANS are important to regulate the 'fight-or-flight' response described by Cannon (1929), the sympathetic nervous system and the parasympathetic nervous system. The sympathetic nervous system initiates the fight-or-flight response after a stressful stimulus on the one hand. On the other hand, the parasympathetic nervous system is designed to return the physiology to a state of homeostasis after the threat.

b) Hypothalamic-pituitary-adrenal axis

When an animal is exposed to more long-term challenge the HPA axis becomes active (Figure 1.2). In this response, the hypothalamus is stimulated and produces corticotrophin releasing hormone (CRH) and vasopressin into the portal blood stream. Via the portal blood stream CRH and vasopressin are transported to the anterior pituitary gland and stimulates the release of corticotropic hormone (ACTH) into the systemic circulation. Circulating ACTH stimulates the adrenal cortex to produce and secrete glucocorticoids such as cortisol into the circulation (Ulrich-Lai & England, 2005). The presence of cortisol in the body causes e.g. increased fat and protein breakdown in the liver, which fuels the production of new glucose. Depending on the strength of the stressor, this action is sustained, and it takes a few hours to return to baseline activity. Prolonged secretion of cortisol will lead to health problems such as the breakdown of the cardiovascular system, digestive system, musculoskeletal system, and the established immune system. To limit over-stimulation, glucocorticoid secreted by the adrenal cortex exerts inhibitory effects including, but not limited, to the negative feedback regulation of the HPA by suppression of the secretion of CRF (de Kloet, 2000; Herman et al., 1996). The negative feedback regulation of HPA occurs acute by inhibition of CRF release and chronically by down-regulating CRF and vasopressin (Keller-Wood & Dallman, 1984).

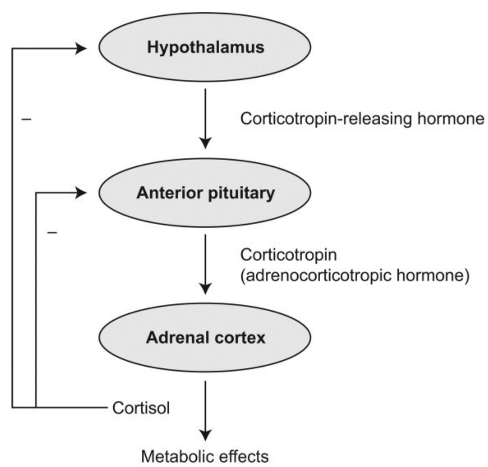

Figure 1.2. Schematic representation of the hypothalamic–pituitary–adrenal axis (Source: Ryan et al., 2007).

1.2.3 Consequences of stress

When an animal has to use reserve resources to cope with prolonged or severe stress, this has a negative impact on other bodily functions and leads to distress (Moberg, 1999). Allostatic overload occurs. Chronically high overloads can lead to a pathological state (McEwen & Lasley, 2002) resulting in decreased reproduction, changed metabolism, the development of abnormal behaviour such as tail biting (Schrøder-Petersen & Simonsen, 2001) and even death. Tail biting behaviour will be studied in more detail as an experimental model in the next paragraph. Stress also affects meat quality and this aspect will be studied in more detail later on.

1.3 Tail Biting behaviour

Tail biting is a major and unpredictable problem in modern pig production, reducing animal welfare and productive performance (Bracke et al., 2004; Moinard et al., 2003). This abnormal behaviour has been reported since about 1950 (Sambraus, 1985) and is today at least as severe as 50 years ago. Moreover, it appears that the frequency of tail biting continues to increase in the field and no reliable solution is currently available (Schrøder-Petersen & Simonsen, 2001).

Tail biting can be defined as the phenomenon that occurs when one pig takes the tail of another pig into its mouth and manipulates it. When this behaviour is repeated, damages to the skin of the tail and bleeding can occur (Van Putten, 1969). Consequently, infections and spinal abscesses can occur which downgrades carcasses at slaughter. In worst cases, tail biting can lead to death. Most researchers refer to biting behaviour resulting in lesions (Schrøder-Petersen & Simonsen, 2001) or obvious avoidance behaviour by the victim (Holmgren et al., 2004).

1.3.1 Types

Based on descriptions of the biting behaviour and under the conditions they occur, three different types of tail biting can be distinguished (Taylor et al., 2010):

1. Fraser (1987) described the tail biting syndrome as two distinct stages (Two-stage-tail biting) and is currently the most accepted ontogeny of tail biting:
In the pre-injury stage, no visible wounds are present. The pig takes the tail of another pig into the mouth and gently manipulates it by chewing. The victim usually tolerates it. This stage can be followed by stage 2, the injury stage. In this stage, the pigs' tail is wounded and bleeds due to repetitive chewing (Fraser & Broom, 1990). Bleeding tails often encourage biting behaviour by pen mates. Sambraus (1985) reported that in severe cases, the wounded animal gives up its resistance, its effort to flee and becomes apathetic.

The injury stage can be classified as either acute or chronic (Fritschen & Hogg, 1983). Cannibalism or acute tail biting often results in crippling and death. Pigs suffering chronic tail biting are characterized by an open wound and are often reluctant to stand. This results in further assaults of pen mates (aggressor) because the pig (victim) can not flee.

2. A second type of tail biting was defined by Van Putten (1969), Fritschen & Hogg (1983) and Fraser & Broom (1990) i.e. sudden-forceful tail biting. The pig's tail is bitten forcefully, generally without an observed previous manipulation. Sudden-forceful tail biting is a rare, more aggressive form of tail biting and is more commonly seen when pigs are unable to access desired resources (Morrison et al., 2007). This form of tail biting behaviour is not always differentiated from two-stage tail biting (Taylor et al., 2010).

3. Beattie et al. (2005) and Van de Weerd et al. (2005) reported a last type of tail biting, i.e. obsessive tail biting, also known as fanatical tail biting. This type of biting behaviour is characterized by a lot of forceful tail biting expressed by one or a few individual animals, like the sudden-forceful tail biting described above. This behaviour differs with type 2 tail biting because pigs focused or fixated on biting tails, are persistently looking for another tail to bite (Taylor et al., 2010).

1.3.2 Risk factors

It is well documented that tail biting is a multi-factorial syndrome including environmental features. Risk factors for tail biting are listed by Schrøder-Petersen & Simonsen (2001) and Taylor et al. (2010) i.e. genetics, gender, age and weight, health status, rearing environment, climate, stocking density and pen size, floor and other constructions in the pen, feeding systems, food, rooting materials and toys. The effect of genetics, gender, age and weight, climate, stocking density and pen size are studied in this thesis.

a) Genetics

Some studies reported that certain breed types are more predisposed towards tail biting but no clear pattern was observed (Schrøder-Petersen & Simonsen 2001). However, Taylor et al. (2010) reported that the influence of genetics on tail biting may be more relevant at line/strain level rather than between breeds. Further, the heritability of tail biting can be positively correlated with performance parameters such as lean tissue growth rate and negatively with back fat thickness (Breuer et al., 2005). These parameters are widely included in selection programs of pigs and make it difficult to solve the biting problem. Behaviours such as foraging, exploration, feeding, and motivation to feed are heritable in pigs (Baumung et al., 2006; Breuer et al., 2003; Renaudeau et al., 2006). As a result, Taylor et al. (2010) suggested that motivation to manipulate objects, and potentially the tail, are likely to follow similar trends.

b) Gender

A review of Schrøder-Petersen & Simonsen (2001) reported that male pigs (boars and barrows) are bitten more frequently than females. Possible explanations why castrated males are more bitten than females are:

- Female pigs are more willingly to fight for food (Wallgren & Lindahl, 1996).
- Female pigs become more active and more interested in ano-genital exploration and anal massage, when reaching sexual maturity, and therefore being more eager tail biters (aggressor) (Sambraus, 1985).

However, Penny et al. (1981) showed a higher frequency of tail biting when boars were penned together than when females were penned together. Moreover, Taylor et al. (2010) stated that there is no clear pattern relating to which gender is most likely to tail bite or to become tail bitten (e.g. Breuer et al., 2003; Moinard et al., 2003; Kritas & Morrison, 2004; Schrøder-Petersen et al., 2003). This review reported that differences in dietary needs between the gilts, barrows and boars could be a reason to perform tail biting behaviour. When only one diet is provided for all animals, this diet will not be optimal for both sexes. As a result, one group can increase explorative behaviour to compensate. When this dietary imbalance is still present, two stage tail biting behaviour can occur.

c) Age and weight

Tail biting behaviour does not occur with the same frequency throughout the pig's life (Schrøder-Petersen & Simonsen, 2001). As soon as the pigs start to "fill their pens", tail biting can be observed (Blacksaw, 1981; Penny, 1981). Literature describes that tail biting is a common problem when the pigs weigh 40-50 kg (Haske-Cornelius et al., 1979; Sambraus, 1985) possibly because gilts are more aggressive at approximately that weight (Hansen et al., 1979). It has been suggested that small pigs seem to start the tail biting behaviour (Fraser & Broom, 1990; Sambraus, 1985). Schrøder-Petersen & Simonsen

(2001) concluded in their review that the frequency of tail biting seems to increase with age and weight, but further research is needed.

d) Climate

Inappropriate indoor temperatures (e.g. non conform with Table 1.1) (Haske-Cornelius et al., 1979; Geers, 1989; Schrøder-Petersen & Simonsen, 2001), high concentrations of certain gases such as ammonia (e.g. > 3000 ppm) and carbon dioxide (e.g. > 10 or 20 ppm) (Boussery, 2009; Jongman et al., 2000; Smith et al., 1996; Van Putten, 1969), high or low humidity (> 80 % or < 50 %) (Colyer 1970; Van Pütten, 1969; Van Gansbeke et al., 2009), etc are aversive to pigs and can cause chronic stress which may lead to two-stage and sudden-forcefull tail biting (Taylor et al., 2010). Consequently, appropriate thermal regulation and ventilation is of fundamental importance. Ventilation systems must allow air movement in all areas of the pig house without creating draughts. Draughts can inflict tail biting (Colyer, 1970). Ventilation systems are temperature controlled.

Table 1.1. Thermal neutral zone in function of animals' body weight and pen infrastructure (Source: Van Gansbeke et al., 2009).

Category	Weight (kg)	Pen infrastructure	Thermal neutral zone (°C)
Piglets	1	Concrete	26-32
	1	Straw	20-27
	5	Concrete	22-30
	5	Metal grid	20-29
	5	Straw	16-26
	20	Concrete	16-28
	20	Straw	11-25
Pigs	40	Concrete	13-26
	40	Straw	7-24

Season also has an effect on tail biting. During spring and autumn, there are large daily temperature fluctuations which are often blamed for outbreaks (Taylor et al., 2010). Moreover, these fluctuations have an effect on the ventilation pattern in the pig house which may result in creating draughts and aversive atmospheres (Smith & Crabtree, 2005).

e) Stocking density and pen size

Schrøder-Petersen & Simonsen (2001) and Taylor et al. (2010) concluded in their review that tail biting is associated with high stocking densities. High stocking densities can interfere with normal social interactions such as avoidance behaviour (Moinard et al., 2003). Further, an increase in frustration can appear, caused by the inability of pigs to reach resources when they wish, and sudden-forceful tail biting can occur. Stocking density is defined by law (EU Council Directive 91/630/ECC; implemented in Belgium as RD of 15 may 2003) (Table 1.2).

Table 1.2. Stocking density proposed by law (EU Council Directive 91/630/ECC).

Weight (kg)	Minimal surface/animal (m²)
< 10	0.15
10-20	0.2
20-30	0.3
30-50	0.4
50-85	0.55
85-110	0.65
> 110	1

f) Other risk factors

Increased tail biting behaviour can be caused by: a suboptimal diet (e.g. low tryptophan diet) or not enough food provision, the method of food supply (e.g. absence of ad lib feeding systems), gastro-intestinal discomfort (e.g. pelleted food which are associated with gastric ulcers), a poor health status (e.g. respiratory disease), environmental stressors (e.g. unpredictable feeding, disturbance), floor and other constructions in the pen (e.g. open connection to the manure pit) (Schrøder-Petersen & Simonsen, 2001; Taylor et al., 2010). These factors contribute to increased stress, frustration and/or foraging behaviour, and will consequently affect pigs' biting behaviour.

1.3.3 Solutions

In an attempt to control tail biting, many farmers dock the tails of all new-born piglets. During this procedure the end of the piglet's tail is removed without anaesthetics, leaving about almost the complete tail up to 1 cm of the tail stump. As a result pig's tails are less attractive to other pigs and the tail stump is more sensitive inducing avoidance behaviour when bitten (Simonsen et al., 1991). The effectiveness of this action is questionable because the procedure does not eliminate the problem (Bracke et al., 2004; Chambers et al., 1995; Moinard et al., 2003; Paul et al., 2007; Schrøder-Petersen & Simonsen, 2001). Moreover, in the European Union routine docking of piglet tails is illegal (Comission Directive 2001/93/EC of 9 November 2001), thus alternative preventive strategies are needed. Other strategies, such as coating of bitten tails in substances with an aversive taste such as wood tar (Arey, 1991) or the alteration of the social environment such as isolation of the tail biter can be efficiently against an outbreak of tail biting (Colyer, 1970; Schrøder-Petersen & Simonsen, 2001). Wallgren & Lindahl (1996) did not find any effect of the use of tar on tail biting. Isolation of the tail biters is not practical with larger outbreaks because extra room is needed in the pig house. Moreover, Zonderland et al. (2008) reported only a temporary reduction in blood scores when the biter was removed. Once tails are bitten and covered with blood, biting behaviour is likely to escalate (McIntrye et al., 2001; Van Putten 1968) even when the offender is removed. The simultaneous removal of the biter and wounded pigs could be an effective treatment (Zonderland et al., 2008).

In order to reduce/solve tail biting behaviour, i.e. improving animal welfare, the risk factors for biting must be considered. Moreover, it is well documented that environmental enrichment can reduce biting behaviour and this is studied in the next paragraph.

1.4 Environmental enrichment

Exploratory behaviour represents an important need in pigs (Studnitz et al., 2007). Even in intensive husbandry systems, pigs seem to be highly motivated to explore, even when feed is available ad libitum (Lyons et al. 1995; Scott et al. 2006b; Van Putten & Dammers 1976). Intensive husbandry systems are often barren environments with slatted floors. Such environment limits the expression of some key behaviours which domestic pigs exhibit under less constrained conditions (Van de Weerd & Day, 2009). In such cases, the behaviour can be directed at the limited number of substrates available, namely pen mates (Beattie et al., 2000; Kelly et al., 2000; Schrøder-Petersen & Simonsen 2001; Scott et al., 2006a). As a consequence, adverse, harmful, manipulative, social behaviours as ear, tail and flank biting often occur at high frequencies (Fraser et al., 1991; Scott et al., 2006b; Van de Weerd et al., 2005). Through the provision of enrichment objects more species-relevant needs of pigs will be provided and allows the animal to express behavioural opportunities to express control over their environment (Van de Weerd et al., 2003, 2006). Consequently stress/biting behaviour will decrease and animal welfare (behaviour, health and physiology, animal performance…) will improve. Environmental enrichment may help to reduce tail biting (two-stage) as tail biting has been suggested to be a redirected foraging behaviour (Beattie et al., 2001; Day et al., 2002; Fraser et al., 1991; Taylor et al., 2010;

Van de Weerd et al., 2005;). Van de Weerd & Day (2009) reported in their review that successful enrichment should meet the needs of four criteria:

(1) Increase species specific behaviour;
(2) Maintain or improve levels of health;
(3) Improve the economics of the production system;
(4) Practical to employ.

The provision of environmental enrichment to pigs of all ages is mandatory since 3 January 2003 throughout the EU (Directive 2001/93/EC). This Directive requires that: 'To enable proper investigation and manipulation activities, all pigs must have permanent access to a sufficient quantity of material such as straw, hay, wood, sawdust, mushroom compost, peat or a mixture of such which does not adversely affect the health of the animals'. However, the legislation does not define proper investigation and manipulation activities, and is more a guidance (Van de Weerd & Day, 2009). Different types of enrichment are reported in the next paragraph.

1.4.1 Types of enrichment

Straw is one of the most studied and successful forms of environmental enrichment and offers advantages for animal welfare due to its use as bedding (thermal and physical comfort), gut fill and by stimulating foraging and manipulating activities in indoor-housed pigs (Arey et al., 1993; Fraser et al., 1991; Tuyttens, 2005). Moreover, Van de Weerd and Day (2009) concluded that straw has the highest potential to meet the four enrichment criteria. It has been shown that the presence of straw reduced pen mate directed behaviour (Beattie et al., 2000; Kelly et al., 2000). Although the significant positive effects of providing straw, it is quite expensive and labour demanding and is not universally adopted as it is not compatible with slatted flooring and liquid manure handling systems (Day et al., 2002). Furthermore, straw has a negative effect on hygiene and promotes the carry-over of diseases (Tuyttens, 2005). The rearing of pigs in straw-based systems is more expensive than in slatted systems (Bornett et al., 2003). Consequently, slatted floors are the most common husbandry systems in the EU (Hendriks et al., 1998). Other substrates such as peat, sawdust and mushroom compost are also very effective types of enrichment (Beattie et al., 1995), but blockage of the slurry systems can occur as well. As a result, other types of environmental enrichment, which improve pig welfare to the same or greater extent as straw, are required (Day et al., 2002).

Alternative forms of enrichment were developed for slatted systems, such as point-source enrichment objects, and can be effective in reducing tail biting behaviour (Sambraus & Kuchenhoff 1992; Van de Weerd et al., 2006). Foraging and exploratory behaviour need to be stimulated (Van de Weerd et al., 2003). Not every object is equally feasible as an enrichment object (Bracke et al., 2006; Studnitz et al., 2007). Moreover, Van de Weerd et al. (2006) suggested that some objects can induce tail biting behaviour (e.g. the Bite Rite). Important characteristics for intense use are complexity, ingestibility, odour, chew ability and destructibility (Studnitz et al., 2007; Van de Weerd et al., 2003) or a combination of flexibility and destructibility (Zonderland et al., 2001, 2008). Accessibility of an object is also very important as limited access to a particular object can lead to competition and aggression (Day et al., 2008) and can induce sudden-forceful tail biting (Taylor et al., 2010). It has been suggested that enrichment should be present at floor level to allow natural rooting behaviours. However, Scott et al. (2009) reported that a hanging object tends to provide better occupation than an equivalent at floor level. A hanging toy can not be soiled with faeces and is consequently more visible and accessible to the pigs. Novelty of an object is another important characteristic involved in initiating and maintaining exploration (Gifford et al., 2007; Trickett et al., 2009), and can be attained by replacing familiar objects (Trickett et al., 2009). However, habituation still occurs when objects are encountered repeatedly. Little is known on how long pigs remember a particular object (Gifford et al., 2007).

Literature indicated that various enrichment items provide less occupation than substrates such as straw (Day et al., 2002; Scott et al., 2006a). However, Trickett et al. (2009) found that the provision of a rope caused interaction levels comparable to straw. The choice of enrichment for pigs is rather based on economy or health-related factors, than on the requirements of the animal themselves (Van de Weerd et al., 2003).

Literature indicates that the provision of environmental enrichment could have an effect on performance, carcass quality and meat quality, but this statement is ambiguous (Van de Weerd & Day 2009). The effect of environmental factors on meat quality will be summarised in the next section.

1.5 Meat quality

1.5.1 Definition

Throughout the years, pork quality has been defined in different ways. Overall, two types of quality can be distinguished: functional and conformance quality. Functional qualities are the desirable attributes in a product. For example, we might want red meat to be tender. Conformance qualities include producing a product that meets consumer's specifications exactly. For example, we want pork chops to be trimmed so there is exactly 5 mm of fat overlying the lean. Both types are important (Warriss, 2000). The components defining meat quality are listed in Table 1.3 (Warriss, 1996).

Table 1.3. Major components of meat quality (from Warriss, 1996).

Yield and gross composition	Quantity of saleable product Ratio fat to lean Muscle size and shape
Appearance and technological characteristics	Fat texture and colour Amount of marbling in lean (intramuscular fat) Colour and water holding capacity of lean Chemical composition of lean
Palatability	Texture and tenderness Juiciness Flavour
Wholesomeness	Nutritional quality Chemical safety Microbiological safety
Ethical quality	Acceptable husbandry of animals

Several components can be influenced by the way the pigs are being handled before slaughter. In the next section concerning meat quality, we will focus on appearance and technological characteristics.

1.5.2 From muscle to meat

The primary role of a muscle is to provide a means of locomotion through carefully orchestrated contraction and relaxation cycles and is modulated by fluctuations in cytosolic calcium (Ca^{2+}) levels (Bowker et al., 2000). When the contraction-induced level is reached, myosin binds to actin molecules. Further contraction is performed through a series of events in which ATP breaks the actin-myosin bond by binding to the myosin head, and ATP hydrolysis resulting in the reattachment of the myosin head to an adjacent actin molecule (Huxley, 1974). Muscle relaxation is coordinated via an ATP-dependent Ca^{2+} pump.

Exsanguination after sticking results in circulatory failure which causes a lack of oxygen in the muscles. Consequently, anaerobic glycolysis is responsible for the generation of ATP but is less efficient than the aerobic metabolism. During this process, lactic acid is produced and accumulates in the muscle, resulting in a lower pH of the muscle. In addition, glycogen and ATP levels decline. As a result, pH measured 24 h after slaughter (pH_u) is approximately 5.4 to 5.7 in the *M. longissimus dorsi* (Briskey & Wismer-Pedersen, 1961). At a certain point, ATP is no longer available in the muscle and irreversible cross-bridge formation occurs between myosin heads and actin ("actomyosin bridges"), resulting in *rigor mortis* (Bendall, 1951). After 24 h, enzymatic degradation of muscle tissue occurs and results in an increase in tenderness. Depending on the rate of post-mortem glycolysis, two main meat quality defects can be defined, i.e. Pale Soft Exudative (PSE) and Dark Firm Dry (DFD) meat. Both conditions are significant causes for financial loss. Normal meat is also called Red Firm Nonexudative (RFN) meat.

1.5.3 PSE, RSE, RFN and DFD meat

PSE meat is a major quality defect in pig meat and is associated with an abnormal high acidification rate caused by acute stress before slaughter (Figure 1.3). Pigs that are genetically sensitive to stress (Gispert et al., 2000; Guàrdia et al., 2004), or normal pigs that are subjected to stressful conditions before slaughter (Honkavaara, 1989) have a high risk to develop PSE meat. An accelerated rate of glycolysis, early post-mortem, develops while carcass temperatures are still high. The lower pH at elevated temperatures immediately after slaughter results in an increased protein denaturation in the meat, leading to reduction of water holding capacity (WHC) and higher light-scattering properties (Offer & Knight, 1988; Offer, 1991). WHC can be defined as 'the ability of meat to hold its own or added water during application of any force' (Hamm, 1986). Consequently, PSE meat is pale meat with a low water holding capacity (Bendall & Swatland, 1988; Bendall & Wismer-Pedersen, 1962; Offer & Knight, 1988; Scheffler & Gerrard, 2007). The potential mechanisms resulting in an aberrant glycolysis may be categorized in four areas and are not completely independent of one another: Ca^{2+} regulation, muscle ATPase activity, glycogenolytic enzymes, and substrate regulation (Bowker et al, 2000). Moreover, not every muscle is equally sensitive for PSE because muscle fiber type has also an effect. In general, whiter muscles have predominantly glycolytic fibres and are consequently more susceptible to PSE (Warner et al., 1993). In contrast, red muscles have predominantly oxidative fibres and are less susceptible to PSE (Briskey, 1964).

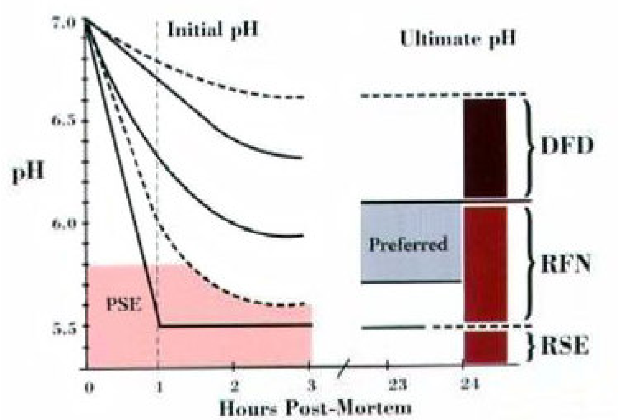

Figure 1.3. Post-mortem pH decline for DFD, RFN, PSE and RSE meat (Source: modified from Warriss, 2000).

DFD meat is a less common quality defect in pig meat and is associated with an abnormal low acidification rate caused by chronic or long term stress before slaughter (Figure 1.3). Long term stress before slaughter leads to depletion of stored glycogen, affecting the normal acidification process and keeping the pH of meat high. The high pH of DFD meat results in almost no denaturation of proteins, a high WHC and dark colour (Scheffler & Gerrard, 2007; Warriss, 2000).

Much less is known about Red Soft Exudative (RSE) meat, an intermediate form of PSE meat. RSE meat has the colour of RFN meat but the exudation of PSE meat. RSE meat is also related to abnormally low pH levels (Figure 1.3) and can result from pre-slaughter animal stress. Like PSE meat, the occurrence of RSE pork is related to certain genotypes (O'Neill et al., 2003; Van Laack & Kauffman, 1999; Warner et al., 1997). This thesis will limit to PSE meat.

1.5.4 Meat quality measurements

The variation in the rate and extent of acidification in the muscles mainly influences meat colour and water holding capacity. Information about the acidification can easily be quantified by measuring the pH. Because the pH drop for PSE meat occurs very fast immediately after slaughter (Figure 1.3), PSE meat is assessed 25-60 minutes after slaughter. Consequently, PSE quality can be defined when the pH is less than 6.0, if measured at 45 minutes after slaughter (pH_i) (Warriss, 2000). However, many different pH ranges are used in literature depending on e.g. the PSE prevalence of the country (Adzitey & Nurul, 2011). In contrast with PSE meat, DFD meat is characterised by a slow decrease in pH immediately after slaughter (Figure 1.3). As a result, DFD meat is often assessed if pH is higher than or equal to 6.0, if measured at 24 hours after slaughter (pH_u) (Warriss, 2000). Again, different pH ranges can be used (Adzitey & Nurul, 2011).

The determination of PSE, normal and DFD meat can also be achieved by measuring meat colour and drip loss (WHC). Different objective and subjective methods are used (Warriss, 2000). The following methods are described in this thesis: measuring the electrical conductivity with a pork quality meter (PQM-meter) and the filter paper method for determining the WHC of the meat; the Japanese colour reference scale and the colorimeter to determine the colour of the meat. A muscle has certain electrical characteristics such as conductivity and can be measured by a PQM-meter. Warriss (1991) reported that the PQM-meter has potential value to identify PSE meat. PSE meat has a low WHC and consequently high conductivity/drip loss. PQM values of more than 4.0 mS can be associated with PSE meat, but different ranges can be used. Drip loss can be measured directly by the filter paper method (Kauffman et al., 1986). A disc filter paper is applied for 2 seconds at 15 minutes after cutting. The paper absorbs juice and can be weighed. High weights (low WHC) are associated with PSE meat. The Japanese colour scale is a subjective method to identify PSE and DFD meat (Figure 1.4) (Van Oeckel et al., 1999). In contrast, measurements with the Colorimeter provide 3 coordinates i.e. L* (lightness), a* (red-greenness) and b* (yellow-blueness), resulting in more objective measurement. Light scattering of meat surface is probably due to difference in refractive indices of the sarcoplasm and myofibrils. A large difference involves a higher scattering and paler meat (Warriss, 2000). PSE meat is characterised by a high L* (more pale meat), low a* (less red meat) and high b* (more yellow). Overall, the best instrumental measure, related to visual colour is the L* value (Brewer et al., 2001). A low L* value (e.g. < 54) is a characteristic for DFD meat (Warriss & Brown, 1993). Meat colour is influenced by several variables (e.g. duration of blooming) and different thresholds can be used as a consequence.

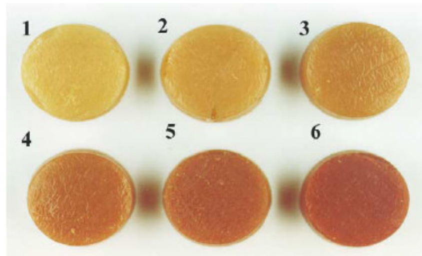

Figure 1.4. Japanese colour comparison scale for fresh meat: Blocks 1-2: PSE; Blocks 3-4: Normal; Blocks 5-6: DFD (Source: O'Neill et al., 2003).

1.5.5 Risk factors for poor meat quality

Handling on farm, time of the year, genetics and pre-slaughter handling are important factors affecting pork quality and yield and will be reported in the next paragraphs (O'Neill et al., 2003; Tarrant, 1989).

Appropriate handling on farm is very important. Literature suggests that an animal's early life rearing environment is important because it may determine its reaction to stressors later in life (Beattie et al., 1995; Simonsen, 1995). Additionally, Terlouw (2005) reported that pigs of the same breed and rearing system, which are slaughtered under similar conditions can show different stress reactivity, due to different social and other experiences. Although, the recent review of Van de Weerd & Day (2009) stated that the effect of e.g. environmental enrichment on meat quality was minimal.

The time of the year can also affect meat quality and is frequently described in literature. Guàrdia et al. (2004) reported that in summer the risk of PSE is almost twice the risk in winter. As pigs do not have working sweat glands, they are sensible for high environmental temperatures and temperature fluctuations affecting the animal's ability to maintain body temperature. This results in stress, a higher post-mortem muscle temperature and poorer meat quality (Guàrdia et al., 2004; Santos et al., 1997; Warriss, 1991). Heat stress can be overcome to a certain extent by e.g. good transport practices and showering during lairage.
In contrast, the probability of occurrence of DFD pork is higher in winter than in summer (Galwey & Tarrant, 1978). At low temperatures pigs tend to maintain deep body

temperature by huddling together or by shivering at the expense of energetic reserves in the muscle (Hrupka et al., 2000).

The presence of the halothane (HAL1843) gene in pigs has also an important effect on meat quality (Gispert et al., 2000). Halothane positive (nn) pigs have a higher lean content and increased susceptibility to pre-slaughter stress (PSE) than its heterozygous (nN) or homozygous negative conspecifics (NN) (De Smet et al., 1996; Nanni Costa et al., 1999; Fisher et al., 2000). The higher prevalence of PSE meat of nn pigs is caused by a defective Ca^{2+} release (Bowker et al., 2000). In Belgium, most pigs are nN i.e. crossbreds between homogenous halothane positive Piétrain boar and homogenous halothane negative hybrid sows. However, Cassens (2000) stated that only 4% of inferior quality is due to genetics, the remainder by pre-slaughter and post-slaughter treatment.

Pre-slaughter handling such as loading, transportation, unloading, lairage and driving to the stunning line are commonly known as being important stressors and consequently responsible for the development of aberrant pork quality (Brown et al., 2005; Fraqueza et al., 1998; Geverinck et al., 1998a; Hambrecht et al., 2004; Lambooy & van Putten, 1993; Pérez et al., 2002; Santos et al., 1997; Warriss & Brown, 1994; Guàrdia et al., 2004; Velarde et al., 2000). Attempts to run away, stop moving forward and vocalization could be used in the stress assessment (Broom, 2000). Although the occurrence of PSE and DFD meat is related to pre-slaughter stress, there is no consistent association between indices of stress and meat quality parameters (Warriss et al., 1998a).

a) Loading, transportation and unloading

Loading at farm needs to be performed calmly and is considered to be the most critical stage of the transport stage as pigs are forced to move from their familiar environment into the truck. Moreover, pigs have difficulties with ascending/descending the loading ramp (Warris et al., 1991). A possible solution is the use of a lift or the use of a ramp with an angle lower than 20° (Christensen et al., 1994; Warris et al., 1991). However, no difference in meat quality was found between loading at < 20° or > 20° (Nanni Costa et al., 1999; Warris et al., 1991).

Stressors for transport are transportation time, loading density, vibrations, movement and noise. In most EU countries the majority of transports are less than 3 hours duration with an average distance of 100 km or less (Barton-Gade & Christenson, 1998; Ramantanis et al., s.a.). Gispert et al. (2000) showed that short transports (i.e. < 2h) increased PSE meat prevalence, whereas long transports (i.e. > 2h) increased DFD meat prevalence. Short deliveries can provoke acute stress when glycogen levels are still high, which results in more PSE meat. Longer transport times may deplete glycogen content in the muscles causing DFD meat (Gispert et al., 2000).

In EU countries, stocking densities range from 0.35-0.39 m^2/100 kg (Barton-Gade & Christenson, 1998) and go up to 0.43-0.50 m^2/100 kg (Ramantanis et al., s.a.). European Directive 95/29/EC states that "loading density on truck should not exceed 235 kg/m^2 (0.425 m^2/100 kg) and a maximum increase of 20% (0.510 m^2/100 kg) may also be required depending on the meteorological conditions and journey time". However, in terms of PSE, stocking densities of 0.425 m^2 per 100 kg are only appropriate for journeys longer than 3 h (Barton-Gade & Christenson, 1998; Guàrdia et al., 2004). In general, little effect of stocking density on the prevalence of PSE or DFD meat was found in literature (Barton-Gade & Christenson, 1998; Guise & Warriss, 1989; Nanni Costa et al., 1999, 2002; Schütte et al., 1994). However, Guàrdia et al. (2004) reported that the effect of transportation time on the risk of PSE interacts with stocking density and need to be considered in future. The highest risk of PSE occurs during short transits carried out at lower densities. The first 3 h of transport the pigs tend to remain standing up, a high stocking density prevent them thrown around as a result of movement of the truck (Barton-Gade & Christenson, 1998).

Driving style (vibrations and movement) can also influence animal welfare during transport by affecting the risk of injury (Cockram et al., 2004) and can consequently affect meat quality (Peeters, 2006). This aspect will not be detailed further in this introduction.

Smooth unloading of pigs is necessary after stressful transport. The angle of the slope must not exceed 20° (Warris et al., 1991). Lifts can be used (Brown et al., 2005) but prolong the unloading time. Brown et al. (2005) also suggested the use of a container which resulted in a quick loading and unloading of pigs, but elevated stress levels were observed which could be a problem for long transports. Other factors such as noise (Geverinck et al., 1998b; Lippmann et al., 1999), shadow/dark areas (Grandin 1990; Tanida et al., 1996) and narrow passages (Grandin 1990; Lambooij, 2000) can affect the unloading process.

b) Lairage and stunning

As stated above, loading, transport and unloading can be very traumatic for the animals and a period in lairage could allow some recovery from previous stressful handling. The objective of lairage is to relieve the stress caused during delivery to the abattoir, and for this reason an improvement in meat quality is expected. It is well documented that a lairage time longer than 1 h reduces the incidence of PSE meat while a prolonged resting time can increase DFD occurrence (De Smet et al., 1996; Grandin, 1994; Malmfors, 1982; Milligan et al., 1998; Nanni Costa et al., 2002; Nielsen, 1981; Warriss et al., 1998a). A lairage time of 2-3 h is recommended (Warriss, 1995) but can vary due to practical issues. Lairage time is mostly a compromise between animal welfare, skin blemish score, meat quality and abattoir economics (Warriss et al., 1998a).

After a period in lairage, pigs are driven to the stunning point, which is very stressful. Appropriate driving of pigs, by e.g. no large groups, minimal/no use of electric goads and the use of automatic push gates results in less stress and consequently better meat quality (Barton Gade et al., 1992; Bertol et al., 2005; Hamilton et al., 2004; Hemsworth et al., 2002). Two different stunning methods are used in commercial abattoirs i.e. electrical and carbon dioxide (CO_2) stunning, and may affect meat quality. Electrical stunning can lead to a higher degree of petechial haemorrhages, bone fractures and PSE meat than CO_2 stunning (Channon et al., 2002; Gregory, 1989; Larsen 1983; Velarde et al., 2000). The higher frequency of PSE meat is caused by a higher increase in physical stress just before electrical stunning (Troeger & Woltersdorf, 1990, 1991).

c) Other risk factors

Other stressors such as the effect of nutrition, feed deprivation period (Guàrdia et al., 2004; Nanni Costa et al., 1999), vehicle characteristics (Dalla Costa et al., 2007; Ritter et al., 2008), mixing animals from different groups (Barton Gade & Christensen, 1998; Nanni Costa et al., 1999; D'Souza et al., 1999), chilling method … could also have an effect on meat quality, but are not reviewed in this study.

1.5.6 Effect of poor meat quality on cooked ham

PSE trait is internationally recognized as a major economic risk factor for fresh and processed pork, mainly because of its poorer ability to bind water (Kuo & Chu, 2003; O'Neill et al., 2003). PSE meat gives rise to poor quality meat products (ham, bacon, dried

sausage, finely comminuted sausage,…) having lower processing yields, aberrant colour (higher L*, lower a* and higher b*), increased cooking/weight losses and reduced juiciness compared to normal meat (Hendrick et al., 1994; Honkavaara, 1988; Müller, 1991; O'Neill et al., 2003; Van der Wal et al., 1988;). If PSE meat is brined, it absorbs more salt than normal meat resulting in e.g. a poor quality cooked ham. Moreover, during industrial slicing of cooked hams, the PSE-zones crumble easily, making holes or splits in the slices (lower slice ability) which are very detrimental to the presentation of the product (Franck et al., 1999; O'Neill et al., 2003). O'Neill et al. (2003) reported that, based on drip loss, cooking loss and slice ability tests, the value of a ham manufactured from PSE meat is worth half the price of a ham manufactured from normal meat. In addition, due to its appearance and shelf life, PSE hams can devalue down to 15%.
DFD meat causes bacterial spoilage in fresh meat and gives important technological problems in dry-cured products (Wirth, 1985). Because of the bacterial spoilage, processing DFD meat causes more problems than PSE meat. Moreover, DFD meat absorbs almost no salt during immersion in brine (Claeys et al., 1998).

Most studies suggest limiting the quantity of lower quality muscles included in processed meat formulations in order to maintain a high quality end product (Everts et al., 2010). However, different processes are investigated that allow the incorporation of increased quantity of lower quality meat, whereas maintaining the end quality of the product e.g. injection of ammonium hydroxide solution (Everts et al., 2010).

Because we are living in an economic driven environment it is essential to deliver a good final pork product, even more since the availability of alternatives such as poultry, meat replacers… (O'Neill et al., 2003). Therefore, it is very important that meat quality can be predicted early in the slaughter line in order to match the observed quality with the right application.

1.6 Aim and outline of the thesis

Through manipulation of the genetics of pigs and careful selection of breeds, meat industry is aiming towards the production of animals that are efficient feed converters, fast growing and having a high lean meat content with minimal production costs. As a result of this selection, pigs are more susceptible to stress which can cause quality defects/variation in pork (Adzitey & Nurul, 2011). However, the behavioural and physiological way of responding to various potentially stressful situations (stress reactivity) of each individual is unique and consequently affects meat quality (Terlouw, 2005) (Figure 1.5).

Prior experience (Figure 1.5) is affected by the pigs' rearing environment and may also influence reactivity to stress factors. Moreover, stress during fattening can result in abnormal behaviour such as tail, ear and flank biting, reduced animal welfare and productive performance (Bracke et al., 2004). In order to reduce this adverse behaviour, the risk factors need to be taken into account. Taylor et al. (2010) reported that the influence of genetics on tail biting may be more relevant at the line/strain level than between breeds. More research is needed to confirm this theory and is studied in Chapter 2. The case study was performed in a pig house with a severe biting problem. The effect on biting behaviour of (1) pigs originating from two different boars (same breed, but different line/conformation), and (2) ventilation characteristics was observed in two experiments carried out in two identical rooms on the same farm.

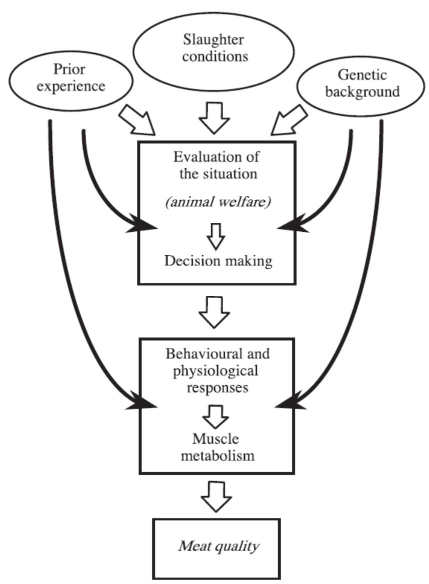

Figure 1.5. Diagram representing relationships between stress reactivity, slaughter conditions and meat quality. (1) Stress levels depend on: (a) characteristics of the situation before slaughter, (b) genetic background, (c) prior experience. (2) Perceived stress causes behavioural and physiological responses. The effect of these responses on muscle metabolism depends on: (a) the level of the responses, (b) the animal's genetic background, (c) the animal's prior history. (Source: Terlouw, 2005).

Studies suggest that environmental enrichment can partly reduce tail biting by means of the provision of substrates (Arey 1993; Beattie et al., 2000, Petersen et al., 1995) which is in consensus with the current EU legislation (Directive 2001/93/EC). Point-source enrichment objects can be an effective alternative for substrates to reduce tail biting behaviour (Van de Weerd et al., 2006) and no blockage of slurry systems occur. However, Van de Weerd et al. (2006) described that in some cases point-source enrichment objects can stimulate tail biting behaviour. Moreover, habituation to point-source objects can occur very quickly in pigs (Van de Weerd et al., 2003). Grandin et al. (1983) and Trickett et al. (2009) reported that rotation of enrichment objects can increase novelty. However, the effect of a continuous repeated sequence of several toys over the complete fattening period has not been investigated yet, to our knowledge. Therefore, Chapter 3 focuses on the sequential application of seven different 'point-source' enrichment objects in relation to pigs' toy contact and biting pen mate behaviour.

As stated above, literature suggests that an animal's early life rearing environment is important because it may determine its reaction to stressors later in life and has consequently an impact on meat quality (Beattie et al., 1995; Simonsen, 1995; Terlouw, 2005). However, the recent review of Van de Weerd & Day (2009) stated that the effect of environmental enrichment on meat quality was minimal. This could be explained by the importance of pre-slaughter stress. Stress in the period during transport and around slaughter is known to influence the physical and biochemical processes in pigs (Adeola & Ball, 1992). Literature also tended to focus on the previously described meat quality influencing factors separately or in groups but these risk factors were never analyzed together. Therefore, the effect of several pre-slaughter parameters measured during unloading, lairage and stunning on meat quality based on pH measurements in the slaughterline is investigated in the fourth chapter. The effect of season and parameters involving transport are also considered.

The occurrence of PSE and DFD meat are significant causes of financial loss i.e. poor processing characteristics, reduced yield and high potential of spoilage compared to normal meat. The determination of PSE, normal and DFD meat can be achieved by measuring meat colour, pH and drip loss. Different limits for PSE and DFD meat can be used (Adzitey & Nurul, 2011). Also, not every muscle is just as susceptible to develop PSE or DFD meat and needs further research. In the fifth chapter, (1) the relation between the biophysical parameters pH, PQM, colour and WHC, measured in the *M. longissimus dorsi*, *M. gracilis* and *M. semimembranosus* on different times post-mortem, and the quality of fresh pork meat and cooked ham, (2) the influence of season on PSE/DFD prevalence/characteristics and (3) the influence of PSE characteristics on ham quality are studied.

As a result of this thesis, guidelines will be formulated so that slaughterhouses can decrease pre-slaughter stress through good management of the proposed critical points, which will positively affect meat quality and processing characteristics. Consequently, the effect of good rearing practices (e.g. provision of appropriate enrichment) might be measured through meat quality measurements.

Following research hypotheses were investigated in order to demonstrate the novelty of this thesis:

(1) Biting behaviour of pigs within the same breed, but different genetic line can differ significantly (Chapter 2).
(2) Differences in sensitivity to ventilation between pigs of different genetic lines might occur (Chapter 2).
(3) A continuous repeated sequence of different toys can stimulate pigs' playing behaviour on the one hand, and reduce biting pen mate behaviour/wounds on the other hand (Chapter 3).
(4) The provision of a continuous repeated sequence of different toys can improve the growth performance (Chapter 3).
(5) Pre-slaughter stress has an important effect on meat quality and different critical points can be revealed (Chapter 4).
(6) Noise produced before slaughter has an effect on meat quality. A cut off value can be provided (Chapter 4).
(7) The biophysical parameters pH, PQM, colour and WHC, measured in the *M. longissimus dorsi*, *M. gracilis* and *M. semimembranosus* on different times post-mortem, and the quality of Meesterlyck cooked ham are related (Chapter 5). As a consequence, meat quality of Meesterlyck cooked ham can be predicted.

Chapter 2: A case control study of an outbreak of biting behaviour in pigs

2.1 Introduction

Tail biting is an abnormal and unpredictable behaviour in modern pig production (Moinard et al., 2003). This behaviour, which reduces animal welfare and productive performance, has been a major problem associated with industrial pig farming (Sambraus, 1985). To control this problem, many farmers tail dock all new-born piglets, but the effectiveness of this method is debatable (Chambers et al., 1995; Moinard et al., 2003; Paul et al., 2007). Moreover, in the European Union the routine docking of piglet tails is illegal (Commission 2001/93/EC of November 9th 2001). Other strategies such as the provision of environmental enrichment can reduce tail biting and are mandatory by law (Directive 2001/93/EC).

Tail biting is a multi-factorial syndrome, and there is not a clear solution to this problem (Moinard et al., 2003). Some studies have reported that certain breed types are more predisposed to tail biting, but no clear pattern has been observed (e.g. Schrøder-Petersen & Simonsen, 2001). Moreover, Taylor et al. (2010) reported that the influence of genetics on tail biting may be more relevant at the line/strain level than between breeds. Furthermore, Breuer et al. (2005) reported that the heritability of tail biting can be significantly correlated with performance parameters such as lean tissue growth ($r = 0.27$) rate and back fat thickness ($r = -0.28$). These parameters, which are widely included in pig selection programs, make it difficult to solve the biting problem. Much more research is still needed to study the effect of genetics on tail biting (biting behaviour) at the line/strain level. The climatic environment can also play an important role. Temperature, humidity, ventilation and the concentration of certain gases are all claimed to be important parameters and are reported in Chapter 1 (Geers et al., 1989; Schrøder-Petersen & Simonsen, 2001; Taylor et al., 2010; Van Putten, 1969). It is important that the ventilation system is able to create air movement to all pens within the pig house without creating draughts (Colyer, 1970). Other risk factors for tail biting include diet, method of food supply, gastro-intestinal discomfort, health status, gender, environmental stressors, group size (stocking density), age and weight, floor and other constructions in the pen, rooting materials and toys (Schrøder-Petersen & Simonsen, 2001; Taylor et al., 2010). These factors are also explained in Chapter 1.

In this study, the effect on biting behaviour of (1) pigs originating from two different boars (same breed, but different line) and (2) ventilation characteristics was observed within two experiments carried out in two identical rooms on the same farm.

2.2 Material and Methods

2.2.1 Animals and housing

Experiment 1

Between September 2008 and December 2009, a total of 355 crossbred Piétrain (P) x Belgian Landrace Negative (BN) pigs, heterozygous for the halothane gene, were followed up. The animals were housed under common conditions in two identical rooms, each with 8 pens (265 x 160 cm) and fully slatted floors, on a farm in Belgium. In both rooms, the female pigs and male castrated pigs were housed together from the age of 26 days (weaning age) until the age of 15 weeks. The pigs were followed up for 11 weeks.

All the pigs originated from one of two Piétrain boars, were randomly divided over the rooms. Those pigs originating from boar 1 (69) were tagged with an ear mark having one colour, and the pigs originating from boar 2 (286) were all tagged with another colour. Ear tagging is mandatory in Belgium, because of food safety and traceability. Boar 2 originated from a different genetic line and was characterized by producing more weaned pigs with better conformation and lean meat content than boar 1. All the pigs from the same boar were housed in the same pens. Pigs of boar 1 were evenly represented in the pens i.e. 17-18 pigs/pen (0.24-0.25 m²/animal) over both rooms. Pigs of boar 2 were evenly represented in the pens over the rooms i.e. 19-20 pigs/pen (0.21-0.22 m²/animal) for room 1 and 27-28 pigs/ pen (0.15-0.16 m²/animal) for room 2. After three weeks the pigs descending of boar 2 were rearranged in room 2 to 19-20 pigs/pen. The experimental setup is summarised in Figure 2.1.

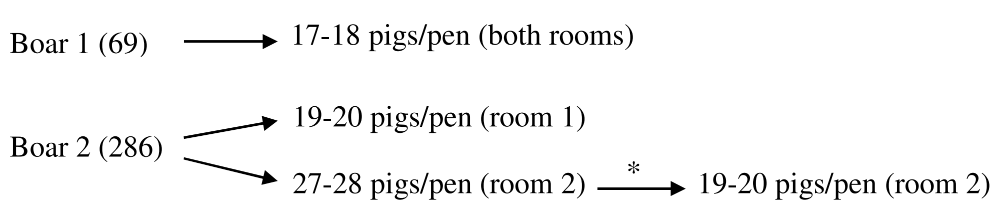

Figure 2.1. Experimental setup of experiment 1. In brackets beside boar number, there is the number of pigs investigated. * Rearrangement after 3weeks.

Pen density was managed by the farmer. Water and feed were available ad libitum. Both rooms had door ventilation. The forced ventilation was temperature controlled throughout the trial in order to maintain dry air temperature within the pigs' thermal neutral zone. A forty cm fan was present in both rooms (4500 m³/h). Chains (1 m long) provided with a wooden stump were fixed to a metal bar at the side wall in every pen as environmental enrichment.

Each pig's tail was almost completely (± 1 cm remaining) docked because there was a severe biting problem on the farm. Although tail docking is routinely prohibited (it is allowed by law before the age of 7 days to prevent chronic tail biting problem), most of the farmers in Belgium and many other countries still use this technique (Comission Directive 2001/93/EC of 9 November 2001). Tail docking was performed 3 days after birth.

Experiment 2

Between December 2008 and January 2009, a total of 314 crossbred P x BN pigs, heterozygous for the halothane gene, were followed up for 11 weeks. The experimental setup was identical to experiment 1 but all the pigs originated from the same boar (boar 1) and were housed together in groups with 19 to 20 animals per pen, equally divided over the two rooms. Pen density was managed by the farmer.

2.2.2 Experimental design

Experiment 1

Smoke tests performed before and at the start of the experiment, showed a ventilation problem in both rooms. The air inlet surface and the air flow rate were shown to be insufficient. There was also not enough air movement in all areas of the room, creating a heterogeneous air refreshment in the room (Figure 2.2).

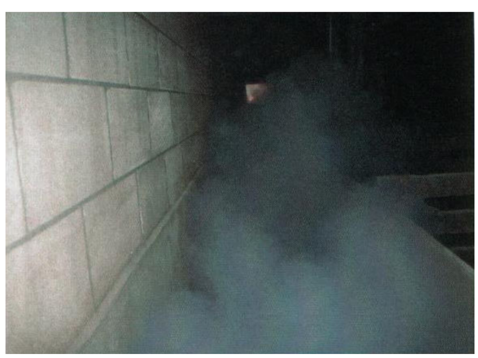

Figure 2.2. Smoke test (Source: Steensels Machteld).

The original climate setup scheme is shown in Table 2.1. In room 2, the air inlet surface was adjusted for the last 5 weeks so that the air could enter at a velocity of 1 m/s, which was calculated on the basis of the total living weight of the pigs in the pig house i.e.

1 m³/kg.h. In addition, the minimum ventilation capacity was adjusted to 0.25 m³/kg.h. These adjustments are in conformity with those of Van Gansbeke et al. (2009). Multiple smoke tests were performed every week to validate the set objectives for air and ventilation patterns. In room 1, the air inlet surface and minimum ventilation capacity was not changed as a reference for standard practice by the farmer. After 11 weeks, the pigs were moved to another pig house.

Table 2.1. Climate setup scheme for pigs between 5-40 kg.

Weight (kg)	Temperature (°C)	Minimum ventilation rate (%)*	Bandwidth (°C)**
5	29	6	8
10	26	10	8
20	24	16	8
30	23	20	8
40	23	20	8

* Written as % of the maximum ventilation rate.
** Bandwidth is the difference in temperature between minimum and maximum ventilation level.

Experiment 2

The experimental setup was identical to that of experiment 1, except that the ventilation pattern was adjusted from the start of the trial. Additional measurements were performed in the middle of the room at pig level; namely, mean temperature (°C), relative humidity (%), CO_2 concentration (ppm) and air pressure (hPa) (Testo 435, Testo NV, Ternat, Belgium). These measurements were performed in both rooms on the observation days.

2.2.3 Behavioural measurements

For both experiments, the number of different pigs that showed tail, ear, flank, and feet biting were continuously observed per minute, every Monday for 2 times 10 minutes per pen. These behaviours were expressed as percentages (i.e. the percentage of pigs that performed tail, ear, flank, and feet biting in a pen during a minute). The observations started at 13.00h, since studies had indicated that pigs are more active in the afternoon (Olsen et al., 2000), and disruption by the farmer is minimal at that time of day. On every observation day, behavioural measurements were performed 15 min after entering the room by the same trained observers (2). This time was taken to restore pig's normal behaviour after having entered the room. Both rooms were always observed in the same order. Biting behaviour was defined as chewing or biting the tail, ear or feet of another pig.

For each pen, the total mean biting behaviour percentage per minute, pen and observation day was calculated as the sum of the means of the total observed percentages for tail, ear, flank, and foot biting per minute and pen of that observation day. In the following text, the total mean biting behaviour percentage per minute, pen and observation day is referred to as the 'total mean biting percentage'. The number of pigs with ear lesions (presence of fresh blood) was counted per pen.

2.2.4 Statistical analysis

The data were analyzed using SAS software (version 9.2, SAS Inst., Inc., USA, 2008). The data were checked for normality, and the summary statistics (means and standard deviations) were explored. The biting behaviour data were analyzed per observation period using linear mixed models with pen as random factor and interaction terms where needed. The wound percentage did not meet the distributional assumptions and was therefore dichotomized using the third quartile (Q3) as cut-off value. Every observation lower than Q3 was considered 0 and higher was considered 1. Differences in wound percentages between rooms 1 and 2 were analyzed per observation period using logistic mixed models with pen as random factor and interaction terms where needed. The differences between the rooms concerning climate measurements (temperature, CO_2 concentration, relative humidity and air pressure) were analysed using multiple ANOVA models.

2.3 Results

Experiment 1

Of all biting behaviours, ear biting was noted the most frequently per minute (3.72% ± 0.08 SEM), followed by feet (0.83% ± 0.04 SEM) and tail biting (0.26% ± 0.02 SEM). No flank biting was observed. As described in the behavioural measurements, these biting parameters were combined into a new parameter, namely the total mean biting percentage (4.81% ± 0.09 SEM). During the study, 38.07% ± 0.63 SEM of the observed pigs had ear biting marks.

Genetics had a significant ($P < 0.05$) effect (Table 2.2). Pigs originating from boar 2 showed significantly more biting behaviour than pigs descending from boar 1, both for the period before the ventilation change (period 1) and for the period after the ventilation change (period 2), except for room 2 during period 2. The difference in biting behaviour between rooms 1 and 2 was significant for the periods both before and after the ventilation change for the pigs descending from boar 2.

Table 2.2. Biting percentage (lsmeans ± SE) before (period 1) and after (period 2) ventilation change for experiment 1.

	Room 1	Room 2
First 6 weeks (no ventilation change)		
Boar 1	2.72 ± 0.68 ^{ax}	3.81 ± 0.76 ^{ax}
Boar 2	5.86 ± 0.41 ^{bx}	7.16 ± 0.46 ^{by}
Last 5 weeks (ventilation change)*		
Boar 1	2.39 ± 0.61 ^{ax}	2.03 ± 0.62 ^{bx}
Boar 2	4.27 ± 0.36 ^{bx}	2.79 ± 0.36 ^{by}

* The ventilation was only adjusted in room 2.
^{ab} Scores in the same column and period with different superscripts differ significantly ($P < 0.05$).
^{xy} Scores in the same row and period with different superscripts differ significantly ($P < 0.05$).

The decrease over age in biting behaviour was significant for the pigs originating from boar 2 for period 1, but not for those originating from boar 1 (Figure 2.3). A significant decrease in biting behaviour for pigs originating from boar 2 was found only for period 2 (Figure 2.4).

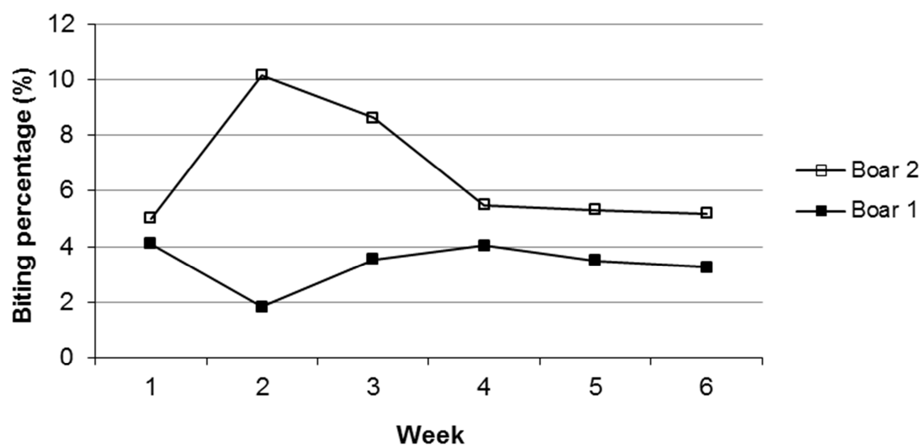

Figure 2.3. Mean biting percentage of pigs originating from boar 1 and 2 before ventilation change (period 1) for both rooms together.

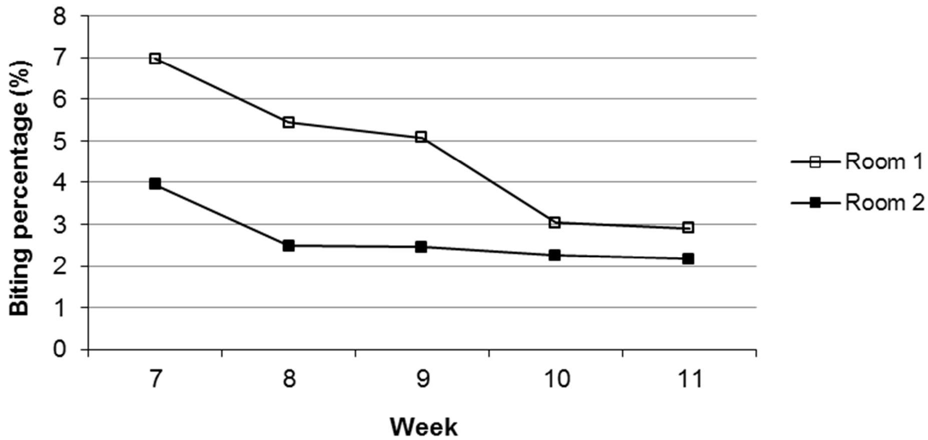

Figure 2.4. Mean biting percentage of pigs originating from boar 2 after ventilation change (period 2) for both rooms seperately.

The differences in wound percentages were significant (Table 2.3) between the rooms for period 2, but not for period 1. Pigs originating from boar 2 had significantly more wounds than those originating from boar 1 for both periods.

Table 2.3. Wound percentage (means ± SEM) before (period 1) and after (period 2) ventilation change for experiment 1.

	Room 1	Room 2
First 6 weeks (no ventilation change)		
Boar 1	7.68 ± 5.13 ^{ax}	17.62 ± 6.23 ^{ax}
Boar 2	28.27 ± 7.04 ^{bx}	35.63 ± 4.82 ^{bx}
Last 5 weeks (ventilation change)*		
Boar 1	4.94 ± 1.99 ^{ax}	18.50 ± 7.39 ^{ay}
Boar 2	45.15 ± 8.46 ^{bx}	84.50 ± 3.58 ^{by}

* The ventilation was only adjusted in room 2.
^{ab} Scores in the same column and period with different superscripts differ significantly ($P < 0.05$).
^{xy} Scores in the same row and period with different superscripts differ significantly ($P < 0.05$).

Experiment 2

Of all biting behaviours, ear biting was noted the most per minute (1.87% ± 0.12 SEM), followed by feet (0.31% ± 0.04 SEM) and tail biting (0.06% ± 0.02 SEM). No flank biting was observed. These biting parameters were combined into a new parameter, namely the total observed mean biting percentage (2.23% ± 0.13 SEM). During the study, 9.18% ± 1.30 SEM of the observed pigs had ear biting marks. The mean values of temperature, CO_2 concentration, relative humidity and air pressure per room are presented in Table 2.4. CO_2 concentration was in both rooms below the maximal allowed level. A significant difference between rooms 1 and 2 was found for mean relative humidity. The mean observed temperatures were lower than the temperature set on the thermostats.

Table 2.4. Temperature, CO_2 concentration, relative humidity and air pressure (lsmeans ± SE) per room for experiment 2.

	Room 1*	**Room 2****
Temperature (°C)	22.2 ± 0.3^{a}	22.4 ± 0.4^{a}
CO_2 concentration (ppm)	2038 ± 62^{a}	1999 ± 62^{a}
Relative humidity (%)	83.3 ± 1.5^{a}	75.8 ± 1.5^{b}
Air pressure (hPa)	1012 ± 3^{a}	1011 ± 3^{a}

^{ab} Scores in the same row with different superscripts differ significantly ($P < 0.05$).
* Room 1: No ventilation adjustments.
** Room 2: Ventilation adjustments.

The pigs in Rooms 1 and 2 did not differ significantly in biting behaviour, either with or without the ventilation adjustment (Table 2.5). The number of wounds differed significantly between the two rooms.

Table 2.5. Biting (lsmeans ± SE) and wound percentage (means ± SEM) per room for experiment 2.

	Room 1*	**Room 2****
Biting percentage (%)	2.34 ± 0.20^{a}	2.27 ± 0.20^{a}
Wound percentage (%)	14.68 ± 2.08^{a}	2.79 ± 0.88^{b}

^{ab} Scores in the same row with different superscripts differ significantly ($P < 0.05$).
* Room 1: No ventilation adjustments.
** Room 2: Ventilation adjustments.

2.4 Discussion

Biting behaviour in pigs is a major problem in modern pig production because it reduces animal welfare and productive performance. The farm studied in these two experiments was selected because a severe tail biting problem was present. In order to reduce tail biting, the farmer was docking the pigs' tails almost completely (Hunter et al., 2001). Consequently, the biting problem moved from the tail mainly to the ears, which resulted in multiple wounds. Fraser and Broom (1990) also reported that the attention of tail docked pigs may be redirected to other body parts of pen mates. Tail docking in order to prevent (tail) biting behaviour is questionable (Chambers et al., 1995; Moinard et al., 2003; Paul, 2007).

In the first experiment, the pigs originated from two different boars, originating from two different lines. In general, pigs originating from boar 2 showed significantly more biting behaviour and consequently more biting wounds than the offspring of boar 1 (Tables 2.2 and 2.3). This result agrees with Taylor et al. (2010), who concluded that the influence of tail biting i.e. biting behaviour may be more relevant at the line/strain level than between breeds. Boar 2 was characterized by producing more weaned pigs with better conformation and higher lean meat content than those from boar 1. Breuer et al. (2005) also associated greater tail biting predisposition with increased lean tissue growth rate. The high prevalence of biting behaviour was reduced by improving the air quality through adapting the ventilation (Table 2.2) and is in consensus with literature (Geers et al., 1989; Schrøder-Petersen & Simonsen, 2001; Taylor et al., 2010; Van Putten, 1969). The pigs originating from boar 2 were more affected by the ventilation pattern than the pigs originating from boar 1. After the ventilation adjustments, the biting behaviour was not significantly lower for pigs of boar 1 than for pigs of boar 2. It is plausible that the pigs originating from boar 2 were more susceptible to stress (poor ventilation) and consequently had a higher level of biting behaviour which disappeared when the ventilation pattern was improved. Smoke tests showed air movements in all areas of the rooms without the creation of draughts, which had a positive effect on avoiding biting behaviour. Draughts seemed to encourage tail biting (Colyer, 1970). Although the biting behaviour decreased due to the ventilation change, a higher proportion of wounds were observed in room 2 after the change for pigs of boar 2 (Table 2.3). These findings could be due to the room effect for biting behaviour observed in period 1 and the difference in time between the observation of the biting behaviour and the severity of the wounds because 'the injury phase' is preceded by the 'pre-injury phase' (Fraser, 1987). During the first three weeks, the pen density for pigs of boar 2 in room 1 was 19-20 pigs/pen and 27-28 pigs/pen for room 2; after the first three weeks, it changed to 19-20 pigs/pen for room 2. Both a higher stocking density (Schrøder-Petersen & Simonsen, 2001) and the removal of pigs from a group (both of which were the case for room 2) can result in higher levels of biting behaviour and numbers of wounds. The removal of pigs from a pen will rearrange the hierarchy in the pen, which will lead to increased fighting and wounds.

Because a room effect was observed due to the difference in pen density during the first three weeks of experiment 1, a second experiment was performed with the same pen densities (19-20 animals/pen). All the pigs involved were descendants of boar 1. Also, the ventilation was adjusted from the start in room 2. The ventilation adjustment had a significant positive effect on the percentage of observed wounds, but not on the observed biting behaviour (Table 2.5). The percentage of observed wounds is of greater significance because it is a cumulative factor. In contrast, the biting behaviour is only a snap-shot of the situation. Consequently, the biting percentage in room 2 of Table 2.5 could possibly be overrated. On the other hand, the observation of pigs' biting behaviour is still appropriate, definitely when little or no wounds are present (e.g. Chapter 3). Moreover, a biting problem can be present at a pighouse without visible wounds. It could also be possible that only a few agressors caused the wounds (i.e. low biting percentage and high percentage of wounds) (Table 2.5). In that case, the removal of the offender(s) is applicable. This hypothesis could also explain the higher percentage of wounds in room 2 after the change for pigs of boar 2 in Table 2.2. The ventilation improvements resulted in a better ventilation pattern (no draughts), as visualized by the smoke tests, and a significantly lower relative humidity. The observed temperatures were lower than expected (Table 2.1), due to the fact that it was winter. In general, all the observed climate parameters (excluding mean humidity: > 80%) were within the standard ranges, and did not differ much between rooms 1 and 2. Nevertheless, it can be assumed that suboptimal levels of temperature, CO_2 concentration etc. can contribute to creating an aversive environment, even when no single factor may be markedly different from the standard acceptable values (Done et al., 2005; Wathes et al., 2004). It should also be noticed that climate parameters are not homogeneous in time and space and was not taken into account (Van Gansbeke et al., 2009). Extra measurements such as the continuous logging of the temperature on several places could give more information.

In general, a decreasing trend ($0.05 < P < 0.08$) was observed for the prevalence of biting behaviour over time in both experiments. This effect was greater for the pigs originating from boar 2 (experiment 1) because they could have been more stress susceptible and therefore needing more time to adapt to the new situation compared to the pigs originating from boar 1. The activity of pigs decreases over age (Stolba & Wood-Gush, 1989) and might be the reason for the decreasing biting and wound percentages over age in this survey.

In each pen, a chain provided with a wooden stump was present during the whole observation period and was not changed. Due to this lack of novelty, little reduction in biting behaviour has to be expected (Van de Weerd et al., 2003). Appropriate use of environmental enrichment can reduce biting behaviour. For example, the use of a continuous repeated sequence of different toys (novelty) can enhance pigs' playing behaviour and consequently reduce biting pen mate behaviour and wounds. This topic will be studied in the next chapter.

More research is needed to investigate the effect of different pen densities on biting behaviour of pigs descending from different boars (same breed different line) because the pen densities in this research were rather high.

2.5 Conclusions

Biting lesions are considered to have a detrimental implication on animal welfare, productive performance and a clear solution for this problem is not unambiguous. Tail docking was not a good solution for avoiding biting behaviour in pigs in the present study as the tail biting behaviour moved to other body parts.

Genetics, age and ventilation were found to have a significant impact on the biting behaviour/wounds in case pigs are housed at a rather high stocking density. In detail:

- Pigs descending from boar 2 were more eager biters than pigs from boar 1.
- Biting behaviour decreased with age.
- Appropriate ventilation (e.g. air inlet at a velocity of 1 m/s) had a positive effect on pigs' biting behaviour and wounds.

Chapter 3: Comparison of pig behaviour when given a sequence of enrichment objects or a chain continuously

Chapter redrafted after Van de Perre, V., Driessen, B., Van Thielen, J., Verbeke, G., & Geers, R. (2011). Comparison of pig behaviour when given a sequence of enrichment objects or a chain continuously. *Animal Welfare*, *20*, 641-649.

3.1 Introduction

Exploratory behaviour represents an important need of pigs (Studnitz et al., 2007). Even in intensive husbandry systems, pigs seem to be highly motivated to explore, even when feed is available ad libitum (Lyons et al., 1995; Van Putten & Dammers 1976). When it is difficult or impossible to express this behaviour, the pig may redirect it towards its pen mates (Beattie et al., 2000; Kelly et al., 2000; Scott et al., 2006a), possibly resulting in tail and ear biting behaviour (Scott et al., 2006b; Van de Weerd et al., 2005). Tail biting is rarely observed under extensive, semi-natural or feral conditions and can consequently be defined as 'abnormal' behaviour (Moinard et al., 2003).

Tail biting is a major problem in modern pig production, reducing animal welfare and productive performance (Bracke et al., 2004). This abnormal behaviour is a multi-factorial syndrome and influencing factors have often included environmental features. Risk factors for tail biting are genetics, gender, age and weight, health status, rearing environment, indoor climate, stocking density and pen size, floor, feeding systems, food, rooting materials and toys (Schrøder-Petersen & Simonsen, 2001).

In an attempt to control tail biting, many farmers dock the tails of all new-born piglets. Controlled experiments show that docking is effective in reducing tail biting, although, as these surveys show, not in eliminating it (Chambers et al., 1995; Moinard et al., 2003; Paul et al., 2007). Moreover, in the European Union routine docking of piglet tails is illegal (Comission 2001/93/EC of 9 November 2001), thus alternative preventive strategies are needed.

Studies suggest that environmental enrichment can partly reduce tail biting through the provision of substrates (Arey, 1993; Beattie et al., 2000, Petersen et al., 1995) which is in consensus with the current EU legislation (Directive 2001/93/EC). This Directive requires that pigs must have access to a sufficient quality of these materials to enable proper investigation and manipulation activities. Different forms of enrichment have been used in the past. Substrates such as peat, straw, sawdust and mushroom compost are effective types of enrichment (Beattie et al., 1995). However, when these 'rooting' substrates are combined with slatted floors, potential problems with blockage of slurry systems occur

(Van de Weerd et al., 2006). Slatted floors are the most common husbandry systems in the EU (Hendriks et al., 1998). Therefore, other types of enrichment are needed. On the one hand, point-source enrichment objects can be an effective alternative to reduce tail biting behaviour (Van de Weerd et al., 2006). On the other hand, Van de Weerd et al. (2006) also described that in some cases point-source enrichment objects can stimulate tail biting behaviour. More studies are still needed to investigate the kind of composition material of the objects, the position of the enrichment object in the pen and the playing behaviour in relation with the object. Habituation to point-source objects can occur very quickly in pigs (Van de Weerd et al., 2003). Grandin et al. (1983) and Trickett et al. (2009) reported that rotation of enrichment objects can increase novelty. However, the effect of a continuous repeated sequence of different toys over the complete fattening period has not been investigated yet, to our knowledge. Therefore, this study focused on the sequential application of seven different 'point-source' enrichment objects (commercial and non-commercial) in relation to pigs' toy contact and biting pen mate behaviour with age starting from 20 kg till slaughter weight.

3.2 Material and methods

3.2.1 Animals and housing

One hundred and eight crossbred (Piétrain x Hypor) pigs, heterozygous for the halothane gene, were used. Animals were housed under common conditions in a room with 12 pens and fully slatted floors at the Zootechnical Centre of the K.U.Leuven (Belgium) from 18 June till 13 October 2008. The female piglets (56) and male castrated piglets (52) were housed together in equal groups of 8 to 10 piglets per pen (195 x 295 cm) at the age of 10 weeks (20 kg) until an age of 14 weeks (40 kg). Every pig was ear tagged for individual identification. Water and feed were available ad lib. At the age of 14 weeks the pigs were moved to a larger room (195 x 400 cm) until slaughter weight (110 kg). The pigs were maintained in their existing groups. Forced ventilation was temperature controlled until the end of the trial in order to maintain dry air temperature within the pig's thermoneutral zone. Chains (1 m long) were attached to the side wall in every pen in the middle of the walking path between the drinking nipple and the feeder. Studies have shown that this is the best place to attach objects so that the resting area in the pen is preserved (Geenen et al., 2009). The chains were present from weaning till slaughter.

3.2.2 Environmental enrichment objects

Seven different materials (Figure 3.1), commercial (rubber bar and rubber ball) (supplied by Schippers BVBA, Arendonk, Holland) and non-commercial (yellow ribbon, orange rope, yellow garden hose, purple ribbon and grey garden hose), were tested. The non-commercial materials were prepared by the technicians of the Zootechnical Centre. These toys were selected because of their different characteristics. The geometric properties of every toy are shown in Table 3.1.

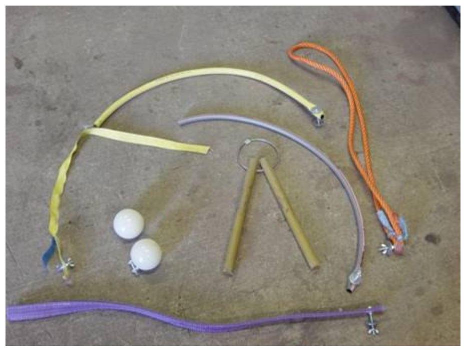

Figure 3.1. Used enrichment materials (Source: Van de Perre Vincent).

Table 3.1. Dimensions and mean destructibility of the used commercial (c) and non-commercial (nc) objects.

	Length (cm)	Width (cm)	Thickness (cm)	Diameter (cm)	Destructibility*	Sort
Yellow ribbon	83	2.3	0.1	-	3	nc
Orange rope	80	-	1	-	3	nc
Yellow garden hose	96	-	1.6	-	2	nc
Purple ribbon	86	3.3	1	-	3	nc
Rubber bar	34	14	4	-	1-2	c
Grey garden hose	89	-	1.6	-	2	nc
Rubber ball	-	-	-	7.5	1	c

* Score 3: More than 50% of the toy was destructed after 1 week of presentation, Score 2: 10-50% of the toy was destructed after 1 week of presentation, Score 1: Less than 10% of the toy was destructed after 1 week of presentation.

3.2.3 Experimental design

The experiments started at different weights (20 kg, 40 kg or 70 kg) and were continued till slaughter weight. Pigs were presented seven different enrichment objects successively. At a weight of 20 kg, three pens were equipped with the first toy (Table 3.2.). The object was attached to the chain in every pen. Pen 1 was taken as a control pen (only a chain). One week later, the toy was replaced by another type of toy. After 7 weeks, when the seven toys were used, a new round of administering the same sequence of toy presentation was repeated in order to standardise the procedure in view of statistical power requirements.

This action was repeated till the pigs reached their slaughter weight. Furthermore, at a weight of 40 kg, three other pens also received seven different enrichment objects successively, starting with the same object as given that week to the group of pigs who received toys starting at 20 kg. Pen 5 was the control pen. Finally, starting at a weight of 70 kg, three last pens received the same enrichment objects as the other pens one by one. Pen 12 was taken as a control pen.

At the age of 10, 14, 20 and 24 weeks the pigs were weighted and daily growth and feed conversions were calculated. Feed conversion was calculated on pen level.

Table 3.2. Presentation order of the toys (1-7) per pen over the weeks.

	Fattening period 1*				**Fattening period 2***							**Fattening period 3***					
Week	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Pen 2, 3, 4	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3
Pen 6, 7, 8					5	6	7	1	2	3	4	5	6	7	1	2	3
Pen 9, 10, 11												5	6	7	1	2	3

Pen 1, 5 and 12 had a chain throughout.
* Fattening period 1: 20-40 kg; Fattening period 2: 40-70 kg; Fattening period 3: 70-100 kg.
1: Yellow ribbon, 2: Orange rope, 3: Yellow garden hose, 4: Purple ribbon, 5: Rubber bar, 6: Grey garden hose, 7: Rubber ball.

3.2.4 Behavioural measurements

Toy contact behaviour for the seven different enrichment objects (Table 3.3) was recorded individually per pig every 2 minutes during 1 hour, from 1300h till 1400h as studies indicated that pigs were more active in the afternoon (Olsen et al., 2000), without interference from the farmer. During toy contact observations, biting pen mate behaviour (Table 3.3) of the pigs in the enriched pens and their respective control pen(s) was also recorded.

Table 3.3. Ethogram of the observed behaviours.

Behaviour	**Description**
Toy contact behaviour	Nose or mouth in contact with an enrichment device
Biting pen mate behaviour	Chewing or biting tail, ear of another pig

The toy contact and biting pen mate behaviours were observed on the day of introduction of the environmental enrichment and 5 days after introduction. On the first (respectively fifth) observation day, behaviour measurements were performed 15 min after installing of the toys (respectively entering the room). This time was taken to restore pig' normal behaviour after having entered their pen or room. No cameras were used because direct visual observations are more precise and detailed than observation via a camera.

3.2.5 Lesion scores

During the entire experiment each pig's tail (Zonderland et al., 2008) and ears were scored for all 12 pens using 2 parameters: tail/ear damage (3 classes) and blood freshness (4 classes) (Table 3.4). All pens which did not receive any toys were treated as control pens. These observations were taken at the end of observation days 0 and 5.

Table 3.4. Scores for the two tail/ear parameters (Zonderland et al., 2008); tail /ear damage and blood freshness (Zonderland et al., 2008).

		Description
Tail/ear damage		
1	**No**	No tail/ear damage visible
2	**Bite marks**	Small damages/bite marks are visible. These individual bite marks have the size of a pinhead
3	**Wound**	Clearly visible wound
Blood freshness		
1	**No**	No blood visible
2	**Dried**	Old dried black blood in the form of a scab
3	**Sticky**	Sticky dark red blood, mainly a half day to day old
4	**Fresh**	Fresh bleeding wound

3.2.6 Statistical analysis

The data were analyzed using SAS software (version 9.2, SAS Inst., Inc., USA, 2008). Data were checked for normality and univariate analysis was performed. The behavioural data (toy contact and biting pen mate) did not have a normal distribution and were dichotomized. The third quartile (Q3) was used as the cut off value. Every observation lower than Q3 was appointed 0, higher was appointed 1. This cut off value yielded a classification that corresponded best with the original behavioural observations.

The data were analyzed using mixed models which accounts for the clustering of the data within pens and pigs and hence do not assume measurements from pigs within pens to be independent. Indeed measurements from the same animal, or measurements from different animals in the same pen are not independent and social facilitation is to be expected. Similarly, Fraser (1978) analyzed the behaviour of group housed piglets with the individual piglets being regarded as the experimental unit. In the analysis of differences in behavioural data, logistic mixed models were used with animal and pen as random factors to cover the potential correlation of the observations within animals and pens. For toy contact behaviour, the fixed effects were observation day, type of toy, fattening period (20-40 kg, 40-70 kg and 70-110 kg) (age) and observation period (first, second or third 20 minutes of the observation hour). The fixed effects for biting pen mate behaviour were the presence of toys, gender, observation day, type of toy, fattening period (age). For both behaviours, the number of times the toy was presented was also taken into account and was nested within the fattening period when the toys were given first. For the analysis of differences in frequency scores, when only a chain or a sequence of toys was present, again logistic mixed models were used with pen as well as animal as random effects. The reported mean percentages in this survey are from the original non-transformed data. Significant differences were obtained after analysis of the dichotomized data. Associations between growth and feed conversion on the one hand, and presence of enrichment (sequence or only permanent chain) on the other hand were studied using linear mixed models, with fixed effects the presence of toys, gender and fattening period, and with pen as a random effect. Animal was also added as extra random effect for the growth analyses. These data had a normal distribution and lsmeans values ± SE are reported.

3.3 Results

During the experiment, tail and ear biting wounds were rarely observed. However, in 1 of the 12 pens there was a biting problem during approximately 3 weeks, which resulted in multiple wounds. Ear and tail biting behaviour, i.e. biting pen mate behaviour, was observed in all pens. In general, the two presentation orders (Table 3.2) used in this study had no significant effect on the mean values of toy contact behaviour (respectively biting pen mate behaviour) observations for the different toys. This was in contrast with the other included fixed effects which all had a significant ($P < 0.05$) contribution to the statistical model.

The numeric ranking of the toys for toy contact and biting pen mate behaviour was not a statistically supported difference in most cases. Therefore, Figure 3.2 and 3.3 need to be consulted.

3.3.1 Toy contact behaviour

Results of toy contact behaviour for the seven different types of environmental enrichment, for both observation days separately, are shown in Figure 3.2. Differences between the toys were found (*P* < 0.05). In general, toy contact behaviour was significantly higher (*P* < 0.0001) for observation day 0 (18.19%) than for observation day 5 (6.27%). Overall, the following numeric ranking for the different toys in toy contact behaviour could be made: the orange rope was the most popular toy followed by the yellow ribbon and the purple ribbon for observation day 0, while the yellow garden hose was the least popular. The same numeric ranking could be made for observation day 5: the orange rope was the most popular toy followed by the purple ribbon and the grey garden hose, while the rubber ball and rubber bar were the least popular.

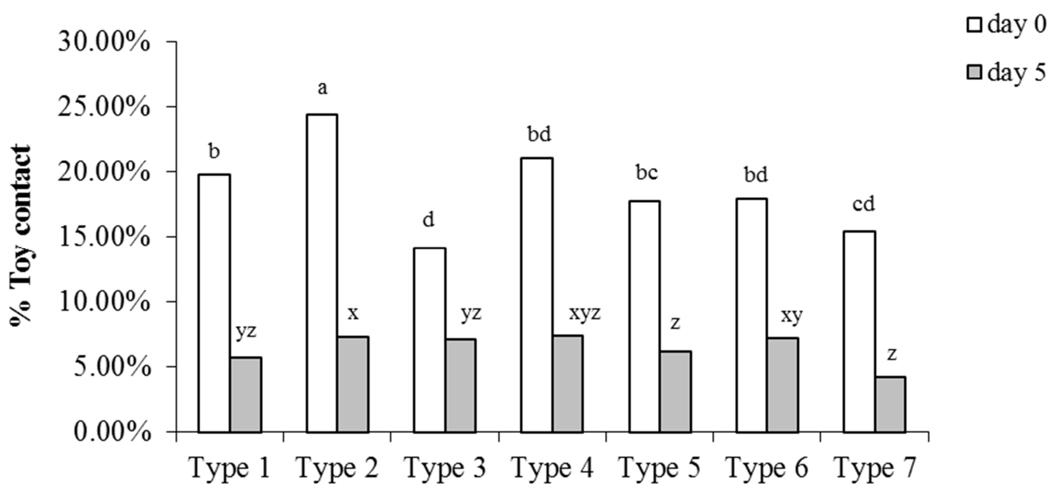

Figure 3.2. Mean percentage toy contact behaviour at observation day 0 and 5 for the seven different types of environmental enrichment during the complete experiment for all pens and fattening periods together. Toy contact behaviour was always significant higher (*P* < 0.0001) for observation day 0. Type 1 = yellow ribbon; Type 2 = orange rope; Type 3 = yellow garden hose; Type 4 = purple ribbon (no observations during fattening period 1: 20-40 kg and 3: 70-100 kg); Type 5 = rubber bar (no observations during fattening period 1); Type 6 = grey garden hose (no observations during fattening period 1); Type 7 = rubber ball (no observations during fattening period 1). Statistical comparisons are carried out on the dichotomized data. ^{abcd}: significant differences (*P* < 0.05) for day 0; ^{xyz}: significant differences (*P* < 0.05) for day 5. Bars with the same superscript are not significantly different.

For both observation days the following results were found. The percentage of toy contact behaviour decreased (*P* < 0.001) from period 1 (0-20 min) (14.94%) to period 2 (20-40 min) (12.03%) and period 3 (40-60 min) (10.58%). No significant difference (*P* > 0.05) between period 2 and 3 was observed.

Pigs received a sequence of enrichment objects at 3 different weights. Most toy contact behaviour was observed when the pigs received a sequence of enrichment toys for the first time at fattening period 1 (20-40 kg) compared to fattening period 2 (40-70 kg) or 3 (70-110 kg) (*P* < 0.05) (Table 3.5). Toy contact behaviour decreased significantly (*P* < 0.05) with age and repetition of the sequence. Presenting a toy and consequently the sequence of toys for the first time caused a significant ($P < 0.001$) increase in toy contact behaviour compared to pigs of the same age who received the toy(s) for a second or third time. No differences in toy contact behaviour were found when pigs of 70 kg to 110 kg received toys since 20 kg and/or 40 kg body weight. No differences in toy contact behaviour between gilts and barrows were found.

Table 3.5. Mean percentage of biting pen mate and toy contact behaviour of pigs per fattening period for both observation days together when a sequence of toys or one single toy (chain) was present.

	Period 1 (20-40kg)	**Period 2 (40-70kg)**		**Period 3 (70-110kg)**		
Start the sequence of toys at	20 kg	20 kg	40 kg	20 kg	40 kg	70 kg
% Biting						
Sequence of toys	1.10 [a] (n=25*)	0.28 [ax] (n=24)	0.34 [ax] (n=27)	0.22 [ax] (n=24)	0.15 [ax] (n=27)	0.12 [ay] (n=30)
Only chain	2.03 [b] (n=8)**	0.59 [bx] (n=17)**	0.59 [bx] (n=17)**	0.28 [ax] (n=27)**	0.28 [ax] (n=27)**	0.28 [bx] (n=27)**
% Playing						
Sequence of toys	17.93 (n=25*)	12.16 [x] (n=24)	15.05 [y] (n=27)	6.67 [x] (n=24)	9.53 [x] (n=27)	14.34 [y] (n=30)

Statistical comparisons are carried out on the dichotomized data.
* One pig died.
** Mean percentage of biting pen mate behaviour of the pigs in the control pen(s) (1 pen for period 1, 2 pens for period 2 and 3 pens for period 3).
[ab] Scores in the same column, for a sequence of enrichment toys and only one toy (chain), with different superscripts differ significantly ($P < 0.05$).
[xy] Scores in the same row, within the same fattening period, with different superscripts differ significantly ($P < 0.05$).

3.3.2 Ear and tail biting behaviour

Biting pen mate behaviour for the seven different types of environmental enrichment, for both observation days separately, are shown in Figure 3.3. Differences between the toys were found ($P < 0.05$). More specifically, biting pen mate behaviour was significantly ($P < 0.001$) higher for observation day 5 (0.49%) than for observation day 0 (0.24%). However, no differences between observation day 0 and 5 were found for the yellow ribbon, orange rope and the rubber bar. Overall, the following numeric ranking for the different toys in biting pen mate behaviour can be made: the rubber bar, orange rope, yellow ribbon and purple ribbon could be associated with the most biting pen mate behaviour for observation day 0, while the rubber ball and the grey garden hose were associated with the least biting pen mate behaviour. The same numeric ranking can be made for observation day 5: biting pen mate behaviour was highest for the purple ribbon and the yellow garden hose, while the grey garden hose and the rubber ball caused the least biting pen mate behaviour.

For both observation days the following results were found. The percentage of biting pen mate behaviour was not influenced by the observation period ($P > 0.1$). Biting percentage was 0.43% for period 1, 0.46% for period 2 and 0.35% for period 3.
The presence of a continuous sequence of toys being attached to a chain reduced the risk of biting pen mate behaviour significantly (0.36%) versus one single toy (chain) (0.58%) ($P < 0.05$). Most biting pen mate behaviour was observed ($P < 0.0001$) when the pigs received a sequence of toys for the first time at fattening period 1 compared to fattening period 2 or 3 (Table 3.5). Biting pen mate behaviour decreased significantly ($P < 0.001$) with age. Presenting a toy and consequently the sequence of toys for the second or third time to pigs of the same age caused a significantly ($P < 0.001$) increase in biting pen mate behaviour compared to presenting the toy(s) for the first time at that same age. No differences in biting pen mate behaviour were found when pigs of 70 kg to 110 kg received toys at 20 kg and/or 40 kg body weight. During the first 4 weeks of biting pen mate observation only one control pen was used. The mean percentage of biting pen mate behaviour for this control pen (0.56%) did not differ ($P > 0.1$) with the other control pen (0.62%) during fattening period 2. Also no differences ($P > 0.05$) in biting pen mate behaviour were found between the control pens (0.25%, 0.32% and 0.26%) during fattening period 3. The mean values of biting pen mate behaviour of the pigs in the control pens per fattening period are presented in Tabel 3.5. During the experiment the gilts expressed significantly higher biting pen mate behaviour (0.47%) than the castrated males (0.35%) ($P < 0.05$).

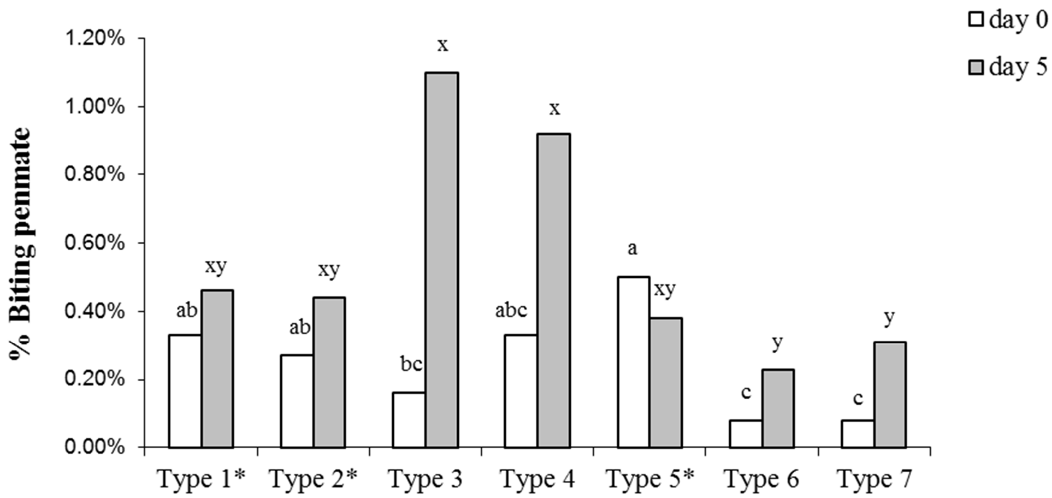

Figure 3.3. Mean percentage biting pen mate behaviour at observation day 0 and 5 for the seven different types of environmental enrichment during the complete experiment for all pens and fattening periods together. Type 1 = yellow ribbon; Type 2 = orange rope; Type 3 = yellow garden hose; Type 4 = purple ribbon (no observations during fattening period 1: 20-40 kg and 3: 70-100 kg); Type 5 = rubber bar (no observations during fattening period 1); Type 6 = grey garden hose (no observations during fattening period 1); Type 7 = rubber ball (no observations during fattening period 1). Statistical comparisons are carried out on the dichotomized data. [abc]: significant differences (P < 0.05) for day 0; [xy]: significant differences (P < 0.05) for day 5; *: no significant (P < 0.05) difference between observation day 0 and 5. Bars with the same superscript are not significantly different.

3.3.3 Tail and ear damage

Overall, lower tail and ear damage scores were found when a sequence of enrichment toys was offered in the pens (Table 3.6) (P < 0.05).

Table 3.6. Percentage of pigs observed with score* 1, 2, 3 (or 4) for tail and ear damage/blood freshness for a sequence of toys versus only one toy (chain).

	Score 1	Score 2	Score 3	Score 4
Tail damage				
Only chain [a]	94.63	3.71	1.66	
Sequence of toys [b]	97.34	2.15	0.5	
Ear damage				
Only chain [a]	91.05	3.32	5.63	
Sequence of toys [b]	98.96	0.74	0.30	
Tail blood freshness				
Only chain [a]	97.63	0.77	0.32	1.28
Sequence of toys [a]	98.07	0.90	0.24	0.78
Ear blood freshness				
Only chain [a]	91.3	4.35	2.75	1.6
Sequence of toys [b]	98.92	1.02	0.06	0.00

* Scores are ascending with severity.
[ab] Scores in the same column, within the same parameter, with different superscripts differ significantly (P < 0.05).

The same results were found for the presence of blood on the ears ($P < 0.0001$). No significant relationship was found between the presence/absence of a sequence of toys and the freshness of blood on the tails ($P > 0.05$). The highest tail/ear damage wounds scores 2 and 3 and blood scores 2, 3 and 4 were seen in fattening period 2 when only a chain was present in the pen ($P < 0.05$), as seen in Table 3.6.

3.3.4 Growth and feed conversion

No difference ($P > 0.05$) in growth was found when a sequence of toys (0.707 kg/day ± 0.013 SE) or only a chain was present (0.707 kg/day ± 0.011 SE). Growth was highest ($P < 0.05$) during fattening period 2 (0.794 kg/day ± 0.011 SE). In general, growth was higher ($P < 0.05$) for barrows (0.734 kg/day ± 0.009 SE) than for gilts (0.680 kg/day ± 0.010 SE). No difference ($P > 0.05$) in feed conversion was found when a sequence of toys (3.033 ± 0.055 SE) or only the chain was present in the pens (2.953 ± 0.044 SE). No associations were found between growth or feed conversion and tail/ear damage or tail/blood freshness.

3.4 Discussion

Current EU legislation (Directive 2001/93/EC) requires that pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities. Studies indicated that 'point-source' materials can reduce pen mate-directed behaviours (Sambraus & Kuchenhoff, 1992; Van de Weerd & Day, 2009). In the present study, a continuous sequence of enrichment objects being attached to a chain reduced biting pen mate behaviour in comparison to only a single toy (chain). Consequently, the presence of a sequence of enrichment materials decreased the number of damage/blood scores (Table 3.6). The highest wound and blood scores were seen between 40 kg and 70 kg, while most biting pen mate behaviour was observed between 20 kg and 40 kg (Table 3.6). The difference in time between the observation of the biting pen mate behaviour and the severity of wounds is because "the injury phase" is proceeded by the 'pre-injury phase' (Fraser 1987). Also, the gilts expressed a significantly higher biting pen mate behaviour than the castrated males and is in consensus with literature (Taylor et al., 2010).
At the first observation day, for every toy, there was a significantly higher proportion of animals showing toy contact behaviour compared to observation day 5 (Figure 3.3). Habituation to point source objects occurs very quickly in pigs (Van de Weerd et al., 2003). This can consequently reduce their usefulness in stimulating exploration

(Wemelsfelder & Birke, 1997). An exposure time of less than 2 days may help to preserve the exploratory value of the objects (Gifford et al., 2007). Moreover, not every object is equally feasible as an enrichment object. Important characteristics for intense use are complexity, ingestibility, odour, chew ability and destructibility (Studnitz et al., 2007; Van de Weerd et al., 2003) or a combination of flexibility and destructibility (Zonderland et al., 2001, 2008). The outcomes of the present study confirm these results for both observation days, namely that the most interesting enrichment objects (orange rope, yellow ribbon, purple ribbon and grey garden hose) were ingestible, chewable, flexible and destructible. The commercial toys were hardly destructible. Colour might also play a role in the popularity of the toy and could explain the significant difference in toy contact behaviour between the grey and the yellow garden (least popular) hose for observation day 0. More experiments are needed to test the impact of the colour characteristic. In general, no effect of presentation order on pigs' behaviour was found for the different toys. The toys were always presented in the same sequence relative to each other, although the first one presented could be different (Table 3.2). Consequently, there could be a confounding effect of toy and sequence such that the attractiveness of individual toys could be questioned (a toy presentation after a boring one is likely to receive more attention). The difference in attractiveness was only known at the end of the experiment, so that the original design of experiment was not changed. Therefore, more research is needed to examine the effect of changing the presentation order as only two presentation orders were used in this survey. As reported by Docking et al. (2008), no differences in toy contact behaviour between gilts and barrows were found.

The higher biting pen mate behaviour at observation day 5 may be explained by the reduced interest for the applied objects (Figure 3.3). Moreover, studies showed that some enrichment objects could be responsible for stimulating biting behaviour (Van de Weerd et al., 2006; Wemelsfelder & Birke, 1997). A numeric high (respectively low) biting pen mate behaviour for observation day 0 (respectively day 5) was observed when the rubber bar (respectively grey garden hose) was present in the pens despite the numeric low (respectively high) toy contact behaviour (Figure 3.2 and 3.3). However, the most popular toys mostly caused the highest biting pen mate behaviour in our study for observation day 0, probably due to spatially- limited access to the particular object (Docking et al., 2008). Simultaneous presentation of multiple high value toys might reduce competition and consequent redirected biting behaviour between pen mates. The presence of fewer pigs per enrichment device (Scott et al., 2007) and/or the presentation of the toy in a more central position in the pen allowing more access to the object might also be a solution. For observation day 5, the same trend as for observation day 0 was found but this was less explicit. At fattening period 3, the orange rope was sometimes completely destroyed after three days and was replaced. The almost completely destroyed orange rope stimulated toy contact behaviour less at observation day 5 and resulted in lower than expected biting behaviour. However, Trickett et al. (2009) showed that replacement of a used object with an identical new one still stimulates a significant increase in interest, so that the interpretation of the results must take into account a possible replacement effect. Again, a difference between the grey and yellow garden hose was observed for observation day 5.

Most toy contact and biting pen mate behaviour was observed during fattening period 1 followed by fattening period 2 and 3 (Table 3.5). This might be caused by an age effect, as the activity of pigs decreases over age (Stolba & Wood-Gush, 1989). Toy contact (respectively biting pen mate) behaviour was significantly higher (respectively lower) when the sequence of toys was provided for the first time compared to pigs of the same age who received the toys for a second or third time (Table 3.5). When the same toys were provided for a second or third time, toy contact behaviour decreased and did not affect biting pen mate behaviour much. These findings suggest that, next to age, novelty might also play an important role. Pigs probably recognized the repeatedly encountered object and did not interact as they would normally do with a novel object (Gifford et al., 2007). Pigs might retain a memory for the 7-day sample object. A longer sequence of different toys, faster change of toy and/or change in presentation order might maintain novelty. Renewing of the same toy within the sequence could also boost novelty (Trickett et al., 2009). More experiments are needed.

Generally, the percentage of toy contact and biting pen mate behaviour tended to decrease with the observation periods. This could be explained by the fact that when the observer had to enter the room, pigs became more active. This activity decreased over the observation hour and the pigs became calm resulting in less toy contact and biting pen mate behaviour.

Conflicting results of earlier studies on the influence of environmental enrichment on productive performance were found (Beattie et al., 2000; Blackshaw et al., 1997; Day et al., 2002; Horrell 1992; Morgan et al., 1998; Pearce & Paterson 1993; Schaefer et al., 1990; Van de Weerd & Day, 2009). In our study growth and feed conversion did not differ in relation to the presence of a single enrichment object (chain) or a sequence of toys during the 3 fattening periods. This is probably due to the low levels (less than 1% for both observation days together) of pen mate-directed behaviours in the pens. Consequently, no associations between growth or feed conversion with tail/ear damage or tail/blood freshness were found. High levels of pen mate-directed behaviours (e.g. biting pen mate behaviour) can result in more wounds and in negative effects on the productivity (growth, feed intake, feed conversion) (Ruiterkamp, 1987; Wallgren & Lindahl, 1996). More detailed information about feed conversion could be found when an individual electronic pig feed monitoring system was used, so feed conversion could be calculated on pig level.

The economic implications of enrichment strategies are also important to consider. Because the provision of a continuous sequence of enrichment objects did not affect animal performance in this study, this may hamper its implementation on a commercial scale. Additionally, frequent replacement of toys is labour demanding and costs to make/buy the toys need to be considered. However, there is an increased public demand for meat originating from welfare-friendly systems (Peeters et al., 2006). If the consument is willing to pay a premium, the cost can be recuperated.

Together with literature, this study demonstrated the positive effects of environmental enrichment on animal welfare, e.g. less stress. Consequently, a positive effect on meat quality has to be expected. However, the recent review of Van de Weerd et al. (2009) stated that the effect of enrichment on meat quality was minimal. This might be explained by pre-slaughter stress. Stress in the period during transport and around slaughter is known to influence the physical and biochemical processes in pigs, which determines meat quality, and is studied in detail in the next chapter.

3.5 Conclusions

Biting lesions are considered to have a detrimental implication on animal welfare and can be reduced by environmental enrichment. Therefore, knowledge about the proper use of environmental enrichment is very important. The results of this study show that a continuous sequence of seven different enrichment objects reduced biting pen mate behaviour, which resulted in less severe damage/blood scores on ears and tails, compared to providing one single toy (chain). However, not every object was feasible as an enrichment object. Moreover, very popular toys seem to induce competition for toy contact enhancing biting pen mate behaviour, which stops when the toy is removed, while pigs do not compete for less popular toys. Next to age, the novelty of the toy also determined pigs' behaviour. The ideal sequence of toys should maintain toy contact behaviour in order to avoid biting pen mate behaviour. Therefore, further work should focus on the impact of e.g. a longer sequence of different toys, a faster replacement of toys within the sequence, colour of the toy, renewing the same toy within the sequence and/or a change in presentation order on pigs’ behaviour.

Chapter 4: Effect of unloading, lairage, pig handling, stunning and season on pH of pork

Chapter redrafted after Van de Perre, V., Permentier, L., De Bie, S., Verbeke, G., & Geers, R. (2010). Effect of unloading, lairage, pig handling, stunning and season on pH of pork. *Meat Science*, *86*, 931-937.

4.1 Introduction

Appropriate pre-slaughter handling of pigs is very important, not only from a welfare point of view, but it also affects pork quality and is consequently linked to economic implications. When pigs are stressed, glycolysis rate increases (Fernandez et al., 1995; Jensen et al., 1999) which can result in poor meat quality after slaughter i.e. Pale Soft and Exudative (PSE) meat. DFD (dark, firm and dry) meat might be associated with a long period of stress. Pigs suffering from chronic stress deplete body energy reserves before slaughter which results in a high final pH value 24 h after slaughter (Tarrant, 1989). PSE meat occurs when pigs suffer acute stress before slaughter and is a major problem in the pork industry. Sufficient energy reserves in the muscles cause a very fast drop of the initial pH post-mortem resulting in pale meat with a low water holding capacity (Offer & Knight, 1988). In the present study only PSE meat was considered as synonymous with poor pork quality.

Actions such as handling on farm, loading, transportation, unloading, lairage and driving to the stunning line are commonly known to be responsible for the development of aberrant pork quality (Brown et al., 2005; Fraqueza et al., 1998; Geverinck et al., 1998a; Hambrecht et al., 2004; Lambooy & van Putten, 1993; Pérez et al., 2002; Santos et al., 1997; Warriss & Brown, 1994). These handling procedures are associated with stress and therefore influence meat quality. The literature also describes genotype (De Smet et al., 1996; Gispert et al., 2000; Guàrdia et al., 2004), stunning method and season as being important risk factors influencing meat quality. Generally, the halothane gene (n) tends to increase the lean meat content in a carcass and involves the risk of development of PSE meat. In order to reduce the prevalence of PSE meat, heterozygous pigs (Nn) for the halothane gene are used, but this does not solve the problem completely. De Smet et al. (1996) reported that the PSE prevalence of Nn pigs is intermediate between nn and NN pigs. Electrical stunning can lead to a higher degree of petechial haemorrhages, bone fractures and PSE meat than carbon dioxide (CO_2) stunning (Channon et al., 2002; Gregory, 1989; Larsen, 1983; Velarde et al., 2000). The higher frequency of PSE meat is caused by a greater increase in physical stress just before electrical stunning (Troeger & Woltersdorf, 1990, 1991). Guàrdia et al. (2004) reported that in summer the risk of PSE is almost twice the risk in winter because pigs are sensitive to high temperatures.

Previous research has tended to focus on the described meat quality influencing factors separately or combined. However, it remains unclear how meat quality is influenced by the complete sequence of events within the pre-slaughter process. Therefore, the aim of this survey was to investigate the combined effect of several pre-slaughter parameters measured during unloading, lairage and stunning on meat quality based on pH measurements on the slaughter line. The effect of season and parameters involving transport were also considered. Cut off values for the measured level of decibels, in relation to PSE occurrence, were based on information collected during additional slaughter house visits. Hence, critical control points can be inferred to improve meat quality in chain organized production systems.

4.2 Materials and methods

4.2.1 Experimental design

Between March 2009 and February 2010, a total of 90 transports of slaughter pigs (with an average number of 141 pigs per transport) were followed up in one to three visits at 17 Belgian commercial slaughterhouses (plants 1-17). During each visit, pigs originating from two different farms, i.e. two different transports were randomly surveyed. For the most part, all the pigs on one truck were from the same farm due to sanitary reasons. This was checked by the unique identification number. However, pigs from more than one farm were occasionally transported on one truck. In these cases, only one group of pigs on that truck was observed. In total, 12,725 pigs were included for measuring the pre-slaughter environment. An average number of 48 pigs were selected at random out of a transported group for pH measurements. Table 4.1 summarizes the number of observed pigs and pH measurements sampled per season, visit and slaughterhouse with their respective stunning method. Due to practical reasons, only two visits were performed in winter.

Table 4.1. Number of observed pigs and pH measurements sampled per visit, season and slaughterhouse (plant) with their resp. stunning methods.

	Number of observed pigs					Number of pH measurements					Number of visits					Stunning method
	Spring[1]	Summer[2]	Autumn[3]	Winter[4]	Total	Spring[1]	Summer[2]	Autumn[3]	Winter[4]	Total	Spring[1]	Summer[2]	Autumn[3]	Winter[4]	Total	
Plant 1	366	410	371	-	1147	62	100	100	-	262	1	1	1	0	3	CO_2
Plant 2	320	325	360	-	1005	61	100	101	-	262	1	1	1	0	3	Electrical
Plant 3	272	347	356	-	975	63	100	100	-	263	1	1	1	0	3	Electrical
Plant 4	318	237	165	-	720	82	135	100	-	317	1	1	1	0	3	CO_2
Plant 5	345	-	485	-	830	73	-	201	-	274	1	0	2	0	3	Electrical
Plant 6	227	-	673	-	900	71	-	191	-	262	1	0	2	0	3	Electrical
Plant 7	277	-	447	-	724	60	-	150	-	210	1	0	2	0	3	CO_2
Plant 8	-	260	251	-	511	-	63	100	-	163	0	1	1	0	2	Electrical
Plant 9	-	-	243	-	243	-	-	107	-	107	0	0	1	0	1	Electrical
Plant 10	331	-	639	-	970	100	-	187	-	287	1	0	2	0	3	Electrical
Plant 11	314	-	521	-	835	81	-	200	-	281	1	0	2	0	3	CO_2
Plant 12	322	-	669	-	991	102	-	201	-	303	1	0	2	0	3	CO_2
Plant 13	327	-	395	-	722	186	-	95	-	281	1	0	1	0	2	Electrical
Plant 14	-	135	79	197	411	-	100	50	100	250	0	1	1*	1	3	Electrical
Plant 15	298	-	538	-	836	90	-	201	-	291	1	0	2	0	3	CO_2
Plant 16	146	-	360	-	506	72	-	200	-	272	1*	0	2	0	3	Electrical
Plant 17	-	205	-	194	399	-	100	-	100	200	0	1	0	1	2	CO_2

* Only one transport was observed.

[1] Spring: 21 March-20 June; [2]Summer: 21 June-20 September; [3]Autumn: 21 September-20 December; [4]Winter: 21 December-20 March.

4.2.2 Pre-slaughter measurements

When a truck arrived at the slaughterhouse mean stocking density (m²/100 kg) was calculated before unloading. During unloading the following information was recorded: time elapsing from arrival at the slaughterhouse till start of unloading (min), mean weight of the pigs (kg), multiple farm identification numbers on truck (yes/no), percentage of panting pigs, unloading time (min), the use of a hydraulic lift/ramp (yes/no), angle of the ramp (°), percentage of falling/slipping/vocalizing/turning back pigs during unloading and the (mean, minimum and maximum) noise level (Testo 815, Testo NV, Ternat, Belgium) produced during unloading (dB(A)). Showering (yes/no), the temperature of showering water (°C), duration of lairage (min), number of pigs per pen, mean stocking density (m²/100 kg), produced (mean, minimum and maximum) noise level (dB(A)), presence of drinking nipples and sufficient air supply (personal perception; subjective measurement) were observed for each sampled group of pigs during lairage. The noise level was measured during 15 min next to the pen(s) of the observed pigs. During movement of the pigs to the stunner, the handling behavior of the slaughterhouse staff was observed (frequency of using an electric prod) together with the produced (mean, minimum and maximum) noise level (dB(A)) and the percentage of falling/slipping pigs. The noise level was measured next to the pathway of the pigs going to the stunner during 15 min. Background sounds could not be distinguished from other sounds, but more information was gathered via the minimum and maximum measurements. In every slaughterhouse, the same standard procedure was followed to measure the sounds (e.g. same place). When an electric prod was used, the intensity of using it was recorded. Frequent use of the electric prod was defined as the prod being used on more than 60% of the pigs, an intermediate use as it was used on 10 to 60% of the pigs and no frequent use as less than 10% experienced an electric prod. An electric prod was only used while driving pigs to the stunner. The stunning method, either electrical (manual, head-only/head-to-chest application of electrodes) or CO_2 and the stunning effectiveness, current (A), voltage or CO_2 concentration was noted. Stunning effectiveness was evaluated by corneal reflex and rhythmic breathing observations (Velarde et al., 2000). If more than 15% of the pigs of the same sampled group showed evidence of consciousness after stunning, the stunning was evaluated as not being effective. Table 4.2 summarizes the measurements made per slaughterhouse.

Table 4.2. Overview of the observed pre-slaughter variables per sampled group of pigs.

Variable
Transport
Mean stocking density on truck (m²/100 kg)
Time elapsing from arrival at the slaughterhouse till start of unloading (min)
Mean live weight of the pigs (kg)
Multiple farm identification numbers (yes/no)
Panting pigs (%)
Unloading
Unloading time (min)
Use of a hydraulic lift/ramp (yes/no)
Maximum angle of the ramp (°)
Mean angle of the ramp (°)
Falling/slipping/vocalizing/turning back (%)
Minimum sound level (dB(A))
Maximum sound level (dB(A))
Mean sound level (dB(A))
Lairage
Minimum sound level (dB(A))
Maximum sound level (dB(A))
Mean sound level (dB(A))
Presence of drinking nipples in the pens (yes/no)
Presence of sufficient air (yes/no)
Showering (yes/no)
Showering water temperature (°C)
Mean stocking density (m²/100 kg)
Lairage time (min)
Number of pigs/pen
Stunning
Minimum sound levels during movement of the pigs to the stunner (dB(A))
Mean sound levels during movement of the pigs to the stunner (dB(A))
Maximum sound levels during movement of the pigs to the stunner (dB(A))
Frequently use of electrical prod (yes/no)
Accurate stunning (yes/no)
Stunning method (gas, manual electrical, head-only, head-to-chest)
CO_2 concentration (%)
Current (A) and voltage (V) measured at the electrodes
Slipping/falling (%)
Other
Season (Spring/Summer/Autumn/Winter)

4.2.3 Meat quality measurements

The pH (Hanna HI99163, Hanna Instruments, Temse, Belgium) was measured in the *M. longissimus dorsi* (LD) at the last rib 30 min after slaughter (pH_i). To ensure that the pH was measured 30 min after slaughter, the site in the slaughter line to measure the pH was determined for each slaughterhouse by measuring the time post-mortem till 30 min were expired. The pH was measured by the same person with the same type of pH-electrode. To guarantee the measurements were correct, the electrode was cleaned by a cleaning solution for oils (HI 7077, Hanna Instruments, Temse, Belgium) and proteins (HI7073L, Hanna Instruments, Temse, Belgium) at the start of each visit, and after every 20 measurements as proposed by Hanna Instruments. After cleaning, the electrode reading was checked with standard solutions of pH 7 and 4. If the measured pH had a deviation of more than 0.01 the electrode was recalibrated. This procedure was probably not followed by Franck et al. (1999, 2000) and might be the reason why the authors said that pH was a bad predictor for PSE meat.

Meat was defined to be PSE meat when pH_i was below 6.0. O'Neill et al. (2003) also used this cut off value for PSE in the LD but the pH was measured 45 min post-mortem. In this research, pH was measured at 30 min post-mortem, being related to the slaughter speed of the slaughter line. In practice, allowances can be accepted for pH ranges to define PSE meat. In countries where the incidence of PSE meat is high, a stricter pH value can be used (Adzitey & Nurul, 2011).

4.2.4 Sound level measurements

In order to provide extra information about sound levels and PSE prevalence, the same protocol was repeated for the sound measurements as mentioned in Table 4.2 between March 2010 and March 2011. Together with previous measurements, a total of 189 farm identification numbers of pigs (i.e. 24,922 pigs) were followed of which 8,508 were subjected to pH_i measurements. The result of this experiment is given in a separate section in the results.

4.2.5 Statistical analysis

The data were checked for normality and summary statistics (means and standard deviations) are reported. The data were analyzed using linear mixed models with slaughterhouse and sampled group of pigs nested within slaughterhouse as random factors.

The model building started introducing explanatory variables separately as fixed effects in the model. Variables with a minimum of 80% of the observations present and significant at the 5% level of significance were considered to be included in the multiple model. The second step in the model building consisted of a backwards elimination in which explanatory variables were analyzed simultaneously. Again, only factors significant at the 5% level were kept in the model. Finally, interactions with a biologically meaningful interpretation were tested and introduced in the model if the *P*-value was less than 0.05. In order to avoid multicollinearity problems, all pairwise correlations between variables were computed. In cases of strong associations ($|r| > 0.6$) between covariates, the most significant one or the biologically most meaningful one was selected to be included in the global model.

Furthermore, frequencies of PSE occurrences per season or sound level were calculated. Differences in PSE prevalences between seasons and sound levels were revealed using logistic mixed models with slaughterhouse and sampled group of pigs nested within slaughterhouse as random factors.

All analyses were performed within the SAS statistical software package (version 9.2, SAS Inst., Inc., USA).

4.3 Results

4.3.1 Influencing factors

After introducing all pre-slaughter measurements separately as a fixed effect in the model 10 variables had a significant effect on meat pH_i (Table 4.3) and are explained in this section together with the other main findings. The frequency distribution of pH_i values is shown in Figure 4.1.

Table 4.3. Separate analysis of significant risk factors ($P < 0.05$) in the generalized linear mixed model for meat quality based on pH_i* measurements

Parameter	Level of parameter**	Estimate***	SE	P-value
Season	spring	-0.07476	0.05042	0.0106
	summer	0.017599	0.04834	
	autumn	-0.05531	0.04895	
	winter	0		
Loading density on truck	< 0.39 m²/100 kg or > 0.45 m²/100 kg	-0.04112	0.0184	0.0255
	0.39-0.45 m²/100 kg	0		
Mean noise level produced during unloading (dB(A))	continuous variable (69-99 dB(A))	-0.00707	0.002377	0.003
Percentage of panting pigs observed during unloading	continuous variable (0-6%)	-0.04222	0.0127	0.0009
Water temperature of douche water	continuous variable (10-24°C)	0.01146	0.003769	0.0024
Mean noise level produced during movement of the pigs to the stunner (dB(A))	continuous variable (84-95 dB(A))	-0.00425	0.002319	0.0446
Stunning effectiveness	not effective	-0.07758	0.02756	0.0189
	effective	0		
Gas concentration (%)	continuous variable (70-94%)	0.00704	0.002597	0.0068
Electrical stunning	two electrodes	0.08286	0.03606	0.0216
	three electrodes	0		
Use of electric prods	no frequent use	0.08591	0.02302	0.0003
	intermediate use	0.1747	0.087	
	frequent use	0		

* pH_i: pH was measured in the *M. longissimus dorsi* at the last rib 30 min after slaughter.
** For the continuous variables the minimum and maximum levels are reported.
*** For a discrete variable, the parameter estimates describes an estimated mean difference in response between the specific group and the reference group (estimate = 0). For example, a positive response implies a higher pH compared to the reference group. For a continuous variable, the estimate can be interpreted as a positive or negative association.

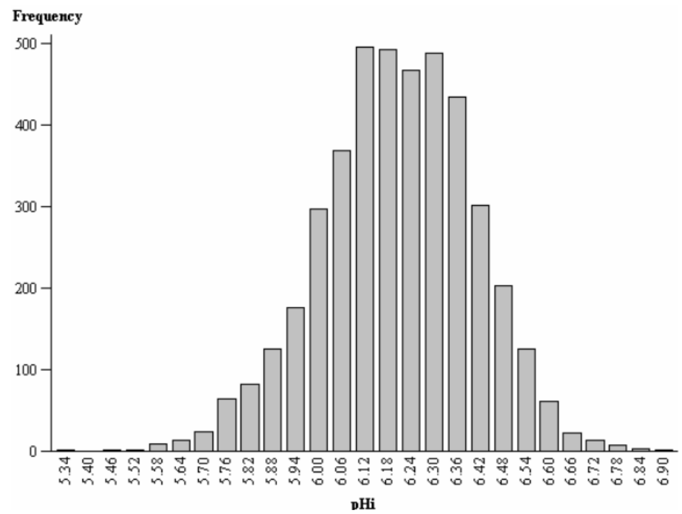

Figure 4.1. Frequency distribution of the pH_i values.

Meat quality was affected by season (Table 4.3). The pH_i was significantly ($P < 0.05$) higher in summer (6.27 ± 0.026 SE) than in spring (6.17 ± 0.022 SE) or autumn (6.19 ± 0.017 SE). Consequently, lowest prevalence of PSE meat was found in summer (Table 4.4). No statement for winter can be made because only 2 slaughterhouses were observed in winter. Water temperature of showering water was significantly ($P < 0.05$) higher in summer (20.28 ± 2.98 SD) than in spring (15.90 ± 1.79 SD) or autumn (15.38 ± 3.06 SD). Trends (0.10 > P > 0.05) of less slipping pigs during unloading (spring: 4.27% ± 5.32 SD; summer: 1.83% ± 1.74 SD and autumn: 6.69% ± 6.81 SD), lower noise levels produced during unloading (spring: 78.99 dB(A) ± 6.10 SD; summer: 75.12 dB(A) ± 3.63 SD and autumn: 77.60 dB(A) ± 3.46 SD) and during movement of the pigs to the stunner (spring: 84.75 dB(A) ± 4.93 SD; summer: 83.81 dB(A) ± 5.92 SD and autumn: 86.51 dB(A) ± 4.12 SD) were observed in summer compared to spring or autumn.

Table 4.4. Observed percentage of PSE meat for spring[1], summer[2], autumn[3] and winter[4].

	Spring	Summer	Autumn	Winter
Number of pH_i* measurements	1103	698	2284	200
PSE (%)	17.59^{a}	6.88^{b}	14.10^{a}	10.00^{ab}

* pH_i: pH was measured in the *M. longissimus dorsi* at the last rib 30 min after slaughter.
[1] Spring: 21 March-20 June.
[2] Summer: 21 June- 20 September.
[3] Autumn: 21 September- 20 December.
[4] Winter: 21 December- 20 March.
[a,b] Percentages without common superscript letter differ significantly ($P < 0.05$).

A higher value of mean noise levels produced during unloading/movement of the pigs to the stunner, percentage of panting pigs during unloading and use of electric prods had a significant negative effect on meat pH_i compared to lower values as indicated by the values of the estimates in Table 4.3. Likewise, a higher temperature of the showering water and higher CO_2 gas concentration during stunning had a significant positive effect on meat pH_i (Table 4.3). No significant effect of pH_i between intermediate use (6.32 ± 0.09 SE) and no frequent use (6.23 ± 0.02 SE) of electric prods was seen in comparison with frequent use (6.15 ± 0.02 SE) ($P < 0.05$). A stocking density between 0.39 m²/100 kg and 0.45 m²/100 kg was significantly ($P < 0.05$) related to a higher pH_i (6.22 ± 0.02 SE) than higher or lower stocking densities (6.18 ± 0.02 SE).

Electrical stunning using three electrodes i.e. head-to-chest revealed a significant ($P < 0.05$) lower pH_i (6.15 ± 0.02 SE) than using only two electrodes or manual electrical stunning (6.25 ± 0.04 SE). A significantly lower noise level was recorded when two electrodes or manual electrical stunning (88.31 dB(A) ± 2.59 SD) was used compared to stunning with three electrodes (90.92 dB(A) ± 3.18 SD). An effective stunning had a positive effect on pH_i values in contrast to an ineffective stunning (Table 4.3). CO_2 concentrations above 80% gave a higher ($P < 0.05$) stunning effectiveness.

No meaningful statistical interactions between the pre-slaughter variables or significant effects of the other variables on pH_i values were found.

4.3.2 Global model

All these risk factors (10) were incorporated in the model with the exception of the factors that represented less than 80% of the observations. The global model contains 96% of the observations and consists of four explanatory variables (Table 4.5) i.e. 4 main risk factors are significantly ($P < 0.05$) associated with pH_i i.e. season, the mean noise level produced during unloading, percentage of panting pigs during unloading and the use of electric prods.

Table 4.5. Final model of significant risk factors ($P < 0.05$) in the generalized linear mixed model for meat quality based on pH_i* measurements.

Parameter	Level of parameter**	Estimate***	SE	P-value
Intercept		6.6184	0.1566	< 0,0001
Season	spring	-0.08868	0.04330	0.0009
	summer	0.007195	0.04216	
	autumn	-0.07003	0.04162	
	winter	0		
Mean noise level produced during unloading (dB(A))	continuous variable (69-99 dB(A))	-0.00525	0.001956	0.0073
Percentage of panting pigs observed during unloading	continuous variable (0-6%)	-0.03445	0.01168	0.0032
Use of electric prods	no frequent use	0.0885	0.02167	< 0.0001
	intermediate use	0.2027	0.07479	
	frequent use	0		

* pH_i: pH was measured in the *M. longissimus dorsi* at the last rib 30 min after slaughter.
** For the continuous variables the minimum and maximum levels are reported.
*** For a discrete variable, the parameter estimates describes an estimated mean difference in response between the specific group and the reference group (estimate = 0). For example, a positive response implies a higher pH compared to the reference group. For a continuous variable, the estimate can be interpreted as a positive or negative association.

4.3.3 Sound level measurements

As reported above, sound had a significant effect on meat quality. Extra measurements were taken to provide reliable cut off values, in terms of risk for PSE occurrence, for the mean level of decibels measured before slaughter. Table 4.6-4.8 shows that, in terms of PSE prevalence, the mean noise level produced before/during unloading and during lairage should be below 80 dB(A). Noise level should be lower than 90 dB(A) during movement of the pigs to the stunner (Table 4.9).

Table 4.6. Mean pH_i and percentage of PSE meat ± SD per mean level of decibels measured before unloading.

	Mean sound level before unloading		
	< 70 dB(A)	***70-80 dB(A)***	***> 80 dB(A)***
N	47	122	20
pH_i	6.19 ± 0.10 ^{a}	6.20 ± 0.10 ^{ab}	6.15 ± 0.11 ^{b}
% PSE	15.60 ± 13.36 ^{a}	14.01 ± 13.84 ^{a}	21.60 ± 17.67 ^{b}

N: Number of observed identification numbers.
^{a,b} Percentages without common superscript letter differ significantly ($P < 0.05$).

Table 4.7. Mean pH_i and percentage of PSE meat ± SD per mean level of decibels measured during unloading.

	Mean sound level during unloading		
	< 75 dB(A)	***75-80 dB(A)***	***> 80 dB(A)***
N	34	104	49
pH_i	6.19 ± 0.09 ^{a}	6.21 ± 0.09 ^{a}	6.16 ± 0.11 ^{b}
% PSE	14.44 ± 12.33 ^{a}	13.45 ± 13.13 ^{a}	19.04 ± 17.14 ^{b}

N: Number of observed identification numbers.
^{a,b} Percentages without common superscript letter differ significantly ($P < 0.05$).

Table 4.8. Mean pH_i and percentage of PSE meat ± SD per mean level of decibels measured during lairage.

	Mean sound level during lairage		
	< 75 dB(A)	***75-80 dB(A)***	***> 80 dB(A)***
N	29	97	51
pH_i	6.23 ± 0.09 ^{a}	6.20 ± 0.09 ^{a}	6.16 ± 0.10 ^{b}
% PSE	11.83 ± 13.54 ^{a}	13.19 ± 12.38 ^{a}	19.18 ± 15.62 ^{b}

N: Number of observed identification numbers
^{a,b} Percentages without common superscript letter differ significantly ($P < 0.05$)

Table 4.9. Mean pH_i and percentage of PSE meat ± SD per mean level of decibels measured during movement to the stunner.

	Mean sound level during movement to the stunner		
	< 85 dB(A)	***85-90 dB(A)***	***> 90 dB(A)***
N	15	80	87
pH_i	6.28 ± 0.08 ^{a}	6.21 ± 0.09 ^{b}	6.17 ± 0.10 ^{c}
% PSE	5.4 ± 6.50 ^{a}	12.68 ± 12.37 ^{a}	17.97 ± 14.59 ^{b}

N: Number of observed identification numbers.
^{a,b} Percentages without common superscript letter differ significantly ($P < 0.05$).

4.4 Discussion

4.4.1 Global model

Independent of the stunning method, the global model demonstrated that meat pH_i was influenced by four risk factors (Table 4.5).

Firstly, meat pH_i was negatively influenced by the mean noise level produced during unloading (Table 4.5). High pitched vocalization has been associated with fear response in pigs and will activate the pigs' defence mechanism (Warriss & Brown, 1994). Sounds may be stressors because of their novelty and intensity, especially when other stressors (e.g. vibrations during transport) are present (Stephens & Perry, 1990). Consequently, higher noise levels produced during unloading will adversely affect meat quality. The same trend ($0.10 > P > 0.05$) for the noise level produced during lairage was observed (data not shown). However, Talling et al. (1996) reported that it is not known if, and how much sound contributes to the stress placed on pigs during transport and while at the abattoir. The extra sound measurements showed significant cut of values that can be used by the slaughterhouses, in order to prevent loss of meat quality (Table 4.6-4.9). The building of of slaughterhouses with sound isolation materials or to provide a 'decibel alarm' when the sound level is too high could be a good tool to control sound.

Secondly, meat pH_i was significant higher in summer than in spring or autumn (Table 4.5). As a result, a significant lower prevalence of PSE meat was found in summer (Table 4.4). In contrast, Guardia et al. (2004) stated that the PSE prevalence is expected to be highest in summer. Both high environmental temperatures in summer and temperature fluctuations affect the animal's ability to maintain body temperature at the required level which results in stress, a higher post-mortem muscle temperature and poorer meat quality (Guàrdia et al., 2004; Santos et al., 1997; Warriss, 1991). In this study, most measurements were performed before noon and not on warm days (> 30°C). Additionally spring and autumn might be characterised by higher temperature fluctuations than in summer and could explain the better meat quality in summer, but was not measured in this research. Heat stress can be overcome to a certain extent by showering during lairage. The water temperature had a significant positive effect on pH_i (Table 4.3) and was highest in summer. However, huddling was observed when showering with cold water, probably resulting in higher body temperature fluctuations and concomitantly more PSE meat. Forrest et al. (1963) reported that the incidence of PSE meat was affected by air temperature fluctuations. Trends ($0.10 > P > 0.05$) such as less slipping pigs during unloading, a lower mean noise level produced while unloading/movement to the stunner during summer might have contributed to less pre-slaughter stress and consequently to a better meat quality in summer. Moreover, O'Neill et al. (2003) reported the highest prevalence of PSE meat in winter and noticed that this prevalence could be influenced by increased slaughter rates. Increased slaughter rates can include more pre-slaughter stress for pigs. In this survey, the slaughter rates were lowest in summer (personal communication).

The percentage of panting pigs is the third factor in the global model. Pigs originating from transports, in which high percentages of panting pigs were observed during unloading, showed a significantly lower meat pH_i (Table 4.5). Dyspnea is a behavioral sign of acute exercise challenge and reflects increased oxygen demand in pigs. This behavior can occur when pigs are stress-challenged during transport or lairage (Ritter et al., 2006). As a result, the percentage of panting pigs of the same sampled transport group can give information about their meat quality. Pigs that are more susceptible to stress because of their genotype might start panting sooner during exercise (Allison et al., 2006). In Belgium more than 90% of the pigs are heterozygous for the halothane gene (Depuydt, 2008) and are consequently less susceptible for stress than nn pigs, but more than NN pigs (Geers et al., 1996).

The use of an electric prod was the last risk factor in the global model (Table 4.5). A negative effect on pH_i was present, when electric prods were used frequently. Frequent use of electric prods results in fear and stress (Brundige et al., 1998) and consequently decreased meat quality. Mostly, electric prods were utilized in slaughter plants with poor ‘driving facilities’ to the stunner.

4.4.2 Other risk factors

Factors that represented less than 80% of the observations, and turned out to be significant after separate introduction as a fixed effect in the global model, are discussed as variables that lost their individual significance after incorporation in the global model together with biologically important factors.

Loading density on the truck can affect pigs’ welfare and consequently meat quality. High stocking densities (< 0.4 m^2/100 kg) cause more fights and lead to a higher incidence of skin blemish because pigs can not lay down to rest (Guise & Penny, 1989). However, low densities lead to injury, because the pigs can fall when standing during inconsiderate handling (Nanni Costa et al., 1999). As a result, the EU Directive 95/29/CE and Council Regulation (EC) No. 1/2005, concerning the protection of animals during transport state that all pigs must at least be able to lie down and stand up in their natural position. In order to comply with these minimum requirements, the loading density for pigs should not exceed 0.425 m^2/100 kg pig. In this research, analysis revealed a significant effect of stocking density on pH_i (Table 4.3). A stocking density between 0.39 m^2/100 kg and 0.45 m^2/100 kg was significantly related to a higher pH_i than higher or lower stocking densities. A higher limit for stocking density (0.39 m^2/100 kg) than defined in the EU Directive (0.425 m^2/100 kg) was used in this survey and did not affect meat pH_i. These results agree with Guàrdia et al. (2004) who showed that, in terms of PSE, a stocking density of 0.425 m^2 per 100 kg pig is only appropriate for journeys longer than 3 h. For journeys shorter than 3 h, like in Belgium, an increased availability of space during transportation raises the risk of PSE pork. The effect of stocking density during transport on meat pH_i disappeared after incorporation in the global model. In the literature, an effect of stocking density on meat quality was not always reported (Barton-Gade & Christenson, 1998; Guise & Warriss, 1989; Nanni Costa et al., 1999; Schütte et al., 1994).

Loading, transport and unloading can be very traumatic for animals and a period in lairage could allow some recovery from previous stressful handling. (De Smet et al., 1996; Grandin, 1994; Malmfors, 1982; Milligan et al., 1998; Nanni Costa et al., 2002; Nielsen, 1981; Warriss et al., 1998a). Nevertheless, no significant effect of lairage time on meat pH_i was observed. Any improvement in meat quality related to resting in lairage, may have been lost due to 'pre-stunning stress'. In most cases, an electric prod was used which leads to high vocalizations while driving pigs to the stunner and consequently affects meat pH_i (Table 4.3). Van der Wal et al. (1999) also found an effect of stress immediately before stunning on pork quality based on pH measurements 45 min post-slaughter in the *M. longissimus lumborum* and *M. semimembranosus*. In view of the sequence of events being studied, further research is necessary to investigate the hypothesis that pigs experiencing stress during transport or unloading might react in a stronger way to pre-stunning stress despite optimal lairage conditions. The benefit of lairage could also be lost due to genetics. The effect of lairage, improving meat quality, is more pronounced in stress-susceptible pigs than in stress-resistant pigs (De Smet et al., 1996).

No effect of stunning method on pH_i was found. Normally, pH_i is higher for CO_2 stunning than electrical stunning. Electrical stunning leads to physical stress and can accelerate post-mortem muscle glycogen breakdown which results in a rapid pH drop (Troeger & Woltersdorf, 1990, 1991). In this study, a possible explanation for this phenomenon was an inappropriate (< 80%) CO_2 concentration in some visits so the pigs were inadequately stunned resulting in a lower pH_i. Stunning effectiveness had a positive effect on pH_i values (Table 4.3), but was found to be less important when combined with other significant variables into the global statistical model. The CO_2 concentration had a significant positive effect on pH_i but also disappeared after incorporation in the global model and represented only 43.50% of the observations. CO_2 concentrations above 80% gave better stunning effectiveness and a significantly higher pH_i indicating a better meat quality. The exact stunning duration of pigs at a certain CO_2 concentration was not measured, but was estimated to be at least 70 s at the level of the measured CO_2 concentration according to the technicians of the slaughterhouses. A concentration of 70% or more CO_2 by volume in air for at least 70 s is in line with the EU Directive 93/119/EC. Moreover, a concentration of at least 80% CO_2 for 70 s which is in consensus with our results has been implemented in, for example Germany (Nowak et al., 2007).

Using three electrodes i.e. head-to-chest gave rise to a significant lower pH_i than using only two electrodes (head-only) or manual electrical stunning (Table 4.3). Following literature, the opposite has to be expected (Velarde et al., 2000). The automatic head-only and head-to-chest system are developed to minimise the incidence of haemorrhages in the carcass and PSE meat. Additionally, the cardiac arrest system prevents the onset of PSE by lowering the degree of muscular activity during the clonic phase. A possible explanation could be a significant lower noise level recorded when two electrodes or manual electrical stunning were used compared to stunning with three electrodes. This result demonstrated the importance of pre-slaughter stress even more. Other factors such as current levels and/or duration of the stun can influence pH_i (Channon et al., 2003) but was not observed in this research.

An overview table with important meat quality affecting factors, based on this survey, is summarized in Table 4.10.

Tabel 4.10. Meat quality affecting factors.

Factor
Season
Loading density
Sound:
- Before unloading
- During unloading
- During lairage
- During movement to the stunner
Presence of panting pigs
Douche water temperature
Stunning effectiveness
Gas concentration
Use of electric prods

This chapter indicated that the incidence of PSE meat is still high and poor processing characteristics and financial losses has to be expected (O'Neill et al., 2003). Additionally, all the pH measurements were performed in the LD, which gave a good image of the total carcass condition, but e.g. different measurements (other muscle or biophysical measurement) in the ham may give more detailed information towards cooked ham production (hypothesis). Consequently, it would be very useful to find 'the ideal measurement' that can predict the end quality of a processed product early in the slaughter line. In the next chapter this was studied for cooked hams and the effect of season was also considered.

4.5 Conclusions

Meat pH_i value was influenced by different pre-slaughter stressors. Stress during transport and/or unloading affected meat quality on a reverse manner and can be measured by the number of decibels produced during unloading, and the percentage of pigs that show panting behavior. Pre-stunning stress, mainly caused by the frequent use of an electric prod while driving pigs to the stunner, had a significant negative effect on pH_i values. The pH_i values in summer were higher than in spring or autumn due to better pre-slaughter conditions. Higher temperature fluctuations in spring and autumn than in summer might also had an impact, but need further investigation.

In order to avoid PSE meat, the mean level of decibels need to be lower than 80 dB(A) produced before/during unloading and during lairage. Sound levels lower than 90 dB(A), measured during forced movement of the pigs to the stunner, were associated with a lower frequency of PSE meat.

Chapter 5: The prevalence of PSE characteristics in pork and cooked ham - Effect of season

Chapter redrafted after Van de Perre, V., Ceustermans, A., Leyten, J., & Geers, R. (2010). The prevalence of PSE characteristics in pork and cooked ham - Effects of season and lairage time. *Meat Science*, *86*, 391-397.

5.1 Introduction

There is a growing demand for quality meat, including the aspect of animal welfare. The quality of fresh pork meat is influenced by many factors (e.g. genetics, nutrition, time of year, stress, handling and processing) and has two main defects: “Pale Soft and Exudative” (PSE) meat and “Dark Firm and Dry” (DFD) meat. PSE meat occurs when the pigs suffer acute stress before slaughter (Warriss, 1995a). When there are sufficient energy reserves in the muscles, the initial pH (pH_i) can drop very fast post-mortem due to the production of lactic acid. This pH drop causes a denaturation of the myosin and sarcoplasmic proteins and results in pale meat with a low water holding capacity (WHC) (Bendall & Swatland, 1988; Bendall & Wismer-Pedersen, 1962; Offer & Knight, 1988). On the other hand, DFD meat occurs when the animals suffer chronic stress and skeletal muscles deplete their energy stores. As a result, there is almost no lactic acid production after slaughter. DFD meat is therefore characterized by a high final pH value 24 h after slaughter ($pH_u > 6$), a dark colour and a high water-holding-capacity (Tarrant, 1989). Consequently, the greatest improvement in pork quality can be achieved by decreasing stress in the immediate pre-slaughter period (Hambrecht et al., 2005). As pigs do not have working sweat glands, they are sensible for high temperatures, hence, abattoirs should be very careful in summer, when the risk on PSE meat is much higher than in winter (Guàrdia et al., 2004). In contrast, the probability of occurrence of DFD pork is higher in winter than in summer (Galwey & Tarrant, 1978). At low temperatures pigs tend to maintain deep body temperature by huddling together or by shivering at the expense of energetic reserves in the muscle (Hrupka et al., 2000). Gender can also affect the risk of obtaining PSE/DFD pork (Guàrdia et al., 2004, 2005; Van der Wal et al., 1999). In general, boars are more prone to develop PSE meat than barrows or gilts (Guàrdia et al., 2004). The greater aggressiveness of the entire males makes them more sensitive to stress factors especially in the period before slaughter. In contrast, gilts and barrows have a higher chance to develop DFD meat than entire males. The difference in energetic metabolism, faster recuperation of chronic stress, the higher energy reserves in the muscles of the boars could be possible reasons (Guàrdia et al., 2005; Van der Wal et al., 1999).

The occurrence of PSE and DFD meat has important economic implications. PSE trait is internationally recognized as a major economic risk factor for fresh and processed pork (Kuo & Chu, 2003). It is associated with lower processing yields, increased cooking losses and reduced juiciness (Hendrick et al., 1994; Honkavaraa, 1988; Müller, 1991). Moreover, during industrial slicing of cooked hams, the PSE-zones crumble easily, making holes or splits in the slices which are very detrimental to the presentation of the product (Franck et al., 2000). On the other hand, DFD meat causes bacterial spoilage in fresh meat and gives important technological problems in dry-cured products (Wirth, 1985). Therefore, it is very important that meat quality can be predicted early in the slaughter line in order to match the observed quality with the right application and will be studied this chapter.

Meat quality or "fitness for use" can be defined by the application of different measurements, including pH, Pork Quality Meter (PQM), temperature, sensory characteristics, colour and water holding capacity (WHC). Generally, PSE/DFD meat is identified on the base of pH value, colour (L^* value) and drip loss (Warner et al., 1997), while in some cases PSE meat is identified only on the base of colour and drip loss (Joo et al., 1999). Nevertheless, there is no golden standard to define PSE/DFD meat. Most quality measurements are performed in the *M. longissimus dorsi* (LD) while very little attention has been given to other muscles; i.e. the muscles of the ham.

In order to reduce PSE/DFD meat, a new (quality) label "Certus" was established in Belgium. Certus pigs are heterozygous negative (nN) for the halothane gene and are consequently less susceptible for stress which results in less PSE meat than pigs homozygous for the halothane gene (Barton-Gade et al., 1988). The occurrence of DFD meat is little affected by the halothane genotype and results from poor pre-slaughter handling (Fisher et al., 2000; O'Neill et al., 2003)). The pH_u is determined by the muscle glycolytic potential before slaughter (Monin et al., 1981). Certus pigs are regularly controlled within the labeling procedure, which is not the case for the control pigs. Each participant within the Certus system (and pigs' descending from no label system) must also adapt to both European and Belgian legislation concerning production, processing and handling of pigs. Meeting the Certus standard implies specific regulations concerning feed and accommodation of the pigs, animal welfare and health, identification of the pigs, transport and slaughterhouse operating aspects and meat processing (Belpork, 2006). For example, the lairage time for Certus pigs is placed on 2-4 hours to recover from stress during transport. Consequently, a positive effect on meat quality has to be expected.

However, lairage time is not always respected because of practical reasons (e.g. no other pigs in lairage).

The aim of the present study was to investigate (1) the relation between the biophysical parameters pH, PQM, colour and WHC, measured in the *M. longissimus dorsi*, *M. gracilis* and *M. semimembranosus* on different times post-mortem, and the quality of fresh pork meat and Meesterlyck cooked ham, (2) the influence of season on PSE/DFD prevalence/characteristics and (3) the influence of PSE characteristics on ham quality.

5.2 Material and methods

5.2.1 Experimental design in summer

Between April and September 2007, a total of 120 pigs was followed up after slaughter in four different trials each time at the same Belgian commercial slaughterhouse belonging to the COVAVEE group. During each trial 15 conventional pigs and 15 pigs from a quality assurance scheme (Certus) were chosen at random. Lairage times were recorded. The slaughterhouse used an electrical head-to-chest stunning method (HTC 400 V head, 2.4 A head for 2.8 s and 265V chest, 1.1 A chest for 2.3 s at a frequency of 50 Hz) and the line speed was 420 pigs/h. The preferred weight of the pigs was between 94 and 97 kg in order to have hams weighing between 11 and 12 kg (important for the standardization of the cooking process). Meat quality measurements were carried out 30 min, 24 and 35 h after slaughter. Approximately 40 min after slaughter the carcasses were stored at 1°C. After removing of the bones (femur, patella, tibia and fibula), 24 h after slaughter, the hams were prepared with a Cobourg cut and were transported (cooled) to a meat processing company. During and after processing, hams were followed up. A sample at the last rib of the LD (about 400 g) was transported to the lab for further analysis.

5.2.2 Experimental design in winter

Between January and March 2008, a total of 60 pigs were followed up after slaughter in two different trials each time at the same Belgian commercial slaughterhouse as in the trial of 2007. During each trial 15 conventional pigs and 15 pigs from a quality assurance scheme (Certus) were chosen at random. The same analyses as during the summer trial were carried out.

5.2.3Meat quality measurements

The pH (Hanna HI99163, Hanna Instruments, Temse, Belgium) and the electrical conductivity (PQM) (PQM-I/KOMBI, Intek Klassifizierungstechnik, Aibach, Germany) were measured in two different muscles of the ham (*M. gracilis* (MG) and *M. semimembranosus* (MS)) and in the LD at the last rib. The measurements were carried out 30 min (pH_i and PQM_i) and 24 h (pH_u and PQM_u) after slaughter. The pH electrode was cleaned (HI700630, Hanna Instruments, Temse, Belgium) and calibrated on daily basis. The MS and LD are predominantly red fiber type muscles while the MG is predominantly a white fiber type muscle. The LD is one of the most sensitive muscles to determine PSE, and is taken as a reference muscle in this study. The MG was chosen to have another fiber type muscle. Little is known about the MG being a good marker muscle.

The samples, taken of the LD from the different pigs, were examined 24 h after slaughter for colour (Japanese colour scale and Minolta Chromometer, Osaka, Japan) and water-holding capacity (filter paper method of Kauffman). In the lab, these pieces of the loins were examined 24 h later for pH (pH_{u48}), PQM (PQM_{u48}), tenderness (Warner-Bratzler Meat Shear, G-R Manufacturing Co, Manhattan, USA) and cooking loss (percentage weight loss). The same protocol as De Smet et al. (1996) was applied to determine tenderness, but five pieces of every sample were used and the mean shear force was calculated.

At the meat processing company, colour (Japanese colour scale and spectrocolorimetric), pH and electrical conductivity were measured 35 h post-mortem by the same trained person. After cooking, the cooking loss (percentage weight loss) was determined. During slicing of the ham slicing loss/yield, water-holding capacity (filter paper method of Kauffman) and colour (Japanese colour scale and spectrocolorimetric) were determined. Afterwards, several slices of each sample were preserved at -18°C for further analyses on protein and dry matter content.

5.2.4 Cooking of the ham

The ham was cooked following the recipe defined by the "Meesterlyck" label. Meesterlyck is a quality label that is only awarded to traditionally prepared Flemish hams. Certain standards must be followed (Belpork, 2005). Just like the Certus label (fresh meat), the Meesterlyck label (processed meat) is established to improve meat quality.

In winter a new cooking facility was made operational and will be discussed later on.

5.2.5 Kjeldahl analysis

Protein analysis was determined according to the Kjeldahl procedure with $CuSO_4$ as a catalyst [Association of Official Analytical Chemists (AOAC, 1990)]. Each sample was run 2 times. The mean protein content was determined.

5.2.6 Statistical analysis

The data were checked for normality and summary statistics (means and standard deviations) explored. Meat quality responses were analysed using multiple ANOVA models with season, lairage time and origin as factors, with interaction terms where needed. Differences in prevalence of PSE and DFD were analyzed using logistic regression models with the same factors as described before. Pearson correlation coefficients were calculated between the various meat quality variables. All analyses were performed within the SAS statistical software package (version 9.1, SAS Inst., Inc., USA), and P-values less than 5% are considered to be significant.

5.3 Results and discussion

5.3.1 Quality measurements

a) Slaughterhouse

During winter and summer a total of 180 pigs (90 Certus and 90 conventional pigs) was examined. Meat quality measurements performed 30 min and 24 h post-mortem revealed a significant higher pH in summer indicating a better meat quality compared to winter (Table 5.1). Opposite results were found for the electrical conductivity measured 24 h post-mortem in the MS and, for the L* value measured in the LD in summer. The differences in measurements between the muscles might be explained by the fact that the 3 examined muscles have a different fiber type composition (Cassens & Cooper, 1971). Normally, muscle pH_i (PQM_i) and pH_u (PQM_u) are higher (lower) in winter than in summer (Küchenmeister et al., 2000; Santos et al., 1997), because pigs are more sensitive to high temperatures. The use of glycogen for thermoregulation would result in lower levels in the muscle and, therefore reduced production of lactic acid and higher pH (Homer & Matthews, 1998). According to studies of Franck et al. (1999, 2000) pH appears to be a bad predictor. It was suggested that L* value could be a more appropriate and accurate parameter than pH value and like in our study, higher L* values were observed during summer. In contrast, Warriss (2000) reported that pH measurements are good, easy to measure and widely used to determine meat quality but need to be performed accurately. In this study, the pH electrode, which was new in summer, was probably more worn out at the start of the winter measurements, which resulted in little scratches on the glass electrode, insufficient electrolyte solution in the probe, more difficult cleaning and calibration. Accordingly, lower pH values will be measured but the same trends will be seen (Hanna Instruments, 2010). Moreover, the pH electrode was cleaned on daily basis with a universal cleaning solution, followed by calibration. More frequent cleaning and calibration can improve the pH measurements as performed in Chapter 4. The pH measurements were also lower than in Chapter 4 because this slaughterhouse was characterised by a very high pre-slaughter stress (e.g. frequent use of the electric prod, high overall sound levels…). Although, no good comparison with Chapter 4 can be made as too little transports were followed during winter in Chapter 4. No significant differences were found for drip loss.

Table 5.1. Means (± SD) of the initial (30 min post-mortem) and ultimate (24 h post-mortem) meat quality variables (pH, electrical conductivity, colour and drip loss) for winter[1] and summer[2] in the MS[3], MG[4] and LD[5].

Variable	Winter	Summer
Measurements 30 min post-mortem (i)		
pH_i MS	5.83 ± 0.23 ^{a}	6.09 ± 0.21 ^{b}
pH_i MG	5.78 ± 0.25 ^{a}	6.06 ± 0.26 ^{b}
pH_i LD	5.80 ± 0.31 ^{a}	6.09 ± 0.26 ^{b}
PQM_i MS (mS/cm)	2.34 ± 0.53 ^{a}	2.44 ± 0.81 ^{a}
PQM_i MG (mS/cm)	3.80 ± 0.86 ^{a}	4.12 ± 1.26 ^{a}
PQM_i LD (mS/cm)	3.60 ± 0.88 ^{a}	3.53 ± 0.92 ^{a}
Measurements 24h post-mortem (u)		
pH_u MS	5.55 ± 0.24 ^{a}	5.73 ± 0.22 ^{b}
pH_u MG	5.38 ± 0.17 ^{a}	5.59 ± 0.20 ^{b}
pH_u LD	5.39 ± 0.14 ^{a}	5.61 ± 0.18 ^{b}
PQM_u MS (mS/cm)	4.09 ± 1.13 ^{a}	4.89 ± 1.51 ^{b}
PQM_u MG (mS/cm)	6.18 ± 1.64 ^{a}	6.76 ± 1.90 ^{a}
PQM_u LD (mS/cm)	4.64 ± 1.36 ^{a}	4.77 ± 1.63 ^{a}
L^*_u LD	55.11 ± 4.08 ^{a}	57.50 ± 5.33 ^{b}
a^*_u LD	9.37 ± 4.55 ^{a}	8.73 ± 1.80 ^{a}
b^*_u LD	4.67 ± 1.42 ^{a}	5.07 ± 1.59 ^{a}
Jap_u LD	1.98 ± 0.65 ^{a}	2.16 ± 0.76 ^{a}
Drip loss loin (g)	0.044 ± 0.020 ^{a}	0.051 ± 0.024 ^{a}

[1] Winter: December-March.
[2] Summer: April-September.
[3] MS: *M. semimembranosus.*
[4] MG: *M. gracilis.*
[5] LD: *M. longissimus dorsi.*
[ab] Within a row, means without a common superscript letter differ significantly ($P < 0.05$) .

Prevalence of PSE meat. Meat quality measurements for PSE meat were performed 30 min, 24 and 35 h post-mortem in the MG, MS and LD. Josell et al. (2000) defined a pH value below 6.1, measured at 30 min post-mortem, as an indicator for PSE meat. In this study, meat was defined to be PSE meat when $pH_i \leq 5.9$ or $PQMi \geq 3.9$ because pH_u values can be very low (Table 5.1). Allowances can be accepted for pH ranges to define PSE meat. In countries where the incidence of PSE meat is high, which is the case in this study, a stricter pH value can be used (Adzitey & Nurul, 2011). A pH of 5.9 was a good cut off value to define PSE meat in this research. Consequently, more extreme PSE cases were observed. PSE meat can also be defined when both conditions ($pH_i \leq 5.9$ as well as $PQM_i \geq 3.9$) are met. The prevalence of PSE meat is shown in Table 5.2.

Table 5.2. Percentage of PSE meat based on $pH_i \leq 5.9$, $PQM_i \geq 3.9$ or when $pH_i \leq 5.9$ and $PQM_i \geq 3.9$; $L^*_u \geq 50$ or 57.1 and $pH_i \leq 5.9$ and $L^*_u \geq 57.1$ for winter[1] and summer[2].

	M. semimembranosus		***M. gracilis***		***M. longissimus dorsi***	
	Winter	**Summer**	**Winter**	**Summer**	**Winter**	**Summer**
pH_i	66.67 ^{a}	17.50 ^{b}	68.33 ^{a}	20.00 ^{b}	55.00 ^{a}	23.33 ^{b}
PQM_i (mS/cm)	1.67 ^{a}	7.50 ^{a}	38.33 ^{a}	47.46 ^{b}	20.00 ^{a}	24.37 ^{a}
pH_i and PQM_i (mS/cm)	0.00 ^{a}	4.17 ^{a}	33.33 ^{a}	12.71 ^{b}	18.33 ^{a}	15.13 ^{a}
$L^*(50)_u$	12.61 ^{a*}	18.33 ^{a*}	63.33 ^{a*}	53.78 ^{a*}	93.33 ^{a**}	91.67 ^{a**}
$L^*(57.1)_u$	6.67 ^{a*}	0.00 ^{a*}	36.67 ^{a*}	7.56 ^{b*}	26.67 ^{a**}	50.83 ^{b**}
pH_i and $L^*(57.1)_u$	5.00 ^{a*}	0.00 ^{a*}	28.33 ^{a*}	2.52 ^{b*}	16.67 ^{a**}	15.83 ^{a**}

[1] Winter: December-March.
[2] Summer: April-September.
^{a,b} Within a row, muscle means without a common superscript letter differ significantly ($P < 0.05$).
* L* values taken 35h post-mortem.
** L* values taken 24h post-mortem.

Significantly more PSE meat was found in winter compared to summer for the three muscles when PSE meat is only based on pH_i. O' Neill et al. (2003) also reported more PSE meat in winter and that the incidence of PSE meat is related to increased slaughter rates, but this info was not available for this research. Normally the risk on PSE meat is found much higher in summer than in winter (Guàrdia et al., 2004). On the other hand, Grandin (1994) reported that instead of focussing on high or low temperatures, weather and temperature fluctuations could be more important risk factors. As in Chapter 4, this was not measured. A significantly shorter resting period (in min) in winter (140.7 ± 99.7 SD) than in summer (213.1 ± 68.6 SD) can also result in more PSE meat (O' Neill et al., 2003). However, the results in Chapter 4 indicated that lairage time has no effect on meat pH_i. The differences in PSE prevalences between winter and summer, based on the pH measurements, could also been caused by the 'more worn out electrode' in winter as described above. As pH measurement is not always a good predictor (Franck et al. 1999, 2000), we defined PSE meat based on PQM, L*, pH and PQM, pH and L* (Table 5.2).

L* is probably the best overall indicator for PSE and DFD meat (Brewer et al., 2009). In our study, meat is defined as PSE meat when $L^*_u \geq 50$ (Gaudré & Vautier, 2006) or $L^*_u \geq 57.1$ (Van der Wal et al., 1988) or based on both $pH_i \leq 5.9$ and $L^*_u \geq 50$ or $pH_i \leq 5.9$ and $L^*_u \geq 57.1$ (Table 5.2). The L* value of the LD was taken 24 h post-mortem, while the other values were taken 35 h post-mortem. A cut off value of $L^* \geq 50$ was not a good parameter to define PSE meat in this study for MG and LD as too many pigs had PSE characteristics. On the other hand, an L* cut off value of 57.1 shows a significant double amount in PSE meat in summer than in winter for the LD. This is in agreement with Guàrdia et al. (2004). Again there is a large difference in measurements between the 3 examined muscles. That is why L* is not a good predictor across muscles. Inherent myoglobin differences are present because of different muscle fiber types. This could be a possible explanation for the higher amount of PSE meat in the MG in winter than in summer in contrast with the LD. PSE meat can also be identified on the base of multiple quality measurements (Channon et al., 2002; Joo et al., 1999). Two-point measurements are better than only one. Consequently, it is better to combine pH_i and PQM_i or $pH_i \leq 5.9$ and $L^*_u \geq 57.1$ to define PSE meat. In this research, measuring the LD seemed to be an appropriate method to define carcass quality (Warner et al., 1993).

Prevalence of DFD meat. Meat quality measurements were performed 24 h post-mortem during winter and summer. DFD meat was defined when pHu ≥ 6.4 (Van der Wal et al., 1988), L*u ≤ 35 or pHu ≥ 6.4 and L*u ≤ 35. In this study no significant differences between winter and summer were found in the three muscles as DFD meat was only observed once. Guàrdia et al. (2005) reported that DFD meat does not occur very often and shows up more in winter than in summer. At low temperatures pigs tend to group together in order to prevent heat loss, and in case the heat balance can not be maintained, shivering thermogenesis will increase heat production at the expense of energy stores in the skeletal muscles. On the other hand, a shorter lairage time in winter results in a lesser amount of DFD meat (De Smet et al., 1996; Warriss et al., 1998a). In our study there were only two trials in winter and four in summer which decreases the chance to detect DFD meat in winter.

b) Meat processing plant

When the hams were delivered in the meat processing plant; pH, PQM and colour measurements were taken 35 h post-mortem in the MS and MG (Table 5.3). The colour measurements could not be taken earlier due to practical reasons. A significantly higher pH_{u35} was found for summer compared to winter for the same reason as explained above. In general, the pH or colour will not change much in time after 24 h compared to early post-mortem meat. The PQM measurements, on the other hand, will increase in time due to post-handling of the meat (PLUMA, personal communication). No significant differences between winter and summer were found for the electrical conductivity values. A significantly higher L* was found for the MG in winter compared to summer. This result shows a better meat quality in summer than winter. In contrast, the Japanese colour scale showed a significant higher value for winter than summer for the MS, indicating a paler colour in summer. Although, the Japanese colour scale is not an objective measurement, visual observations are better for some products because sometimes the Lab measurements do not correlate well with visual observations. Additionally, a significant lower a* value was found for the MG in summer, indicating a better meat quality in winter. The contrast between the L* and a* measurements for the MG need to be researched further in similar experiments.

Table 5.3. Means (± SD) of ultimate (35 h post-mortem) meat quality variables (pH, electrical conductivity and colour) for winter[1] and summer[2] in the MS[3] and MG[4].

Variable	Winter	Summer
pH_{u35} MS	5.59 ± 0.21 ^{a}	5.69 ± 0.23 ^{b}
pH_{u35} MG	5.45 ± 0.14 ^{a}	5.60 ± 0.19 ^{b}
PQM_{u35} MS (mS/cm)	8.35 ± 0.91 ^{a}	8.57 ± 1.92 ^{a}
PQM_{u35} MG (mS/cm)	8.66 ± 0.69 ^{a}	8.94 ± 1.89 ^{a}
L^*_{u35} MS	45.54 ± 5.94 ^{a}	44.35 ± 4.79 ^{a}
a^*_{u35} MS	17.91 ± 3.87 ^{a}	15.55 ± 3.22 ^{a}
b^*_{u35} MS	9.20 ± 2.24 ^{a}	8.32 ± 2.34 ^{a}
Jap_{u35} MS	4.66 ± 0.86 ^{a}	4.33 ± 0.83 ^{b}
L^*_{u35} MG	54.52 ± 8.83 ^{a}	50.08 ± 5.30 ^{b}
a^*_{u35} MG	15.33 ± 4.39 ^{a}	12.93 ± 6.21 ^{b}
b^*_{u35} MG	9.43 ± 2.64 ^{a}	7.46 ± 2.19 ^{a}
Jap_{u35} MG	2.92 ± 0.50 ^{a}	3.08 ± 0.64 ^{a}

[1] Winter: December-March; [2]Summer: April-September; [3]MS: *M. semimembranosus*; [4]MG: *M. gracilis*.
[a,b] Within a row, means without a common superscript letter differ significantly ($P < 0.05$).

c) Research on the loins

Forty eight hours after slaughter the loins were examined for shear force and cooking loss (Table 5.4). In theory, PSE meat has a higher shear force (low tenderness) and higher cooking loss (Hendrick et al., 1994; Honkavaara, 1988; Müller, 1991). No significant differences were found between summer and winter for shear force and cooking loss. This was expected because there was not seen any difference in PSE frequency between winter and summer in the LD (pH_i and PQM_i; pH_i and $L^*(57.1)_u$ based) (Table 5.2). The pH and PQM 48 h post-mortem were also taken and will be used later on to determine the correlations between the quality measurements.

Table 5.4. Means (± SD) of meat quality variables (shear force and cooking loss) for winter[1] and summer[2] in the LD[3].

Measurement	Winter	Summer
Shear force LD (N)	32.35 ± 6.77 [a]	30.36 ± 5.70 [a]
Cooking loss LD (%)	27.68 ± 3.75 [a]	28.33 ± 3.33 [a]

[1] Winter: December-March.
[2] Summer: April-September.
[3] LD: *M. longissimus dorsi.*
[a,b] Within a row, means without a common superscript letter differ significantly ($P < 0.05$).

d) Slicing and protein content

Ten days after cooking, the hams were sliced. Cooking loss, drip loss, protein content, dry matter content, slicing yield and colour measurements were performed (Table 5.5). The colour measurements are not shown in the table and are used later on.

In summer, hams had a higher protein and dry matter content, a lower water/protein ratio and a lower slicing yield. These findings, which are PSE characteristics, are in contrast with more PSE meat in winter than in summer based on previous findings e.g. pH_i measurements and $L^*(57.1)_u$ measurements of the MS and MG (Table 5.2). On the other hand, PSE meat identified on PQM_i or $L^*(57.1)_u$ in the LD showed the same trend i.e. more PSE characteristics in summer. This is in agreement with Guàrdia et al. (2004). It should be noticed that the measurements before cooking are only point measurements. The measurements after cooking are measurements of the whole ham and are consequently a better representative for the ham.

Kuo et al. (2003) also reported a higher percent protein in fresh and processed PSE meat (sausages), probably because of another important aspect for PSE meat, i.e. the inability to bind water, so that dry matter content increases. During transit, PSE hams lose more moisture (Kauffman et al., 1978) which results in a higher dry matter content than normal hams. Consequently, the water/protein ratio is lower for PSE meat. Moreover, during industrial slicing of cooked hams, the PSE-zones are more prone to holes or splits in the slices which results into a lower slicing yield (Franck et al., 1999, 2000) (Figure 5.1).

The large difference between summer and winter in protein content might partly be explained by the use of a new cooking facility in winter. A different cooking process (e.g. pre-heating time) can provoke differences in N losses which can affect the protein content, but no detailed information was available for the cooking process.

Table 5.5. Means (± SD) of meat quality variables (Cooking loss, drip loss, protein content and colour) for Winter[1] and Summer[2] in hams.

Measurement	Winter	Summer
Cooking loss ham (g)	13.50 ± 4.10 ^{a}	14.52 ± 3.11 ^{a}
Drip loss sliced ham (g)	0.033 ± 0.015 ^{a}	0.038 ± 0.020 ^{a}
Protein content (%)	20.61 ± 1.52 ^{a}	23.29 ± 1.33 ^{b}
Dry matter (%)	30.35 ± 1.68 ^{a}	32.07 ± 1.48 ^{b}
Water/Protein ratio	3.40 ± 0.28 ^{a}	2.93 ± 0.19 ^{b}
Slicing yield (%)	69.63 ± 23.99 ^{a}	48.80 ± 21.86 ^{b}

[1]Winter: December-March.
[2]Summer: April-September.
[a,b] Within a row, means without a common superscript letter differ significantly (*P* < 0.05).

Figure 5.1. Hole in a slice of ham (Source: Van de Perre Vincent).

5.3.2 Effect of the lairage time on the prevalence of PSE and DFD meat

Loading, transport and unloading can be very traumatic for the animals and a period in lairage could allow some recovery from previous stressful handling. The objective of lairage is to relieve the stress caused during delivery to the abattoir, and for this reason an improvement in meat quality is expected. It is well documented that a lairage time longer than 1 h reduces the incidence of PSE meat while a prolonged resting time can increase DFD occurrence (De Smet et al., 1996; Grandin, 1994; Malmfors, 1982; Milligan et al., 1998; Nanni Costa et al., 2002; Nielsen, 1981; Warriss et al, 1998). A lairage time of 2-3h is recommended (Warriss, 1995b) but can vary due to practical issues.

As reported before, lairage time (min) was significantly higher in summer (213.1 ± 68.6 SD) than in winter (140.7 ± 99.7 SD). More research is needed to investigate the impact of different lairage times on PSE and DFD prevalence in winter and summer as more variance in lairage times and more transports are needed.

5.3.3 Correlations between pH and meat quality parameters

a) Summer

Table 5.6 gives an overview of the correlations between the individual meat quality variables, which are moderate. Many significant ($P < 0.0001$) correlations were found but only the correlations with a $|r| \geq 0.5$ are listed. The pH measurements taken 24 h and 35 h after slaughter correlate well with the colour of the 3 examined muscles. These negative correlations are in agreement with Lindahl et al. (2006). Meat with a fast decrease in pH post-mortem has a low ultimate pH and WHC, what results in a pale colour (high L* value) (Bendall & Swatland, 1988; Offer et al., 1988). The positive correlation between pH_u and the Japanese colour scale is consequently logical. Previous studies have shown that the ultimate pH might be important in meat with high variation in the ultimate pH; e.g. Bidner et al. (2004) found that 70% of the variation in L* and b* values, but only 28% of the a* value in the LD was explained by the ultimate pH. A lower variation in pH_u resulted in a less explained L* variation by pH_u (Brewer et al., 2001).
In this study, the pH_u measured after 24 h in the loin and 35 h post-mortem in the MG gives a good prediction of the colours in the other muscles. The pH_u measured 24 h after slaughter in the MS has a predictive value for the level of the colour measurement in both ham muscles.

Table 5.6. Correlations ($|r| \geq 0.5$) between ultimate pH (24/35 h post-mortem) and colour in the MS[1], MG[2] and LD[3] ($P < 0.0001$).

	L*u LD	**b*u LD**	**Japu LD**	**L*u35 MS**	**b*u35 MS**	**Japu35 MS**	**L*u35 MG**
pH_u MS		-0.53		-0.5	-0.59	0.5	-0.52
pH_u MG					-0.57		-0.5
pH_u LD	-0.55	-0.61	0.56	-0.54	-0.53		
pH_{u35} MS		-0.55		-0.54	-0.59	0.5	-0.54
pH_{u35} MG	-0.51	-0.53	0.53		-0.55		-0.57

[1] MS: *M. semimembranosus.*
[2] MG: *M. gracilis.*
[3] LD: *M. longissimus dorsi.*

Significant correlations ($|r| \geq 0.5$) were found between pH_u and % cooking loss, drip loss, % dry matter and water/protein ratio of the processed ham (Table 5.7). Other correlations were observed, but had no higher $|r|$ than 0.5. The pH_u measurements of the three different muscles showed a negative correlation with % cooking loss and % dry matter of the hams. A significant positive correlation was found with WHC.

Table 5.7. Correlations (|r| ≥ 0.5) between ultimate pH (24/35 h post-mortem) in the MS[1], MG[2] and LD[3] and the meat quality parameters (cooking loss, drip loss, % dry matter and water/protein ratio) ($P < 0.0001$).

	% Cooking loss ham	WHC loin	% dry matter	water/protein
pH_u MS	-0.59		-0.51	0.54
pH_u MG	-0.58			
pH_u LD	-0.50		-0.59	
pH_{u35} MS	-0.65			0.51
pH_{u35} MG	-0.61	0.52		

[1] MS: *M. semimembranosus.*
[2] MG: *M. gracilis.*
[3] LD: *M. longissimus dorsi.*

Meat with PSE characteristics has a lower initial and ultimate pH value (Irving et al. 1989; Offer & Knight, 1988) and results in a lower WHC and a higher cooking loss (Hendrick et al., 1994). The WHC of pork has been reported to be influenced by a number of factors, including ultimate pH (Offer & Knight, 1988). A low WHC (low pH) will result into a lower amount of water than normal meat and a higher dry matter content of the cooked ham. A low ultimate pH results in a higher protein content (Kuo et al., 2003) and lower water/protein ratio. The correlation with ham protein content was low in this study (|r| < 0.5). Meat with more PSE characteristics has more holes or splits in the slices than meat with less PSE characteristics. Holes or splits in the ham are very detrimental to the presentation of the product and results in lower yields (Franck et al., 1999; Müller, 1991). The same results were found in this study, although with a low correlation value (|r| < 0.5 ($P < 0.0001$)).
In this study, it can be concluded that the ultimate pH measurements are valuable to predict meat quality of ham after cooking.

b) Winter

About the same significant correlations (|r| ≥ 0.5) as above were found for winter (data not shown). In general, they were lower for winter than for summer. This could be a result of the lower amount of measurements in winter than in summer. A difference between summer and winter were the higher correlations between pH_i/PQM_i in the MG and L* values of the LD and MG for winter. The negative correlation between pH and PQM is well known. In this study a high positive correlation (r = 0.69 ($P < 0.0001$)) between PQM_i

of the MG and L^*_u in the LD was found. Again, meat with more PSE characteristics will have higher L* values.

5.4 Conclusions

PSE meat is still a common meat quality deficiency and influences the quality of cooked ham. Based on the quality characteristics of the cooked ham, more PSE was found in summer than in winter. This was in consensus with the PQM_i measurements and $L^*(57.1)_u$ measurements in the *M. Longissimus dorsi*. On the other hand, pH measurements are more often used in industry to define PSE meat than PQM or L* measurements. The proportion of PSE meat differed between the three examined muscles and could be explained by a different fiber composition.

From this study, it can be concluded that the ultimate pH measurements (after 24 h and 35 h) in the 3 examined muscles, and certainly the ultimate pH in the *M. gracilis*, are good measurements to predict colour 24-35 h post-mortem and the meat quality of hams after cooking.

Chapter 6: General discussion, conclusions and perspectives for further research

Since pork industry is currently under significant pressure, costs need to be reduced and quality needs to be increased. Moreover, fresh pig meat is one of the most exported food products of Belgium with 702,576 tons (including edible by-products, excluding fat) or 1.17 billion Euros in 2010 which is 68% of the total Belgian pig meat production or 0.7% of the total world production, making this research topic even more relevant (VLAM, Belgian Meat Office, personal communication). Stress experienced by pigs is an important cause of financial loss by affecting pigs' behaviour and meat quality. Consequently, behaviour and meat quality can yield very useful information as indicators for welfare measurements (Fraser & Broom, 1990; Pérez et al., 2002; Tarrant, 1989; Warriss et al., 1998a, b). Additionally, the meat market in Belgium wants a good carcass quality, high slaughter yield and high lean meat content. As a result, most pigs are nN i.e. crossbreds between a homogenous halothane positive Piétrain boar and a homogenous halothane negative hybrid sow, making stress even more important because these hybrids are still carrier of the halothane gene and consequently associated with risk for PSE meat (De Smet et al., 1996; Fisher et al., 2000; Nanni Costa et al., 1999).

During this thesis the effect of stress during rearing (Chapter 2 and 3) and before slaughter (Chapter 4 and 5) was studied. More in particular, this study investigates the effect of (1) stressors such as ventilation (Chapter 2) and environmental enrichment (Chapter 3) on pigs' biting behaviour and (2) pre-slaughter stress on meat quality (Chapter 4) of fresh and processed meat (Chapter 5).

In Chapter 2, the effect on biting behaviour of (1) pigs originating from two different boars (same breed i.e. Piétrain, but different line) and (2) ventilation characteristics was studied on the same farm. The results of this case study showed that biting behaviour in pigs is indeed a multi-factorial syndrome (Moinard et al., 2003; Sambraus, 1985). Genetics and ventilation had a significant effect on the pigs' biting behaviour and consequently wound percentages. Boar 2 originated from a different genetic line and was characterized by producing pigs with a better conformation and a higher lean content than pigs from boar 1. Pigs with a high lean content are heavy muscled and need more resources to maintain their body metabolism compared to pigs that are less muscled. Because of the higher maintenance requirements of the pigs with a high lean meat content, the oxygen demand is higher compared to pigs with a lower lean meat content. Consequently, appropriate ventilation (e.g. air inlet at a velocity of 1 m/s) is needed. This could explain why pigs descendent from boar 2 were more susceptible for ventilation defects, i.e. higher biting behaviour than pigs descendent from boar 1. More research, such as performed in Chapter 2, was needed to verify the hypothesis formulated by Taylor et al. (2010), namely that the effect of genetics on tail biting (biting behaviour) at the line/strain level is significant. However, stocking densities in this case study were rather high and could also be a risk factor (Schrøder-Petersen & Simonsen, 2001; Taylor et al., 2010). Further research is needed to investigate the effect of different pen densities on biting behaviour of pigs descending from different boars (same breeds, different lines). As a consequence of previous results it could be possible that certain breeds/or genetic lines within a breed, which have a high lean content, need improved rearing conditions (e.g. better ventilation, lower pen density,…) compared to pigs with a lower lean content. The research also demonstrated that tail docking was not a good solution to avoid biting behaviour. As reported in literature, the attention of tail docked pigs was redirected to other body parts of pen mates (Fraser & Broom, 1990).

By means of the provision of environmental enrichment, biting behaviour can be decreased (Van de Weerd et al., 2009). Although in the case study, habituation occurred since the same toy was presented during the whole observation period. Due to this lack of novelty, it can be assumed that the reduction in biting behaviour by the toy was minimal (Van de Weerd et al., 2003).

Rotation of enrichment objects can increase novelty (Grandin et al., 1983; Trickett et al., 2009). Nevertheless, only one paper was found reporting the effect of 2 alternating toys (short sequence) over a few weeks after weaning, which increased novelty, but habituation still occurred (Trickett et al., 2009). As a result, the effect of a continuously repeated sequence of different toys was investigated over the complete fattening period (Chapter 3). The continuous sequence of seven enrichment objects reduced biting pen mate behaviour and the number of wounds compared to providing only a single toy (chain). However, no effect on growth and feed conversion of the pigs was seen. This is probably due to the low levels of pen mate-directed behaviours. It would be interesting to repeat this experiment at a pig house with a severe biting problem while all other risk factors, such as described in the introduction, are under control. A positive effect on the performance of the pigs (higher growth and lower feed conversion rate) has to be expected.

Generally, the most toy contact was observed together with the highest biting pen mate behaviour as more popular toys can induce competition behaviour (Docking et al., 2008). Fewer pigs per enrichment device (Scott et al., 2007), presentation of the toy in a more central position or simultaneous presentation of multiple high value toys might reduce competition and need further investigation. As in literature, most popular toys were found to be ingestible, chewable, flexible and destructible. However, more studies are necessary to investigate the ideal point-source enrichment object because all examined objects provide less occupation than substrates such as straw (Day et al, 2002; Scott et al 2006). Green is the favourite colour of pigs (personal communication). Consequently, the provision of toys, which are known to reduce biting behaviour, in the green colour could reduce biting behaviour even more. More research is needed to confirm. Furthermore, habituation still occurred since toy contact behaviour was lowest at observation day 5 or decreased when the same toys were provided for the second or third time. An exposure time of less than 2 days instead of 7 days (Gifford et al., 2007) or a longer sequence of different toys might be a solution.

The effect of changing the order of presentation needs further examination because the toys were always present in the same sequence to each other. Consequently, there could be a confounding effect of toy and sequence. The ideal sequence should maintain toy contact behaviour without competition in order to avoid biting pen mate behaviour and reduced animal welfare.

It should be mentioned that changing a toy can be time consuming and easy to use systems are needed. However, this takes less time than the use of straw.

Good rearing practices will result in less stress and consequently a decrease in abnormal behaviour will occur. Meat quality is influenced by pre-slaughter stress which, in turn is influenced by the rearing environment (Beattie et al., 2000; Geverinck, 1998c) e.g. pigs reared in enriched environments showed less fearfulness towards humans (Pearce et al., 1989) and expressed lower reactivity in response to stressful stimuli than those reared in barren environments (Pearce & Patterson, 1993). Therefore, a positive effect of environmental enrichment on meat quality has to be expected, but was rarely reported in literature (Van de Weerd & Day, 2009). In Chapter 3 it was discussed that toy contact and biting pen mate behaviour decreased over age, and is in consensus with the reduction in biting behaviour as reported in Chapter 2. The activity of pigs decreased over age and was also found in literature (Solba & Wood-Gush 1989). This decreasing activity over age could also explain why environmental enrichment has almost no effect on meat quality, but was not examined in this thesis. Nevertheless, there is also an increased public demand for meat originating from welfare-friendly systems with the expectation that meat from such systems has a better quality (Peeters et al., 2006).

Stress before slaughter might have an enormous effect on meat quality and is the determining factor being multifactorial in its nature (Cassens, 2000). As a result, a straight relationship between stress during fattening and meat quality has not to be expected (e.g. Terlouw, 2005). Therefore, the critical points before slaughter were defined in Chapter 4 and if standardized in all slaughterhouses, good handling practices might improve meat quality even more. The examined critical points before slaughter were transport, unloading, lairage and stunning within the same research sequence, which has not been reported in literature before. The results showed that PSE is still a common problem in Belgium. This can be explained by the nN genetics of the pigs and by pre-slaughter stress (Cassens, 2000). The observed PSE percentages were highest in spring and autumn and lowest in summer, in contrast with literature (Guàrdia et al., 2004). This could be explained by less observed pre-slaughter stress in summer than in spring or autumn. Extra preliminary research reported no effect of temperature on the prevalence of PSE meat. Instead of focussing on high or low temperatures, weather and temperature fluctuations could be more important risk factors (Grandin, 1994). Further, research should measure the weather conditions, the temperature on the day of observation and the temperature the day before. Large differences in temperature might result in a higher chance to observe PSE meat. Next to season, the percentage of panting pigs, the use of an electric prod and the mean noise level produced during unloading were determining factors for meat quality. In order to decrease panting behaviour, pig handling and transport need to be optimal. Moreover, the use of an electric prod needs to be avoided. Electric prods are used in slaughterhouses with poor driving facilities to the stunner which will also result in more noise produced by the pigs. Sound and in particular novel sound is a stressful factor for pigs and will activate the animals' defence mechanism, which consequently affects meat quality (Talling et al. 1996). Sound level was not only important during unloading, but extra measurements revealed significant cut off values for sound level before/during unloading, during lairage and during movement to the stunner. These values can contribute to the development of new management practices which will result in less stress and consequently better animal welfare. An example of a system is the low stress system (LSS), which incorporates smaller pens holding 15 pigs and automatic push gates to move the animals to the stunner. Before the stunner, the groups were divided into small groups of 5 pigs for CO_2 stunning

(Ramantanis et al., s.a.). In Belgium, one slaughterhouse uses this system, which is associated with low sound levels and has a very high meat quality. Another perspective to improve meat quality is to build the stables of slaughterhouses with sound isolation materials or to provide a 'decibel alarm' when the sound level is too high. Actions, such as more animal friendly driving to the stunner (e.g. no electic prod), can be taken when the decibels are too high.

Separate introduction of the pre-slaughter measurements as fixed effects in the statistical model showed six more parameters affecting meat quality. These pre-slaughter parameters were loading density on truck, shower water temperature, mean noise level produced during movement of the pigs to the stunner, stunning effectiveness, CO_2 concentration and electrical stunning. A loading density on the truck of 0.39-0.45 m²/100 kg had a positive effect on meat quality but differs from the one defined in the EU Directive (95/29/CE). The cut off values, used by the EU Directive, were proposed by Lambooij et al. (1985) and were results of transports for long distances (2 days). Consequently, other cut off values, such as proposed in Chapter 4, can be used for short transports (< 3 h). Cold shower water resulted in high body temperature fluctuations and needs to be avoided. Stunning effectiveness was also important and a CO_2 concentration above 80% gave a more effective stunning (Nowak et al., 2007), which both had a positive effect on animal welfare and consequently meat quality.

If the proposed risk factors are taken into account, animal welfare will improve together with meat quality. As a result, financial losses of fresh and processed meat due to poor meat quality will decrease. Based on the encountered risk factors, some adjustments were performed in a few slaughterhouses and the preliminary results showed an improvement in meat quality. These adjustments were:

- An increase in [CO_2] from 70% to above 80%.
- The transition of the manual driving system of pigs too the use of a LSS system.
- The lightening of the driving area to the stunner, especially when the pigs need to make a turn of 90°.
- The removal of the electric prod.
- Smooth unloading and driving to the stunner (e.g. small groups)

The research of the cooked hams also confirmed that PSE meat is still a common problem in Belgium, in contrast with DFD meat. Different biophysical measurements were used to define PSE meat and revealed a higher PSE percentage in summer in the *M. Longissimus* *dorsi* than reported in Chapter 4. Moreover, a lower cut off value for pH_i to define PSE meat was used in Chapter 5. This was necessary, otherwise most samples would be PSE meat and no extra information could be collected. Different cut off values can be used (Adzitey & Nurul, 2011). The pH_i and pH_u values were low and more extreme PSE cases were observed. The higher PSE occurrence can be explained by pre-slaughter stress, which was much higher in this slaughterhouse (e.g. sound levels, frequent use of the electric prod, low stunning effectiveness…) than during summer in the slaughterhouses described in Chapter 4. The contrast between Chapter 4 & 5, concerning the most/lowest PSE prevalence in summer, could also been caused by temperature fluctuations and weather conditions, as described above, but were not measured. It should be noticed that no good comparison between Chapter 4 & 5 could be performed for the winter as not enough measurements were performed in Chapter 4. Furthermore, different cleaning protocols and calibration times were used for the pH electrode in both studies. PH measurements are widely used to determine meat quality (Warriss, 2000) but many mistakes are still made (Hanna Instruments, personal message). To execute more precise pH measurements, 2 cleaning solutions, one for oils and one for proteins, were used every 20 measurements as proposed by Hanna Instruments during following study as reported in Chapter 4. Additionally, the electrode was checked with standard solutions and recalibrated if needed instead of once a day during the ham experiment. Moreover, if the recalibration took too long, a new electrode was used. Measurements such as conductivity and colour can provide more information about meat quality but are not easy to perform on industrial scale. Although, if pre-slaughter stress decreases, PSE occurrence will decrease and no need for complicated measurements is necessary. O' Neill et al. (2003) reported that the retail value of a ham manufactured from normal pork is 11.10 Euros and a ham manufactured from severe PSE pork is only 5.55 Euros.

The pH, measured 24 or 35 h post-mortem in the three examined muscles revealed the most information about the meat quality of the hams after cooking. This information is very interesting for meat industry, because hams can be selected in the slaughterhouse depending on the needs of their customers. However, more research is needed to determine which cut off values should be used for these measurements.

The results of this thesis showed the relevance of good ventilation and the impact of genetics on biting behaviour and wound percentages of pigs. Moreover, the sequential application of different toys also affected biting pen mate behaviour in a positive way. This information, which leads to improved rearing conditions and animal welfare, might positively affect fresh meat quality if pre-slaughter stress is minimized. As a consequence, financial losses will decrease. The pre-slaughter stressors are revealed in this study for the Belgian certificated Certus slaughterhouses. Furthermore, good rearing practices and low pre-slaughter stress might also positively affect the quality of the end product, and can be predicted by pH measurements 24 or 35 h post-mortem in the three examined muscles for cooked ham, as reported in Chapter 5, which reveals another financial benefit. More research is needed to investigate the effect of good rearing practices, in combination with minimal pre-slaughter stress, on the quality of fresh and processed meat. A significant improvement of meat quality has to be expected.

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