Animal welfare and meat quality in pigs as affected by trailer type, travel distance and genotype

Thèse

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Résumé

L'objectif de cette thèse consistait à évaluer les effets de la conception des véhicules *pot-belly* [PB] vs. *flat-deck* [FD] ainsi que les distances des transports (45 min vs. 7 h) sur le bien-être animal et la qualité de la viande des trois croisements Piétrain (Piétrain 50% HAL^{Nn} (50Nn), 50% HAL^{NN} (50NN), et 25% HAL^{NN} (25NN). Les réponses comportementales et physiologiques ainsi que les paramètres de qualité de la viande ont été mesurées. Les relations entre la température corporelle juste avant l'abattage, mesurée par la thermographie infrarouge (IRT), et les paramètres de bien-être animal et de la qualité de la viande ont également été étudiées et comparées.

L'ensemble des résultats obtenus des deux études sur les transports de longue et courte distance ont indiqué que la génétique a un impact plus important sur les paramètres de bien-être animal et de qualité de viande que le type de remorque. Toutefois, les effets négatifs du génotype sur les réponses physiologiques au stress et les paramètres de qualité de la viande sont plus marqués pour le modèle PB lorsque le transport est de courte durée. Les porcs issus de croisement Piétrain 50%, quel que soit le génotype HAL, ont produit des carcasses plus maigres, mais semblent être plus sensibles au stress lié au transport. La qualité de la viande n'a pas été affectée par la proportion de l'héritage Piétrain dans le croisement, mais par la présence du gène HAL.

L'étude de la température corporelle par thermographie infrarouge suggère que l'IRT oculaire peut détecter des changements de la température corporelle associée à l'état physiologique des porcs et peut être considéré comme un outil potentiel pour prévoir les variations de la qualité de la viande dans des conditions commerciales.

Summary

This thesis dealt with the effects of vehicle design (pot-belly [PB] vs. flat-deck [FD]) and transport distances (45 min vs. 7 h) on animal welfare and pork quality of three Pietrain crossbreds (Pietrain 50% HAL^{Nn} (50Nn), Pietrain 50% HAL^{NN} (50NN), and Pietrain 25% HAL^{NN} (25NN). Behavioural and physiological responses and pork quality parameters were measured. The relationship between body temperature as measured by infrared thermography (IRT) in the restrainer before stunning and other animal welfare and pork quality parameters was also studied.

Results obtained from both short and long distance transportation indicated that genetics has a larger impact on animal welfare parameters and pork quality traits than trailer type. However, under short distance transportation the PB trailer model augmented the negative genotype-related defects as measured by physiological responses to stress and pork quality parameters. Pigs with 50% Pietrain crossbreeding, regardless of the HAL genotype, produced leaner carcasses but appeared to be more responsive to transport stress. Pork quality was not detrimentally affected by the proportion of Pietrain inheritance in the crossbred but by the presence of the HAL gene.

The study of IRT body temperature suggests that ocular IRT may detect changes in body temperature associated with the physiological condition of pigs and may be considered a potential tool to predict pork quality variation under commercial conditions.

Table of content

Table List

Figure List

Acronyms and Abbreviations List

AA: amino acid

ACTH: adrenocorticotropic hormone

AD: adductor

ADP: adenosine diphosphate

ATP: adenosine triphosphate

CBT: core body temperature

CK: creatine kinase

CRH: corticotrophin-releasing hormone

CT: computed tomography

CV: coefficient of variation

DFD: dark, firm, dry

DHR: delta humidity ratio

DNA: deoxyribonucleic acid

DOA: dead-on-arrival

DT: delta temperature

DXA: dual energy X-ray absorptiometry

EU: Europe

FD: flat-deck

GIT: gastro-intestinal tract

GITR: mean gastrointestinal tract temperature measured at the restrainer

h^2: heritability

HAL: halothane

HC: halothane challenge

HCW: hot carcass weight

HPA: hypothalamic-pituitary-adrenal

HR: humidity ratio

IROT: ocular infrared thermography

IRST: skin infrared thermography

IRT: infrared thermography

LACR: blood lactate measured at the restrainer

LD: longissimus dorsi
LSA: hand-held lactate analyzer
MRI: magnetic resonance imaging
NAI: non-ambulatory, injured
NANI: non-ambulatory, non-injured
PB: pot-belly
PC: phosphocreatine
PCV: packed cell volume
PFN: pale, firm, non-exudative
IP: inorganic phosphate
PQM: pork quality meter
PSE: pale, soft, exudative
PSS: porcine stress syndrome
QTL: quantitative trait loci
RFN: red, firm, non-exudative
RH: relative humidity
RN: rendement napole
RSE: red, soft, exudative
SA: sympatho-adrenal
SM: semi membranosus
T: temperature
THI: temperature humidity index
WHC: water-holding capacity
WK: week

If you have men who will exclude
any of God's creatures from the shelter of
compassion and pity, you will have men who
will deal likewise with their fellow men.
St. Francis of Assisi

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Foreword

This thesis is composed of 6 chapters. The first chapter is a bibliographic review of the parameters used to measure the stress response and of the preslaughter factors influencing animal welfare and pork quality. In order, chapter 2 outlines the hypothesis and objectives of the experiments presented in this thesis. Chapters 3, 4 and 5 are presented in the form of manuscripts and describe the experiments showing the related results. In chapter 6, general conclusions and future work are presented.

The first manuscript, entitled "Effects of trailer design on animal welfare parameters and carcass and meat quality of three Pietrain crosses being transported over a long distance", was published in the Journal of Animal Science 90: 3220-3231. Authors: Angela V. Weschenfelder, Stephany Torrey, Nicolas Devillers, Trever Crowe, Anna Bassols, Yolanda Saco, Matilde Piñeiro, Linda Saucier and Luigi Faucitano.

The second manuscript "Stress responses and carcass and meat quality variation in pigs of three Pietrain crosses transported by two different trailer types over a short distance" was submitted to Livestock Science in Decembre 2012. Authors: Angela V. Weschenfelder, Stephany Torrey, Nicolas Devillers, Trever Crowe, Anna Bassols, Yolanda Saco, Matilde Piñeiro, Linda Saucier and Luigi Faucitano.

A third manuscript, based on chaper 6: "Correlation between infra-red thermography images as a tool to monitor the physiological condition of pigs prior to slaughter and predict pork quality variation", was submitted to the Meat Science journal. Authors: Angela V. Weschenfelder, Xavier Maldague, Al Schaefer, Linda Saucier and Luigi Faucitano.

The first and second manuscripts had the objective to evaluate the effects of different trailer designs and travel distances on animal welfare and meat quality parameters, in three different Pietrain crossbreds. The objective of the third manuscript was to evaluate the use of infrared thermography as a tool to assess the animal welfare condition prior to slaughter and to predict meat quality.

Angela V. Weschenfelder was in charge of the set up and data collection for all experiments and also generated the idea for experiment 3. She was also responsible for

statistical analysis of data and preparation of the 3 manuscripts. Drs. Linda Saucier and Luigi Faucitano, director and co-director of research, supervised all steps of this work, with Dr. Luigi Faucitano generating the idea for experiments 1 and 2 and being the corresponding author in all manuscripts. Drs. Stephanie Torrey and Nicolas Devillers collaborated in the set up and work planning of the behaviour study and participated in the interpretation of results and articles revision. Drs. Anna Bassols, Matilde Piñeiro and Yolanda Saco are experts in the analysis of acute phase proteins and helped in the laboratory analytical phase and manuscript revision. Dr. Trever Crowe collaborated in the analysis and interpretation of the environmental data collection inside the trailers and participated in the manuscript review. Drs. Al Schaefer and Xavier Maldague collaborated in the analysis of the IRT body temperature images, data interpretation and revision of the manuscript.

Introduction

In Canada, pigs are mostly transported to slaughter plants using pot-belly (PB) trailers. Originally designed for cattle transportation, the PB trailer design is not considered suitable for swine transportation. The main reasons are the large number of steep ramps that result in more stress on the pigs during loading and unloading, with clear consequences on load and unload time and the incidence of deads-on-arrival (DOA) and downers (Ritter et al., 2008; Torrey et al., 2008). Another trailer type commonly used in Canada is the flat deck (FD) trailer (more common in Western Canada and US). Compared to the PB trailer, the FD model requires shorter loading and unloading durations and causes fewer casualties (Ritter et al., 2008). Other factors, such as trip duration, ambient conditions and animal density, may have an additive effect on the incidence of total pig losses (Dewey et al., 2009). To date, the effects of PB and FD trailer designs on stress response and meat quality of pigs of different genotypes, transported at different travel distances has not been evaluated.

Transport conditions in Canada are not ideal, given the extreme climatic conditions, trailer types, and travel distances. Optimizing these conditions is now becoming of paramount importance considering the gradual introduction of Pietrain genetics in the North American pig population.

Pietrain pigs are known for their superior growth performance, but also for having greater susceptibility to stress, which results in higher transport mortality and inferior meat quality (Fàbrega et al., 2002a,b). Whether Pietrain pigs' response to stress is associated with breed or the HAL gene remains unclear. Currently, the increased stress response in Pietrain pigs has been explained by the higher frequency of the HAL gene (*n* gene, considering NN, Nn and nn pigs). Despite these effects, Pietrain pigs carrying the allele *n* are generally leaner, faster growing and have an increased feed conversion efficiency compared to non-carriers (NN; Leach et al., 1996; Gispert et al., 2007). Pigs raised for slaughter are usually heterozygote for the HAL gene (Nn) and thus less affected by extreme stress sensitivity (Fernandez et al., 2002). A major discussion is whether carriers (nn or Nn) have acceptable meat quality compared to animals that are completely free of the gene (NN; de Vries et al., 2000). Several studies showed a clear increase in the incidence of PSE (pale, soft,

exudative) in heterozygote (Nn) pigs (Leach et al., 1996; Gispert et al., 2000; Fàbrega et al., 2002b), whereas others reported meat quality from Nn pigs to be either closer to NN pigs or intermediate between NN and nn (Rundgren et al., 1990; Wolf-Schwering and Kallweit, 1991). According to de Vries et al. (2000), no general conclusion on the impact of the HAL gene on pork quality is possible as it depends on the applied pre-slaughter conditions.

The meat industry seeks new tools to assess animal welfare and predict pork quality variation. The measurement of body temperature using infrared thermography (IRT) images has the advantage of being non-invasive and would allow obtaining real-time thermal profile of animals, providing information related to their physiological status. It was used to predict pork quality in the past; however, it was suggested that more studies in pigs of known genotype would be necessary (Gariépy et al., 1989). Aiming to provide more information about the use of IRT under commercial conditions, preliminary results of two different studies using infrared thermography to estimate animal welfare and to predict pork quality are presented.

Chapter 1: Literature Review

1.1. Ethics, animal welfare and meat production

The concern about cruelty against animals is a primary moral issue, and each person will differ in their values and outlook towards it (Gregory, 1998). Some feel that animals are less important than themselves or other humans and so they warrant less concern.

The utilisation of animals in our society is ethically discussed and debated from four main points of view: animal rights, utilitarianism, anthropocentrism, and from the species-integrity-view (Rollin, 1990; Sandoe et al., 2003). The production of meat and other animal protein source as a result of livestock production are mainly supported by the utilitarianism together with the anthropocentrism views (Sandoe et al., 2003; Haynes, 2008).

Regarding the human animal relationship, the "utilitarianism", as the name suggests, is basically a pragmatic view that is based on on the principle of cost-benefit, i.e. the compromise between what humans can get from animals and the life of those creatures (Francione, 2003; Rollin, 2003; Haynes 2008).

The anthropocentrism view is based on the assumption that humans are superior to other animal species. Despite their condition of superiority, humans do not have the right to be careless in their relationship with animals. Endowed with rationality, humans are expected to treat more vulnerable beings with dignity as a reflex of compassion, one of humanity's communal moral values (Arkow, 1998; Rollin, 1996a,b).

The ethical justification of animal production has to do with the human alleged needs. The necessity to eat is undoubtedly one of the most important human needs and meat has been an important component of the human diet throughout history. From both the utilitarianism and the anthropocentrism point of views, it is justifiable that livestock be bred and raised with the purpose to produce meat. However, as the balance between interests is an asset for the utilitarianism and the care of humans towards animals essential for the anthropocentrism, humans should be committed to grant these animals a good life, free from unnecessary suffering (Sandoe et al., 2003; Rollin 2003). According to both views,

once the conditions are satisfied (e.g. care principles towards the animals used for meat production) the trade is fair and acceptable (Francione, 2003; Rollin 2003).

Complementary to the aforementioned ideas, Gregory (1998) has described three main reasons to be concerned about animal welfare in the meat production:

- *respect for animals and a sense of fair play;*
- *poor welfare can lead to poor product quality;*
- *risk of losing market share products, which have acquired a poor welfare image.*

From this perspective, legislation and codes of practice were created on the basis of the Farm Animal Welfare Council of the United Kingdom's five freedoms (FAWC, 1979), which were identified in order to respect the following animal needs:

- *the freedom from thirst, hunger and malnutrition;*
- *the provision of appropriate comfort and shelter;*
- *the prevention or rapid diagnosis and treatment of injury, disease or infestation with parasites;*
- *the freedom from distress;*
- *the ability to display normal patterns of behaviour.*

To improve and guarantee these freedoms, it is necessary to constantly evaluate animal behaviour and health status. That demands knowledge about their innate and stimulated behaviours to external factors, considering natural and physical aspects. As an example, pig producers use indoor systems for different reasons, such as climate control, ease of cleaning, use of labour-saving machines, protection from predators, animal handling and control of the animals. Because producers are seeking high productivity, the freedom from thirst, hunger and malnutrition, the provision of shelter, the prevention of rapid diagnosis and treatment of injury, diseases and parasites are freedoms easily achieved. The optimisation of husbandry systems through environmental enrichment allowed the achievement of the freedom from distress or at least minimized the effects of the lack of them.

The ability to display natural behaviours appears to be the least respected freedom in intensive husbandry systems. However, it has been suggested that farm animals do not need "natural behaviour" *per se* to achieve good welfare, since "natural behaviour" does not exist in a form of one standard measure (Spinka, 2006). Furthermore, beside the fact that "natural" behavioural pattern "naturally" vary enormously in quantity, there is another even more radical source of variation in natural behaviour which is the existence of behavioural strategies (Spinka, 2006). For instance, animals are capable of adaptations to meet challenges and explore opportunities making it possible to survive and reproduce successfully under different environments (Fraser, 2008). This means that even when a behavioural pattern is performed differently, it does not necessarily mean that the animal is behaving "unnaturally". Indeed, some natural behaviour, such as emergency and fitness behaviours are not necessarily associated with good animal welfare and should be avoided (Spinka, 2006).

1.2. Animal welfare and meat consumption

"Animal welfare" and "health" are concepts directly related to the new consumer profile. The idea that animals raised for food are being treated humanely improves the acceptance of meat for those who like it (Eurobarometer, 2005).

Most modern vegetarians and semi-vegetarians share the opinion that humans, as individuals, are not innately cruel to animals or disrespectful to the environment, but cultural values have forced society towards being cruel and wasteful (Gregory, 1998).

According to a consumer survey run in the EU (Eurobarometer, 2005), EU consumers (52% of the respondents) never or very rarely think about animal welfare when they buy meat. However, 48% of respondents answered that the main reason to buy animal welfare friendly food products would be the better quality of the products. Indeed, Bornett et al. (2003) suggest that consumers would be interested to pay more for meat obtained from animals raised under welfare-friendly conditions. These results are supported by another survey, where 57 % of the interviewed consumers declared to be ready to pay more (up to 25 %) for animal welfare-friendly products (Eurobarometer, 2007). Curiously, the same survey (Eurobarameter, 2007) reported that these intentions might not necessarily convert into a real purchase action.

In North America, a similar survey was conducted with focus on the traceability of red meat (Dickinson et al., 2003). The results of this study showed that North American consumers would pay for traceability, and would accept to pay even more if animal welfare was included in the system (Dickinson et al., 2003).

More recently, Norwood and Lusk (2010) reported that one-third of Americans do not value welfare improvements. That was demonstrated in a survey where the extent to which consumers were willing to pay premiums for animal-friendly pork and eggs was investigated. This survey showed that, although consumers are concerned about animal welfare, this factor influences the consumer choice only when it is associated with other meat attributes, such as food safety, quality and healthiness. For instance, when information on animal welfare and nutritional characteristics are combined, meat acceptability increases (Napolitano et al., 2007).

This growing concern about animal welfare has resulted in three broad areas of interest for consumers: 1) Organic production; 2) Animal welfare-friendly production; and 3) Natural production (without the use of advanced technology; Grunert et al., 2004). Contrary to the results presented by the Eurobarometer survey (2007), consumers who are concerned about animal welfare either avoid buying meat or, when they do not, they buy meat from free range and/or organic production systems (Hoogland et al., 2005).

These consumers need, to trust regulatory systems and farm monitoring practices, as well as food labelling, which is the only way to be assured that animals are not suffering unnecessarily (Frewer et al., 2005; Velarde and Dalmau, 2012). This change in consumers' attitude would fit into the definition of animal welfare as an important attribute of an overall "food quality concept" as proposed by Blokhuis (2008).

1.3. Animal welfare and general principles of stress

1.3.1. Animal Welfare

Animal welfare concepts are based on three main approaches: 1) the environment-inherent nature approach: animals should be able to perform natural behaviours; 2) the feeling-based approach: animals' feelings should be respected; and 3) the functioning-based approach: animals should be able to cope with their environment (Broom, 1991, Dalmau, 2007; Marchant-Forde, 2009). Concepts that, according to Duncan (2005), can be divided into two main schools: the "biological functioning school" and the "feelings" school. The biological functioning school is concerned with the ability of animals to satisfy their biological needs. Failures and difficulty in coping with the environment are indicators of poor welfare. Besides, the "feelings" school believes that welfare is the absence of suffering as a result of a negative emotional state. Despite the different principles covered by these concepts, it is clear the necessity to understand and correlate both physical (behaviour, physiology, health, productivity and pathology) and mental (emotional) parameters to obtain a more global view of an animal's state. Physiological and stress responses are indicatives of adaptation efforts to cope with the environment, and can be measured to reveal an individual condition in a specific time (Boersma et al., 2009). This information can predict the animal welfare state that can vary from very good to very poor (Broom, 2008).

1.3.2. Homeostasis

Homeostasis was defined by Cannon (1935) as the stable state of the internal environment of the body. Under homeostasis approximately constant conditions are required for life maintenance. Naturaly, homeostasis is constantly threatened by different stimuli; which will trigger behavioural and physiological feedback mechanisms to maintain constant internal characteristics. Regulatory systems are activated to supply, process, and waste essential nutrients at cellular levels providing adjustment of body functions in an admissible range of variation (Broom and Johnson, 1993). Problems of maladaptation and poor welfare can be observed when the intensity or duration of a given stimulus prevents animals from coping (control of mental and bodily stability) triggering disease, behavioural problems, and even death (Broom and Johnson, 1993).

Physiologically, a healthy, rested animal of a given species should present biochemical and haematological values of different metabolic systems (e.g., plasma cortisol, lactate) in a determined range (Knowles and Warriss, 2007). Thus, alterations are expected under stressful conditions and basal levels should be restored once the stressor is taken away.

1.3.3. Stress

Stress can be defined as an adaptive response triggered by a stressor, real or perceived, that disturbs physiological homeostasis or psychological well-being (Committee on alleviation of distress in laboratory animals, 2008). It can be associated with neutral or even positive situations, such as mating (Colborn et al., 1991).

Physical (exercise, restricted movement, injury or pain, thermal-related demands, dietary limitations) or psychological stressors (spatial intrusion, fear, restricted movement, frustration and learning limitations) will result in a stress response eliciting a number of coping mechanisms (Ewing et al., 1999) to restore homeostasis.

A stress response causes significant biological changes in the animal. During stress, biological resources will be re-directed from biological activities, which were occurring before the stressor resulting in a "biological cost". The biological cost of the stress is what distinguishes a non-threatening stressor from distress (Moberg, 2000).

Behaviour is the most biologically economical (as related to changes in biological activity) response to a stressor (Von Holst, 1998). However, just adjusting behaviour may not be enough, or appropriate under given circumstances, to cope with a stressor. The autonomic nervous system and neuroendocrine system will play different roles during a stress response, which, if acute or prolonged enough may result in distress (e. g. development of pathology, stereotypic behaviour). For instance, once the animal is exposed to a stressor, simultaneous factors will increase the stress level additively with a cumulative result (Manteca, 2008). The type and duration of the stressor combined with age, genetics, coping patterns, and social status can influence the response of an animal to stress (Von Holst, 1998; Salak-Johnson and McGlone, 2007). A simplified scheme of an animal's physiological reaction to a stressor is presented in Figure 1.

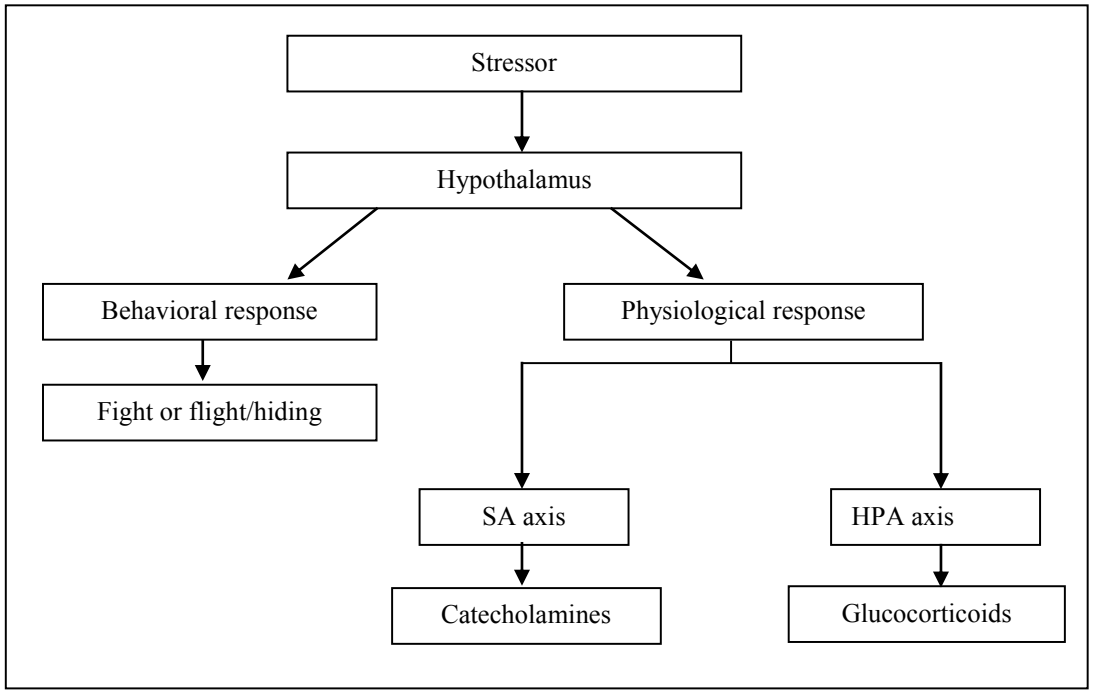

Figure 1. Animal physiological reaction to a stressor. SA: Sympatho-adrenal; HPA: Hypothalamic-pituitary-adrenal (adapted from Carragher and Matthews, 1996).

1.3.4. Behaviour and stress response

Behavioural responses are the primary way of interaction of the animal with its environment. It is a sensitive index of its physiological and health status, the most obvious indicator that an animal is experiencing difficulty (Broom and Johnson, 1993; Mormède, 2008).

A behavioural response to a stressor may be identified by exchanges and incomplete behaviours. Stereotypies, and various forms of undesirable socially oriented behaviours such as fighting, inflicting damages to self or other animals are some examples (Ewing et al., 1999).

1.3.4.1. Normal and abnormal behaviour

Pig's sensory abilities such as hearing, vision, touch, olfaction and taste allow them to interact with the environment, detecting information and responding to a wide range of stimuli (Held et al., 2009). These stimuli can be aversive or not and are translated into behaviour patterns, such as sexual receptiveness and spatial intrusion (Held et al., 2009).

In pigs, “normal” behaviour has been described as follows: 1) animals want to do (e.g. nest building in sows); 2) animals do not want to do, except under specific circumstances (e.g. shivering under cold conditions); and, 3) animals do not want to do at all (e.g. increased vocalization under isolation; Olsson et al., 2011). Thus “abnormal” behavioural response can be observed when the range of “normal” behaviours is known (Keeling and Jensen, 2009).

According to Mormède (2008), in pigs, “abnormal” behaviours can be categorized as: 1) apathy – under adaptation to a novel environment; 2) aggressiveness – during competition for food, mixing, and mainly at loading and in lairage; and, 3) stereotypies – under poor welfare conditions (e.g. sows in crates). Measures of abnormal behaviour are used to assess environmental needs or preferences, health problems, and adaptation to stressful conditions (Keeling and Jensen, 2009).

1.3.4.1.1. Stereotypies

Stereotypies are repetitive, relatively invariate sequence of movements, with no obvious purpose (Broom, and Fraser, 2007). It is prevalent in conditions considered aversive to the animals and it is associated with poor animal welfare (Olsson, et al., 2011).

Abnormal stereotypic behaviour may develop when an animal is prevented from performing normal and strongly motivated behaviour with typical behavioural signs (Keeling and Jensen, 2002). It has been associated with poor environmental conditions such as the lack of stimulation, social isolation, frustration, and feed restriction (Lawrence and Terlouw, 1993; Olsson et al., 2011). Stereotypies have been intensively studied in gestating sows. It appears that feed restriction is the primary cause of these behaviours; however, environmental factors are also involved (Lawrence and Terlouw, 1993). Stereotypies may be prevented or even eradicated by environmental enrichment such as the use of rooting areas (chains, peat, straw dispenser) inside pens or crates where animals are kept (Beattie, et al., 1995; Broom and Johnson, 2007).

1.3.4.2. Fear

Negative emotions, such as fear, have been characterized as physiological and behavioural responses that prepare the animal to cope with a danger, leading to protective reactions

(Jones and Boisy, 2011). It is important, when measuring fear, to take into account the nature of the stimuli, which may include novelty and intense stimuli (Janczak, 2010).

Fear-related behaviours may include active defence (attack, threat), active avoidance (flight, hiding, scape), or passive avoidance (immobility; Jones and Boisy, 2011). Hyper-excitability is also associated with fearful situations (Gregory, 2004).

Pigs cognitive abilities and elaborated social behaviour may cause frustration or fear, which might result in injuries, disease, pain, and physical discomfort (Broom and Fraser, 2007). Fear during pre-slaughter management such as handling and transport may be associated with freezing behaviour, tonic immobility, escape or avoidance from humans, aggression, vocalization, balking, slipping or falling (Broom, 1991; Grandin, 1997; Hemsworth and Coleman, 1998; Hemsworth et al., 2002; Marchant-Forde et al., 2003). For instance, panic-induced injuries are observed in pigs and other livestock species during transportation. The effects of panic-induced injuries result in acute and long term welfare problems such as pain, infection, physical debilitation and even death (Jones and Boisy, 2011). In the long term, chronic fear may lead to behavioural depression as a result of the animal's inability to cope and adapt to its environment, and to interact successfully with its companions (Gregory, 2004; Jones and Boisy, 2011).

1.3.4.3. Pain

Pain may be easily identified by changes in animal behaviour (Gregory, 2004). Indeed, behavioural responses should be especially considered to assess pain, and are important to be recognized in order to provide strategies to alleviate the symptoms (Stafford and Mellor, 2010). Some behavioural responses to pain are more obvious than others and can be measured by a range of observations, such as the level of vocalization (e.g. castration in pigs; Broom and Johnson, 1993), and changes in posture (e.g. lameness and depressed behaviour followed by fever; Viñuela-Fernadez et al., 2011).

As some common procedures in farm animals are known to be painful, to determine whether or not a painful procedure is really necessary is one of the recommendations to improve animal welfare. Alternatives to necessary procedures, such as ear tagging instead

of ear notching in piglets (Viñuela-Fernandez et al., 2011), should be considered to reduce pain response.

During pre-slaughter management, pain was observed as a response to handling procedures in commercial slaughterhouses (Grandin, 1997, 2001). For instance, higher levels of vocalization, which is associated with painful and fearful situations (e.g. falling, physical coercion, electric prod, slipping), is used as an audit criterion in assessing animal welfare at the slaughter plant (Velarde and Dalmau, 2012).

1.3.4.4. Aggression

Aggression, at a certain level, is a normal behaviour. Under farm conditions, piglets within a litter engage in mainly playful behaviour and establish dominance relationships with one another (D'Eath and Turner, 2009). Aggression can also occur from retaliation when an animal is fearful, and it can also be a spontaneous response to sudden pain (Gregory, 2004). Furthermore, aggression may result from situations where animals experience frustration, as reported by Dantzer et al. (1980).

In domestic animals, the most common reason for abnormal aggression results from mixing (Keeling and Jensen, 2002). Indeed, fighting between unfamiliar pigs may occur under confined space, where tolerance through mutual avoidance is not possible, leading to the establishment of new dominance hierarchy (D'Eath and Turner, 2009). No mixing is a practical recommendation during pre-slaughter management in order to prevent animal welfare and meat quality problems.

Allong with environmental conditions, genetics play an important role in animal aggressive behaviour. D'Eath et al. (2009) reported an important heritability ($h^2 = 0.47$) of aggressive behaviour with a positive correlation between genetics and fighting/bullying behaviour (r = 0.41/0.60) in pigs during handling procedures. For instance, 30-40% of variation in aggressive behaviour is related to genetics (Hocking et al., 2011). It is suggested that selection against aggressiveness may be possible by observation of the levels of skin lesions. Indeed, victims and aggressors may present skin lesions on different parts of the body. The recipient of the aggression will be identified as those presenting higher levels of skin lesions on the anterior parts of their body (head and shoulders), whereas the victims

will present lesions evenly on all body parts (Hocking et al., 2011).

1.3.5. Physiology and stress response

According to Le Neindre et al. (2001), for a more reliable assessment of animal welfare, the measurement of behavioural responses should be associated with physiological measures.

Physiological responses to stress are largely controlled by the hypothalamus, which triggers a series of reactions by the activation of the sympatho-adrenal system and the hypothalamic-pituitary-adrenal axis (Spoolder and Waiblinger, 2009).

1.3.5.1.Sympatho-adrenal (SA) system

During stress response, nerve impulses are transmitted to the hypothalamus activating the sympatho-adrenal system. A cascade of events (Figure 2) will physiologically prepare the body to cope with the stressor (the "fight or flight" adaptive response) resulting in metabolic adjustments (Broom and Johnson, 1993; Von Holst, 1998; Keeling and Jensen, 2009).

The SA stimulation produces the neurohormones adrenaline and noradrenaline, two catecholamines, which are released into the blood by the adrenal medulla during the "alarm reaction" (Von Holst, 1998). Because information is transmitted via neurons, catecholamines are released into the bloodstream within 1 to 2 seconds following stress stimuli (Broom and Johnson, 1993; Mormède, 2008). Higher levels of catecholamines indicate an acute sympathetic response and are associated with aversive situations, being extremely sensitive to handling procedures (EU-SCAHAW, 2002). The use of catecholamines is of value for short-term, acute stress assessment provided the animals are catheterized (Broom and Johnson, 1993; Mormède, 2008).

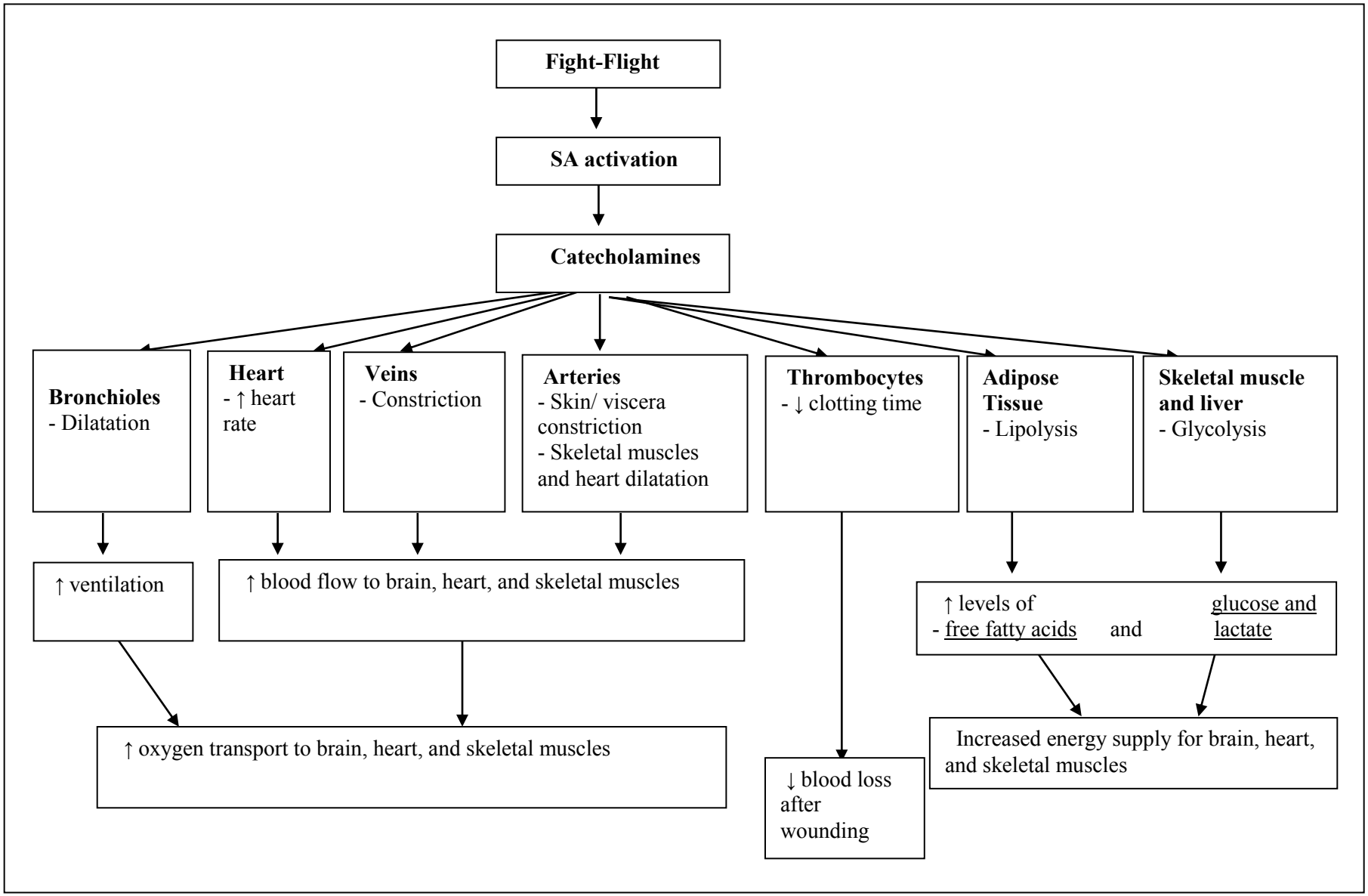

Figure 2. The activation of the Sympatho-adrenal (SA) system during a fight or flight response (adapted from Von Holst, 1998).

Under commercial conditions, in pigs, the assessment of these hormones in bloodstream during pre-slaughter conditions may be masked or affected by different stressors, such as electrical stunning (Hambrecht et al., 2004). As an alternative, the assessment of catecholamines in urine, instead of plasma, has been used to identify differences in neuroendocrine functioning in pigs (Plastow et al., 2005, Terlouw et al., 2004). Besides the fact that blood collection itself may ttrigger acute stress responses, difficulties in obtaining reliable basal levels of catecholamines in blood are the main impediments for this measure on plasma. Whereas, in urine, it can be collected non-invasively, and catecholamines and glucocorticoids are mainly excreted by urine with accumulation over several hours (Hay et al., 2000).

1.3.5.2.Hypothalamic-pituitary-adrenal (HPA) axis

The HPA axis is activated as a preparation for a long-term response,compared with the SA system, where the adrenal cortical hormones (e.g. cortisol) are produced. The hypothalamus secretes the corticotrophin-releasing hormone (CRH) that stimulates the pituitary gland to secrete the adrenocorticotrophic-hormone (ACTH) into the bloodstream, then stimulating the cortex of the adrenal gland to secrete the corticosteroids hormones (Figure 3).

Catabolic (proteolytic and lipolytic) and anabolic (gluconeogenesis and protein synthesis) activities are stimulated by the liberation of cortisol into the blood producing the energy necessary to cope with the stressor (Ewing et al., 1999; Tsigos and Chrousos, 2002; Mormède et al., 2007; Salak-Johnson and McGlone, 2007). Furthermore, cortisol negative feedback is involved in the return of the HPA axis to basal levels after stimulation (Mormède et al., 2007).

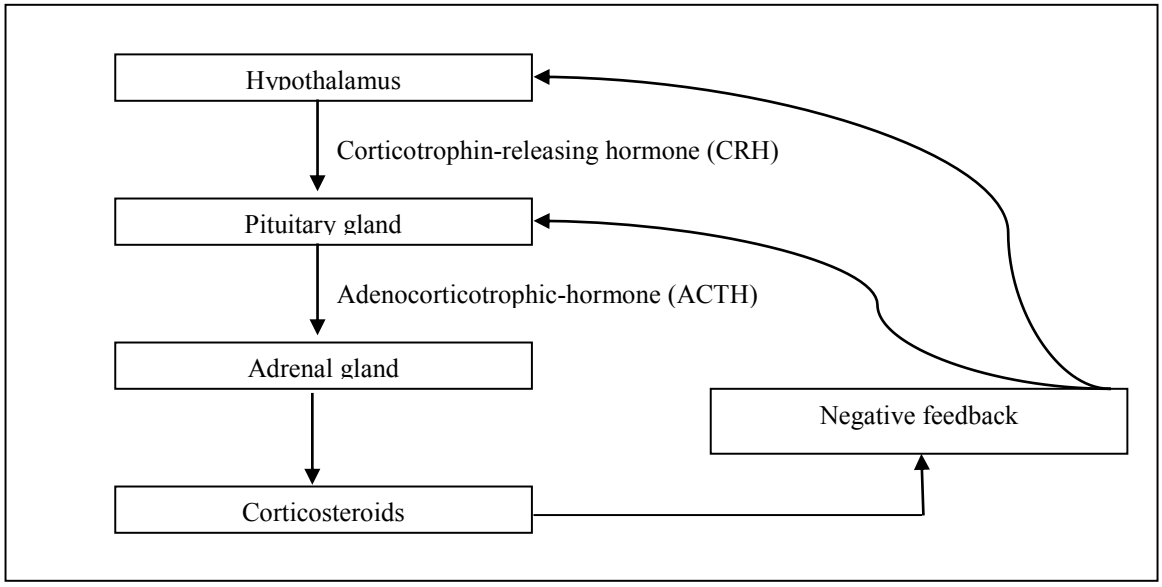

Figure 3. The hypothalamic-pituitary-adrenocortical (HPA) axis. (adapted from Newton, 2000).

Glucocorticoids are largely used to assess animal welfare. The increase of this hormone in blood flow is mostly related to the animals' psychological perception of a situation as well as to the extent of its physiological reaction (Knowles and Warriss, 2007). Cortisol is the glucocorticoid most often used for the assessment of pig welfare. Its levels can be measured in blood, saliva and urine. Cortisol levels have been reported to increase 30 min after loading and remained elevated up to 4.5 h during transport (Bradshaw et al., 1996; Apple et al., 2005). However, cortisol levels may be increased by circadian rhythm and by several environmental factors (e.g. feeding) which implies that this measure must be carefully evaluated in the context of when and where they are taken (Mormède, 2008).

Under chronic stress, the continuous stimulation of the HPA axis results in a chronic enhanced feedback of cortisol on the pituitary gland resulting in lowered or unchanged response (Janssens el al., 1995). However, if repeated exposure to stress occurs, the increased activity of brain regions responsible to regulate HPA axis activity may lead to an increased response with a cumulative effect (Mormède et al., 2007). Due to its role in the regulation of energy fluxes, the intensity of the HPA activity may negatively affect body weight and growth rate, with consequences on the energy balance, feed intake and temperature regulation (Mormède, 2008).

1.3.5.3.Immune system and infection

Different levels of glucocorticoids are also reported to affect immune function. Glucocorticoids, such as cortisol have anti-inflammatory functions (Mormède, 2008). However, physical and psychological stressors have been associated with deficient immuno-competence and increased susceptibility to infections and other diseases (Cockram and Hughes, 2011). According to Broom (2006), the extent of immunosuppression on animals can be measured by changes in T-cell activity, which is supressed as a result of increased glucocorticoids in blood.

The susceptibility of animals to infection during transportation is related to the effects of stress on their immune response, which is strongly affected by the increased levels of corticosteroid hormones, but also, by that of other hormones, such as beta-endorphin and vasopressin (Broom, 2006).

Increased levels of beta-endorphins follow ACTH increases and are related to the regulation of several reproductive hormones. Beta-endorphins are being used in the assessment of animal welfare as complementary information helping to understand changes in ACTH, and helping to interpret cortisol measurements (Geers et al., 1994; Broom, 1996; Bradshaw et al., 1996; EU-SCAHAW, 2002).

According to Knowles and Warriss (2007), the major role of vasopressin is to regulate body water homeostasis. The release of vasopressin into the bloodstream is triggered by increased plasma osmolality, which causes water retention. Indeed, vasopressin levels are reported to gradually raise during exercise in combination with increases in plasma volume and body temperature (Stebbins et al., 1994).

Enhanced secretion of vasopressin is associated with nausea symptoms in different animals (Hall and Bradshaw, 1998). In pigs, lysine vasopressin was related to motion sickness during transportation (Randall and Bradshaw, 1998) as it was observed at the same time as physical signs of nausea and vomiting (Bradshaw et al., 1996).

According to Gabay and Kushner (1999), acute-phase proteins increase (positive acute phase protein) or decrease (negative acute phase protein) by at least 25% during inflammatory disorders. It is an innate reaction in response to the occurrence of an infection

caused by tissue injury, damage or trauma (Baumann and Gauldie, 1994; Lampreave et al., 1994; Heegard et al., 2011).

Chen et al. (2003) compared clinically normal and pigs with growth deficiencies and found lower concentration of acute phase proteins in clinically normal pigs. Furthermore, between these pigs a higher concentration of the acute phase haptoglobin was found in pigs that presented lesions.

The changes in acute phase proteins are largely associated to their production of hepatocytes (Gabay and Kushner, 1999). They are produced in the liver and their synthesis is regulated by pro-inflammatory cytokines (Gabay and Kushner, 1999). During an acute phase response, increased local vascular permeability triggers the release of plasma proteins and leucocytes, which in turns release the cytokines (Cockram and Hughes, 2011). The cytokines then activate receptors on different target cells that trigger a systemic reaction activating the HPA axis. The result is the reduction in growth hormone secretion and clinical signs, such as fever, anorexia, negative nitrogen balance and catabolism of muscle cells (Gruys et al., 2005).

Haptoglobin and pig-MAP, the major acute phase proteins in pigs (Lampreave et. al., 1994), can be measured in blood plasma or meat juice for the assessment of pre-slaughter stress in pigs (Saco et al., 2003; Piñeiro et al., 2007; Piñeiro et al., 2009a,b; Averós et al., 2009).

1.3.5.4. Exercise and physical exertion

Muscular contraction occurs in the presence of ATP, which is also necessary to maintain the ionic balance and to provide heat for the body under resting conditions (Pösö and Puolanne, 2005). ATP synthesis occurs through oxidative and anaerobic pathways depending on the presence or absence of oxygen. During extreme or prolonged exercise, when oxidative energy production is exceeded, energy is anaerobically produced resulting in large amounts of lactate and protons. Increased exercise leads to increased metabolic activity rising oxygen consumption and cardiac output (Pösö and Puolanne, 2005).

1.3.5.4.1. Creatine kinase (CK)

In vertebrates, muscles with a high ATP turnover have the capacity to store PC as free energy to rapidly regenerate ATP by the reaction catalysed by creatine kinase (CK; Voet and Voet, 1995). During high metabolic activity (low levels of ATP), PC acts as an ATP buffer in cells that contain CK. Creatine is rephosphorylated in mitochondria using ATP derived from oxidative phosphorylation, then, CK use the mitochondrial PC to resupply ATP for muscle activity, which will explain why CK levels increase as a result of vigorous exercise (Broom and Johnson, 1993, Knowles and Warriss, 2007; Daroit and Brandelli, 2008).

The changes in membrane permeability generally associated with increased levels of enzymes of intracellular origin such as creatine kinase (CK) will influence metabolic rates during the conversion from muscle to meat (Pösö and Puolanne, 2005; Mormède, 2008). Indeed, at resting conditions the energy is provided to living muscle by the degradation of PC (phosphocreatine; catalysed by CK), thus even a mild stress may influence post-mortem metabolism where reduced amounts of PC will imply earlier glycolysis and faster pH drop (Daroit and Brandella, 2008). During post-mortem metabolism, ATP is produced via glycogenolysis where PC is broken into creatine (C) and inorganic phosphorus (Pi), one proton is bound to oxygen ($PC^{2-} + ADP^{3-} + H^{+} \rightarrow ATP^{4}_{+} + H_2O + C$), and pH increases by the action of CK and by glycolysis (Pösö and Puolanne, 2005). By maintaining a certain ATP levels just after slaughter (by reducing physicial exertion before stunning), CK defers meat acidification by delaying lactate production (glycolysis), and by proton buffering (H^{+}) as a result of CK reaction in the ATP synthesis using CP, ADP and protons. This condition creates an initial buffer effect on the pH fall, which does not occur when PC levels have been exhausted, which explain the rate of pH fall during transformation from muscle to meat (Pösö and Puolanne, 2005).

Serum CK levels are usually measured to indicate level of physical exertion and muscle damage of pigs during pre-slaughter procedures. During transportation, higher CK levels are associated to higher loading densities (Ritter et al., 2009b) and poor lairage conditions (Warriss et al., 1994).

1.3.5.4.2. Lactate

Exercise and physical exertion also contributes to the rise of lactic acid in the blood stream. Different body tissues constantly produce lactate during normal aerobic, but mainly during anaerobic metabolism, where pyruvate is converted to lactate in muscle (Voet and Voet, 1995). Lactate occurs mainly in striated muscles and in the liver and increases with higher levels of activity or disturbing conditions. During vigorous activity, the higher demand of energy results in lactate being transferred from the muscles to the liver to be converted to glucose. Glucose, in turn, is broken down and oxidized to pyruvate. Increases in lactate may take many minutes to occur; depending on the ratio production/clearance which is compromised under vigorous activity as the production of lactate is faster than the tissues can remove it (Broom and Johnson, 1993). There is evidence that lactate concentrations may rise and retrun to basal levels in 2 h (Anderson, 2010). The increased concentration of lactate in the blood will result in metabolic acidosis as showed by the lower blood pH (-0.5), which, when combined with higher body temperature (+1 to 2.5 ºC), may lead to the fatigued pig syndrome (Carr et al., 2005; Ritter et al., 2009a; Anderson, 2010).

1.3.5.4.3. Heart and respiratory rates

Together with endocrine and immune responses to stress, physical exercise raises metabolic activity increasing heart and respiratory rates, together with body heat production (Broom and Johnson, 1993; Pösö and Puolanne, 2005; Lewis et al., 2008). Changes in respiratory rate can be assessed by direct observation and are associated with fatigue in pigs during pre-slaughter procedures (Ritter et al., 2007).

As an indirect measure of the autonomic system, heart rate contributes to the better understanding and assessment of the underlying neurophysiologic processes of stress responses and different welfare states in farm animals (Broom and Johnson, 1993, De Jong et al., 2000; von Borell et al., 2007). Changes in heart rate are associated with metabolic rate as a result of physical activity (Pösö and Puolanne, 2005). Nonetheless, emotional responses may also result in heart rate variations, such as the presence of a predator or human contact (Broom and Fraser, 2007; Terlouw et al., 2004). A common problem when measuring heart rate is distinguishing between the changes caused by metabolic activity and emotional responses (Broom and Johnson, 1993).

In pigs, increased heart rate has been associated with disturbing situations, such as the use of electrical goads (Kuchenmeister et al., 2005; Correa et al., 2010) and negotiating ramps at loading (van Putten and Elshof, 1978), transport (Warriss, 1998b; Geverink et al., 1998) and social stress (de Jong et al., 2000).

1.3.5.4.4. Body temperature

Thermoregulation is crucial in the maintenance of homeostasis (Terrien et al., 2011). Body temperature is controlled by the hypothalamus that acts as a thermostat (Charkoudian, 2003; Campbell, 2011), with core temperature being an indicator of the metabolic rate (Webb, 1995). The hypothalamus then sends impulses via the autonomic nervous system to adjust body temperature and to maintain core temperature (temperature of the blood passing through the brain; Webb, 1995). Indeed, under heat stress, metabolism may be severely affected resulting in cellular damage at the central nervous system level (Campbell, 2011; Terrien et al., 2011).

Pigs are homoeothermic animals and the thermal equilibrium results from the balance between metabolic heat production and heat loss. Sweating (heat stress) and shivering (cold stress) are the main pathways for the control of body temperature (Charkoudian, 2003). However, because pigs do not have functional sweat glands, their control of body temperature under heat stress depends on wallowing, thermal panting (evaporative cooling) and heat dissipation (peripheral blood flow) with associated changes in behaviour (e.g. reduction of feed intake, increase in respiratory rate and greater water consumption; Ingram, 1965).

As the heat produced cannot be dissipated immediately, the difference is stored as heat, causing temperature to rise rapidly with heat balance, which is achieved with increased heat dissipation. Thermal stress is associated with increases in heart rate, rectal temperature, blood pressure and urinary excretion (Patience et al., 2003). It eventually results in dehydration, depletion of glycogen reserves, altered acid-base status, muscle damage, and even death in extreme conditions (Kettlewell et al. 2001; Robertshaw, 2004; Brown et al., 2007).

Besides external conditions, body temperature in pigs is also affected by feed intake and circadian rhythm (Ingram and Dauncey, 1985), oscillating around the mean core temperature with a minimum in early morning and a maximum at late night (Robertshaw, 2004; Andersen et al., 2008; Campbell, 2011).

Body temperature can be assessed by the use of i-Buttons. This device has been used for long-term recording of body temperature in animals (Davidson et al., 2003) and humans (Hasselberg et al., 2011). Tamminga et al. (2009) used i-Buttons to assess changes in core body temperature (CBT) in pigs being transported in two different vehicle types (pot-belly vs. double-deck). It has been demonstrated to be more accurate than rectal temperature in rats (Dallman et al., 2006).

Because the use of i-Buttons implies some level of aversive handling, because its recovery in pigs intestinal tract after slaughter are demanding on personal, and also because the recovery rates of this device may not be satisfactory (30 to 80%) the use of alternative tools, capable of indicating thermal stress in pigs during pre-slaughter procedures, are also being investigated. The infrared thermography being a non-invasive tool, seems to be a promising technique to measure body temperature (Speakman and Ward, 1998; Griffith et al., 2002; Kniskova et al., 2007) and indicate welfare status of animals in a less invasive way (Schaefer et al., 2012). In livestock production, this technique has been used to assess the animal health status (Schaefer et al., 2012), pain (Stewart et. al., 2008b), and meat quality (Gariépy et al., 1989; Nanni-Costa et al., 2007).

1.3.5.5. Energy depletion

Deprivation of food for long periods leads to the mobilization of alternative body energy reserves (mainly in the form of lipids) reducing glycogen reserves and increasing ketones and free-fatty acids (FFA; Broom and Johnson, 1993; Knowles and Warriss, 2007). The mechanism involved is lipolysis, which is controlled by hormones triggered by the decrease of glucose in the blood. Lipase breaks down the triacylglycerols, which are hydrolysed into FFA and glycerol. FFA binds albumin and is carried by the bloodstream to be directly used as an energy source for most tissues in the body (Knowles and Warriss, 2007). Insulin, glucagon and leptin are other hormones that can be measured to indicate the energy depletion level of the animal during long-term challenges (Blache et al., 2011).

1.3.5.5. Osmotic stress

Changes in salt or water intake, long-term water deprivation and increased water loss during heat exposure are some of the conditions that may affect osmoregulation. Water restriction triggers physiological responses in order to prevents water loss (Blache et al., 2011).

The degree of dehydration may be observed by variations in blood volume and fluid overload. Rises in hematocrit or packed cell volume (PCV) levels (the percentage of red blood cells in blood) have been observed as a result of: 1) long transport duration and water deprivation due to the reduced plasma in blood (Berry and Lewis, 2001); and 2) arousal and the increased blood cells release from the spleen (Hall and Bradshaw, 1998; Broom and Fraser, 2007; Knowles and Warriss, 2007). Spleen contraction results from the release of catecholamines during sympathetic stimulation providing a large amount of oxygenated blood cells allowing rises in physical activity (Becerril-Herrera et al., 2010). Dehydration state can be also measured in the urine as water deprivation may affect kidney function, resulting in alteration of the excretion of urea and uric acid, thus affecting the acid-base balance and osmolality of body fluids (Blache et al., 2011).

1.4. Animal losses

Losses during transport are associated with dead-on-arrival (DOA), injured (non-ambulatory, injured or NAI) or fatigued (non-ambulatory, non-injured or NANI) pigs. NAI pigs can be recognized as not being able to move due to a body injury (ex. broken leg), while NANI pigs can be identified as presenting visible symptoms of stress, such as difficulty in moving, open-mouth breathing, skin discoloration and muscle tremors. These pigs generally present a metabolic state of acidosis, which if allowed to, pigs can recover from in 2-3 h (Ritter et al., 2009a). The incidence of DOA or downers is usually recorded on arrival at the plant and reflects the effects of poor handling (on farm fasting, loading and unloading), and transport conditions on the welfare of pigs (Correa et al., 2008; Ritter et al., 2008). The official figure for transport mortality rates in Canada in the last 4 years has been of approximately 0.08 % (approximately 16,000 DOA, per year) and 0.4% NANI pigs, resulting in losses in the order of several millions of dollars annually (CFIA, 2012). The common physiological indicators of stress of livestock during transportation are summarized in Table 1.

Table 1. Commonly used physiological indicators of stress during transportation.

Stressor	Physiological Variable
Measurable in blood or other fluids	
- Energy depletion	- ↑ Free fatty acids, ↑ Beta-hydroxybutyrate, ↓ Glucose.
- Osmoregulation	- ↑ Osmolality, ↑ Hematocrits, ↑ Packed cell volume, ↑ Urea.
- Physical exertion	- ↑ Creatine kinase, ↑ Lactate.
- Fear/arousal	- ↑ Cortisol, ↑ Packed-cell volume.
- Motion Sickness	- ↑ Vasopressin.
Other measures	
- Fear/arousal and physical exertion	- ↑ Heart rate, heart rate variability, ↑ Respiration rate.
- Hypothermia/ hyperthermia	- ↑/↓ Body temperature, ↑/↓ Skin temperature.

Adapted from Knowles and Warriss (2007).

1.5. Pre-transport factors influencing animal welfare and meat quality

1.5.1. Farm of origin

The effect of the farm of origin on pig response to pre-slaughter stressors and on carcass and meat quality has been reported in many studies. An epidemiological study run in Ontario reporting 0.17 % in-transit mortality rate and 0.27 % non-ambulatory pigs on arrival at the plant identified the major source of animal loss variation as the farm (Sunstrum et al., 2006; Dewey et al., 2009). An increase of 0.93% in total losses (DOA and downers) was associated to poor (3 sorts) performing farms, when compared to better ones (1 sort; Fitzgerald et al., 2009). Whereas, a higher incidence of fatigued pigs was associated to the distance pigs were subject to walk during loading (61-90 vs. 0-30m; Ritter et al., 2007, 2008). These effects can be explained by differences in genetic background (Terlouw, 2005; Lepron et al., 2007), raising system (Barton-Gade, 2008), and pre-transport handling of pigs (Faucitano and Geverink, 2008).

1.5.1.1. Genetic background

Genetic variation is the basis of all genetic change as a result of natural selection or breeding program. The purpose of a gene is to regulate all processes in the body and to transfer information to the next generation (Rydhmer and Lundeheim, 2008). The global genetic information of an individual is the genome, which in turn is built of DNA sequences and packaged in chromosomes. In each cell, a pair of alleles, one from the mother and another from the father will determine if the animal is homozygote (same alleles) or heterozygote (different alleles). In many genes, the alleles are neither dominant nor recessive, the expression being an average between the alleles inherited (Rydhmer and Lundeheim, 2008). Qualitative trait is the denomination for characteristics governed by single genes while many genes govern quantitative trait. Major genes are defined as genes whose individual effect on a trait (e.g. meat quality, litter size, etc.) is large in relation to the total variance of this trait, being sufficient by itself to cause a condition (Navajas and Simm, 2004).

Breeding selection uses, then, molecular analysis to identify animals with the desired qualitative traits through the genome map of determined breed (Rydhmer and Lundeheim,

2008). The pig's genome is not completely known and has only been mapped since the late 1980s. Since then, porcine linkage maps based on polymorphic DNA markers, identified by mapping the quantitative trait loci (QTL), have been used to breed pigs (Groenen et al., 2011). The current knowledge on genetic variation allows the development of specific breeds and lines, each with specific traits. For instance, several QTL studies deal with meat quality traits (Plastow et al. 2005; Groenen et al., 2011).

Together with genotype, phenotype plays an important role in pig breeding. Phenotypes are the result of genotype and environment. Genotype has, thus, a high heritability ($h^2 = 1$ meaning no environmental influence), whereas phenotype has low heritability ($h^2 = 0$ meaning environmental influence only). Moderately to highly heritable traits, such as carcass leanness and carcass length ($h^2 = 0.30$ to 0.35; and 0.55 to 0.60, respectively) are easy to change with breeding, while the reverse occurs with low-heritability traits (Sellier, 1998b; Rydhmer and Lundeheim, 2008; Ciobanu et al., 2011).

Breeding pigs for greater feed efficiency and leaner carcasses has been the focus of genetic selection over the past decades (Rauw et al., 1998; Busck et al., 2000). More recently, breeders are also concerned about meat quality (Sellier, 1998a; Ciobanu et al., 2011) and animal welfare (as seen in section 1.3.4.4.; Rauw et al., 1998; Jensen et al., 2008).

The effects of genetic selection for improved production and carcass traits has a negative impact on animal welfare, in terms of higher susceptibility to stress, leg weakness, osteochondrosis and temperament (Carragher and Matthews, 1996; Rauw et al., 1998). The most known effect of genetic selection for muscle development and lean deposition on animal welfare is represented by the increased frequency of the porcine stress syndrome (PSS), which was described by Ewing et al. (1999) as a pathology. Predisposition of pigs to the PSS is clearly inherited through a simple recessive gene, the halothane gene (*n,* HAL).

The HAL gene is considered to be equivalent to the ryr^{-1} gene, which encodes the muscle ryanodine receptor. A mutation of the ryanodine receptor reduces its sensitivity to magnesium leading to uncontrolled contractions when the concentration of intracellular calcium is high (Fujii et al., 1991). The HAL gene is present in several breeds (e.g. Duroc, Landrace and Hampshire), but in Pietrain pigs the frequency (h^2) of the allele *n* is close to

1. The main effects of the HAL gene are increasing risk of death during transport or handling (Fàbrega et al., 2002a). Muscular metabolism in pigs with different halothane status (NN, Nn and nn) were tested by Henning et al. (2000) who reported a significant decrease of PC and a corresponding increase of inorganic phosphate (Pi) in homozygous animals. These results are consistent with the observed intracellular pH decrease from 7.1 to 6.4, with a 100% increase of Mg++ (a calcium antagonist and ATP activator). When triggered, PSS results in shortness of breath, rapid increase in body temperature, patches of blanching and flushing of the skin, collapse, and rapid death followed by almost immediate rigor mortis (Ewing et al., 1999). The higher frequency of PSE meat in HAL pigs is a result of the mutated calcium channel which, as already mentioned, leads to accelerated conversion of glucose to lactate and faster pH fall (Oliver et al., 1993; Leach et al., 1996; Tor et al., 2001, Fernandez et al., 2002).

In pigs that are homozygous for this defect (*nn*), PSS is precipitated by a number of stress stimuli, such as separation at weaning, weaning, fighting, coitus, transport, restraint, vigorous exercise and slaughter. The use of the halothane gas challenge (HC) test as the basis for diagnosis of swine homozygous for PSS gives rise to HAL as the name of this gene (Ball and Johnson, 1993).

Another major gene capable of influencing meat quality is the Napole rendement yield (RN^-) gene (Houde et al., 2001). This gene is responsible for the so called “acid meat” occurrence, which seems to be limited to the Hampshire breed (Monin, 2000). RN gene is responsible for the lower ultimate pH (acid meat) and water-holding capacity with highe cooking losses (Lundstrom et al., 1999; Houde et al., 2001). Pigs that are carriers for the RN defect have higher than normal muscle glycogen stored resulting in extended glycolysis which leads to lower final pH and increased shrinkage during processing and cooking. For instance, the name RN is originated from “Rendement Napole” a measure used to estimate the processed meat yield of cooked hams (Sellier and Monin, 1994, Monin, 2000). A summary carcass and meat quality as affected by the major genes HAL and RN^- is presented in Table 2, and the effects of the breed and genotype on animal welfare and meat quality parameters are summarized in Table 3.

Table 2. Major genes and their effects on carcass and meat quality in pigs

Major Gene	Breed	Effects on carcass and meat quality	Identified gene
RN^-	Hampshire	**Reduces**: processed meat yield; ultimate pH; lean colour intensity **Increases**: drip loss, tenderness	PRKAG3
Halothane	Several (e.g. Duroc, Pietrain, Landrace, Hampshire)	**Reduces**: ultimate pH; water holding capacity **Increases**: pale, soft, exudative (PSE) meat	RYR1

Adapted from: Lambe and Simm (2004).

Table 3. Summary of breed and genotype effect on pork quality and animal welfare.

Author	Breed	Genotype	Meat quality and welfare (Pietrain vs. others)	Meat quality and welfare measure (n vs. N)
Honkavaara (1988)	Landrace, Yorkshire, LxY	nn vs. unkown		↑ CK
Murray et al. (1989)	Lacombe, LacombexPietrain	NN, nn, Nn		↓ pH45, ↓ fat
Zhang et al. (1992)	Pietrain, Yorkshire	NN, nn, Nn	↑ lean yield, ↓ meat quality	↓ meat quality
McPhee and Trout (1995)	LargeWhitexLandrace	NN, nn, Nn		↑ PSE, ↑ lean content
Rempel et al. (1995)	Yorkshire, Pietrain	N		↑ lean yield, ↓ meat quality (colour defect)
Klont and Lambooij (1995)	Landrace	NN, nn, Nn,		↑ Lactate, ↑ CK
De Smet et al. (1996)	Landrace, PietranxLandrace	NN, nn, Nn		↑ PSE
Garcia-Macias et al. (1996)	Large-WhitexPietrain, PietrainxDuroc	nn, Nn		↑ PSE
De Smet et al. (1998)	Pietrain, Landrace, Large-White	NN, nn, Nn		↑ lean content, ↓ pH40
Nanni-Costa et al. (1999)	Duroc, Landrace, Large-White	NN, Nn		↓ pH1, ↓ glycogen, ↑ lactate
Fernandez et al. (2002)	Pietrain, Large-white	NN, nn, Nn		↑ lactate, ↓ pH_{40}, ↑ drip loss, ↑ L*
Fàbrega et al. (2002b)	Large-white, Pietrain	NN, Nn	↑ lean yield,	↑ lean yield, ↑ cortisol, ↑ lactate, ↑ CK, ↑ L*
Fàbrega et al. (2004b)	Pietrain, Landrace, Large-white	NN, Nn	↑ PQM	↑ Heart Rate, ↑ CK,
Gispert et al. (2007)	Large-white, Landrace, Duroc, Pietrain, Meishan		↑ lean yield	
Franco et al. (2008)	Pietrain, Landrace, Large-white	NN, nn, Nn		↑ lean meat, ↑ drip loss
Li et al. (2008)	Erhualian, Pietrain	Not determined	↑ CK, ↑ lactate	
Kaić et al. (2009)	Large-white, landrace, Pietrain	NN, Nn		↓ pH_{45}

1.5.1.2. Housing conditions

According to Lebret (2008), animal growth performance and pork quality result from the combined effects of housing conditions (floor type, space allowance, ambient temperature, outdoor access of free-range rearing, etc.), which can affect physical activity and feed requirements as well as breed, genotype and nutrition (feeding level and feed composition).

With respect to housing systems, Klont et al. (2001) found that by only improving indoor system, which is economically more feasible than various outdoor rearing systems, improved animal welfare and pork quality. This can be achieved by the implementation of specific criteria in the housing system (combined husbandry procedures and housing environment), such as avoidance of injury and disease, generous provision of food and water; small group size, solid flooring for at least part of the pen (necessity to distinguish dunging from resting area), use of bedding; provision of material for oral manipulation (environmental complexity), low density stocking; provision of ample space for growing pigs; individual feeding for breeding sows, stability of animal groups by obtaining constancy of individuals in the group and keeping growing pigs in family (litter) groups until slaughter (O'Connell, 2009).

1.5.1.3. Loading facilities

The design of the farm alleys must be in accordance with the number of pigs being moved at once. However, Kavanagh et al. (2009) reported that alley width of 0.9 to 1.2 m resulted in less turn-back and handling interventions compared with a 2.4 m width one, regardless of the group size. Sloped (> 10°) ramps have been reported to increase heart rate (van Putten and Elshof, 1978; Warriss et al., 1991a). Thus, the use of the hydraulic tail-lifts or moving decks are recommended as they make the pigs easier to handle and reduce loading time, animal losses and the risk of PSE pork (Faucitano, 2001; Guàrdia et al., 2004; Brown et al., 2005). Furthermore the use of dual ramp with a wire panel, allow pigs to see each other (Carr, 2006); and solid laterals will avoid distractions (SCARM, 1997; Grandin, 1999). Loading chutes should also be as wide as the truck door and wide enough to allow 2 to 4 pigs to walk side by side and avoid bottle-necks (Lachance et al., 2005). Furthermore,

loading chute floor should have stair steps covered with rubber to prevent slipping (Christensen and Barton-Gade, 1996).

1.5.1.4. Loading

According to Geverink et al. (1998) and Marchant-Ford et al. (2003), the most stressful task a market pig must endure during transport to slaughter is the pig's initial departure from its finishing pen. At this stage, the position of the handler, the use of a solid board, and moving small groups are important to improve ease of handling and limit stress (Hemsworth, 2000).

Reid and Mills (1962) suggested that animals could be trained to accept some irregularities in management, which reduces strong reactions to novelty. It has been shown that animals that experienced repeated handling at the farm before transport are less reactive to familiar and unfamiliar humans (Terlouw, 2005), and to alleys and ramps (Lewis et al., 2008); making them easier to handle, reducing the workload for stockman (Abbott et al., 1997; Geverink et al., 1998) and loading time (Stewart et al., 2008a). This would result in a significant reduction in the incidence of pigs exhibiting open-mouth breathing and skin discoloration during loading and of total transport losses compared to pigs that were not previously handled ((0.07 vs. 0.38%; Stewart et al., 2008a).

Moving pigs in a group size larger than the farm alley, loading quay or ramp is a common practice at loading. This practice is mistakenly considered effective to speed up the handling procedure. Considering heart rate increase and time to load a transport vehicle, moving groups of 2 to 6 pigs at a time, depending on the farm alley and loading quay or ramp design, is recommended for both producer/transporter savings and animal welfare as it reduces animal losses and injuries (Grandin, 1999; Lewis and McGlone, 2006).

The use of appropriate tools (Hemsworth, 2000; Correa et al., 2010), small group sizes (Lewis and McGlone, 2007), and the respect of pigs' physical needs are reported to improve animal welfare at loading minimizing animal losses during transport. The use of electrical prods has been related to increased heart rate (Correa et al., 2010), higher levels of lactate (Hemsworth et al., 2002) and the incidence of NANI pigs (Benjamin et al., 2001). Furthermore, different studies reported that the use of electric prods, compared with control

and nose snare tests resulted in poorer meat quality (Hemsworth et al., 2002; Kuchenmeister et al., 2005).

When mixing before transport is unavoidable, pigs should be mixed at loading, which minimizes fights, as this behaviour is less intense during transportation and pigs have more time to rest after fighting (Warriss, 1996a). Mixing unfamiliar pigs in lairage will result in increased fighting with consequences for welfare and carcass and meat quality (Warriss, 1996a).

1.5.1.5. Feed withdrawal

In Canada, feed withdrawal is recommended by the codes of practice (AAFC, 1993). Different authors have investigated the importance of feed withdrawal in pigs and some results indicate that this practice is beneficial from an animal welfare point of view. It decreases animal losses during transport (Guàrdia et al., 1996; Ellis and Ritter, 2006; Averós et al., 2008), prevents pigs from suffering motion sickness (Guàrdia et al., 1996; Warriss, 1996a; Viau and Champagne, 1998) and vomiting during transportation (Bradshaw et al., 1996), and increases the ease of handling at the slaughterhouse (Eikelenboom et al., 1991; Chevillon, 1994). Furthermore, this practice reduces carcass contamination due to lower risk of gut contents spillage during carcass evisceration (Saucier et al., 2007), improves pork quality (Guàrdia et al., 2004, 2005), and reduces environmental pollution by reducing waste disposal volume at the abattoir (Warriss, 1992).

Under commercial conditions, Correa (2011) reported that the application of a fasting interval at the farm halved the proportion of animal losses during transport, while its absence (no fasting) produced 77 % condemned carcasses due to contamination.

Despite these potential advantages, however, feed withdrawal is sometimes not used or not applied by producers, resulting in complaints and penalties from the meat-processing sector. For instance, a survey conducted on swine farms in Quebec reported that only 15% of pigs had no access to the feeder until the time of transport (Viau and Champagne, 1998). Some reasons for not withholding feed prior to transport were: 1) lack of a shipping room - to which pigs sorted by live weight (split-marketing) could be transferred from their home pen in order to withdraw feed and allow them to rest until the arrival of the truck; and 2)

concern about body weight losses reducing the economic value of the carcass (Viau and Champagne, 1998).

Chevillon et al. (2006) reported significant carcass weight loss (360 g/pig) only after 24 h of feed withdrawal. This loss resulted in a 0.33-point % difference in dressing yield, which is equivalent to 30 g/h of cold carcass weight loss for a pig weighing 110 kg at slaughter. Kephart and Mills (2005) reported that withholding feed for 24 h of resulted in a savings of 2 kg of feed/pig. Furthermore, feeding pigs until the time of transport may be very costly because feed consumed by pigs takes 4 to 8 h to be absorbed in the small intestine after ingestion and most nutrients enter the blood 9 h after intake; thus, feed provided to pigs in the last 10 h will not be converted to carcass gain and represents a waste that the processing plant needs to deal with (Warriss, 1985).

Based on current data, Faucitano et al. (2010a) considered a period of 24 h between the last meal and slaughter to be an acceptable compromise to obtain optimal carcass yield, pork quality,microbial contamination and safety. Indeed, Bertol et al. (2005) reported that pigs fasted for 24h showed lower muscle glycolytic potential and body temperature compared to pigs. Not fasted pigs also improved colour and water holding capacity in the *post-mortem* muscle. However, certain aspects of the welfare of fasted pigs still need to be studied as pigs arriving at slaughter with empty stomachs are more aggressive when mixed with unfamiliar conspecifics (Guàrdia et al., 2009) and are likely to feel hungry, as suggested by the increased drinking rate in lairage (Brown et al., 1999; Saucier et al., 2007).

1.6. Transporting pigs: inside the vehicle

During transport, pigs are exposed to several stressful situations, such as unfamiliar noises and smells, vibrations and sudden speed changes of the truck, variations of environmental temperature, lower individual social space and handling (Grandin, 1997). The main factors reported to affect animal welfare and/or meat quality at some extent are: vehicle design, placement and microclimate inside the vehicle, loading density, season, duration of the journey, and driving conditions.

1.6.1. Vehicle design

The comfort of pigs in transit is highly dependent on the vehicle design (Bench et al., 2008; Haley et al., 2008a). The design of vehicles used for pig transportation varies around the world from small single deck trucks to large three-deck punch-hole trailers equipped or not with fan-assisted ventilation depending on the geographic location and climatic conditions. In Canada, the centralization of packing plants over the last 15 years increased long distances between farms and slaughterhouses resulting in the need for trailers with a large load capacity, such as the pot-belly (PB) trailer. The PB trailer is the most common vehicle for pig transportation in Canada as it can transport up to 230 pigs on three decks (10-13 compartments) in a single journey.

However, this vehicle features multiple (up to 5) and steep (up to 40° slope) internal fixed ramps and 180° turns, which result in the reduction of handling ease during loading and unloading, increasing the use of electric prods, and extending the load and unload time (Ritter et al., 2008; Torrey et al., 2008). These observations have been associated with a higher proportion of DOA and fatigued pigs in the PB trailer and in European trucks, when compared to other vehicle types (Barton-Gade et al., 2007; Sutherland et al., 2009). Furthermore, the use of fixed decks and ramps may result in a higher incidence of PSE pork (Guàrdia et al., 2004; Lammens et al., 2007). However, Dalla Costa et al. (2007a) and Correa et al. (2008) reported no effect on pork quality when comparing a single- with a double-decked truck and a PB trailer with a compact truck, respectively. However, Lynch et al. (1998) and Dalla Costa et al. (2007b) reported lower pH values and paler colour in pork from pigs transported in a traditional wooden trailer compared to those hauled in a modern single- or double-decked trucks having floating hydraulic decks and air suspension.

1.6.1.1. Vehicle compartment

In a study where a large fixed body truck was compared to a small towed twin-axle trailer, Randall et al. (1996) concluded that vehicle type accounts more for the comfort of pigs during transit than any other transport factor, such as animal location in the vehicle. However, it has been reported that pigs transported on the lower deck of European trucks had higher body temperature and blood cortisol levels and showed a higher degree of dehydration (Lambooij et al., 1985; Lambooij and Engel, 1991; Barton-Gade et al., 1996b). These results are similar to those reported by Tamminga et al. (2009) who also found that pigs loaded onto the top deck compartments of a PB trailer were at greater risk for heat stress than pigs in other areas of the truck. This could be due to higher heat load and/or exertion required to climb a ramp to these compartments. Pigs transported in lower decks had a greater incidence of PSE pork, which may be caused by poor ventilation (Guise and Penny, 1992) and/or by the use of ramps (Ryan, 2007).

The physical effort to remain standing, in order to cope with the high level of vibrations in the lower deck, can also result in increased DFD (dark, firm, dry) pork (Barton-Gade et al., 1996b; Randall et al., 1996). Skin damage score is also higher in these pigs as standing pigs are more subjected to falling or trampling and thus can be injured during transport (Barton-Gade et al., 1996b).

Greater body weight loss and incidence of death in transit have been reported in the front compartment, where the temperature within the truck is usually higher and the ventilation poorer (Christensen and Barton-Gade, 1999; Dalla Costa et al., 2006). Pigs transported in the front and rear compartments produced poorer meat quality (PSE or DFD) and had higher lactate levels compared to pigs traveling in central pens (Guise and Penny, 1989; Barton-Gade et al., 1996b).

1.6.2. Loading density

Amongst the international regulations and guidelines reviewed by Bench (2007), a consensus exists on two key points with regard to loading densities of pigs during transport. The first agreement is on the need to partition animals in order to minimize injury during transport. The second consensus is on the need to reduce pig loading densities (increase

space allowance) during warmer weather (above 24ºC). For instance, a 10 % higher space allowance is recommended for pigs during transport at environmental temperatures higher than 29°C, with more space being allowed to pigs heavier than 120 kg (NFAAC, 2008; Schwartzokpf-Genswein et al., 2012).

Even when loading densities are regulated by legislation, like in the case of the EU directive 95/29/EC, they are hardly met in practice as the chosen densities are frequently adjusted to different transport conditions (weather, road type and distances) and pig genetic background (Christensen et al., 1994). In most EU countries, for example, loading densities vary from 0.35 to 0.50 m^2/100 kg (Warriss, 1998a). Based on the measurements of the space needed for sternal recumbency, it is now suggested that the minimum space required is equivalent to appriximately 250 kg/m^2 (or 0.44 m^2/100 kg) for normal slaughter pigs of 90-100 kg live-weight (Warriss, 1998a). In North America loading densities may vary from 0.36 m^2/100kg in Canada to 0.50 m^2/100 kg in USA (CARC, 2001).

High densities (<0.40m^2/100 kg) are reported to negatively affect microenvironment inside the truck by increasing temperature and decreasing the air circulation (Warriss et al., 1998b). High densities prevent pigs from lying down, while lower densities (>0.50 m^2/100 kg) result in pigs being fatigued due their attempts to keep their balance. In both circumstances muscular fatigue can be expected. Indeed, Guise and Penny (1989; Ritter et al., 2009b) reported an increase of CK levels in pigs transported under high densities. Other studies have reported the incidence of lower welfare indices, such as rectal prolapses (Guise and Warriss, 1989), lameness (Kephart et al., 2010) and higher blood CK and lactate levels (Lee et al., 2000; Chai et al., 2010) when densities are high.

Ritter et al. (2006) reported that the optimal density to prevent death losses during transportation would be of 0.48m^2/100 kg after having observed that densities of 0.40m^2/100 kg resulted in three-fold higher percentage of death losses compared to 0.50 m^2/100 kg. The effect of higher densities on the development of fatigued pigs was reported to be greater following short distance transportation (< 40 min vs. < 2.5 h) by Kephart et al. (2010) and Pilcher et al. (2011). Furthermore, high densities are also related to higher incidences of carcass bruises (Nanni-Costa et al., 1999) and meat quality defects (Barton-Gade and Christensen, 1998; Chai et al., 2010). The negative effects of high densities on

meat quality can be observed alone, as described above, or in association with other effects, as reported by Carr et al. (2008) who found that pigs being handled roughly at loading and transported at high density produced darker pork (lower L* values).

1.6.3. Journey time, distance, and season

In general, the longer the journey, the more fatigued, more in need of water and food, more affected by any adverse condition, more immunosuppressed, more susceptible to disease and sometimes more exposed to pathogens are the animals (Broom, 2008). Transport distances are largely governed by the availability of pigs in the region around the abattoir. Journey times tend to increase (up to 35 h in Canada) with the concentration of the slaughter industry into fewer, larger plants for economic reasons. In the journeys surveyed in Canada, most pigs spent less than 3 h in the truck and 4% spent more than 24 h in transit (Aalhus et al., 1992).

The recommendation of the EU working group on pig transport is that transport durations should be reduced with a maximum acceptable journey limit of 3 h (Warriss, 1996b). In case of long journeys, transport can be prolonged up to 24 h, provided transport conditions (ventilation and density) are good (Brown et al., 1999) and water is available (Bench et al., 2008).

Different studies have demonstrated that pigs may not recover from loading stress under short distance transportation. It is demonstrated that short transportation does not allow pigs to restore homeostasis after loading stress (Bradshaw et al., 1996; Pèrez et al., 2002a), and to acclimate to transport stress (Stephens and Perry, 1990). Pigs transported short distances are more difficult to handle and produce poorer meat quality (Grandin, 1994; Pèrez et al., 2002a). Conversely, long distance transportation may result in depletion of energy with pigs exhibiting dehydration (Lambooij et al., 1985; Averós et al., 2007) with the possible development of DFD pork mainly during the winter (Gispert et al., 2000; Mota-Rojas et al., 2006). Indeed, Guàrdia et al. (2004) reported a two-fold higher risk of PSE pork in summer months compared to winter. Dalla Costa et al. (2007a) reported a higher level of skin bruises in winter and paler meat during summer.

Considerations regarding trailer type might be important under different transport distances. The same density may not be appropriate for short and long distances as pigs will not have enough time to lay down under short trip duration (Barton-Gade and Christensen, 1998). Based on their findings, Chai et al. (2010) suggested that longer transportation as well as high densities should be followed by longer lairage durations allowing pigs to recover.

Transport distances are associated with greater in-transit mortality. It was demonstrated that in-transit losses are lower as travel distances are longer (Haley et al., 2008a). However, Werner et al. (2007) reported that travel distances longer than 8 h and shorter than 1 h, both, negatively affected animal welfare with increased mortality rates. A summary of effects of transport times in meat quality and animal welfare is presented in Table 4.

Higher temperatures increase the number of pigs showing open-mouth (17ºC) and the number of in transit losses (20ºC; Sutherland et al., 2009; Kephart et al., 2010). Furthermore, in transit losses double from 0.15 to 0.30% when temperatures rise from 20 to 35ºC (Grandin, 1994). In Canada, there is evidence that the month of August, when temperatures may go up to 30ºC, is associated with a higher incidence of deaths during transportation (Haley et al., 2008b).

Colder temperatures are also associated with increased total transport losses. Clark (1979) observed an increase in mortality when pigs were transported during winter in Saskatchewan. Similarly, Sutherland et al. (2009) found an increase in the incidence of non-ambulatory pigs transported at temperatures below 5ºC.

Table 4. Summary of the effects of transport time on pork quality and animal welfare.

Author	Transport duration	Meat quality measure (short vs. long)	Welfare measure (short vs. long)
McPhee and Trout (1995)	0.5 h, 10-11 h	↑ PSE, ↓ DFD	
Martoccia et al. (1995)	180, 650 (Km)	↓ pH_{45}, ↓ pHu, ↑ L*	↓ Lactate
Pèrez et al. (2002a)	15 min, 3h	↓ pHu	↑ Lactate;↑ cortisol
Park et al. (2003)	30, 30-60, >60 (min)	↑ PSE	
Vecereck et al. (2006)	50, 300 (Km)		↑ Mortality rate
Gosálvez et al. (2006)	< 50, 50-100, > 100 (Km)		↑ Mortality rate
Becerril-Herrera et al. (2007)	8h, 16h, 24h	↑ normal pH (5.8 – 6.2)	↓ Hyperventilation; ↑ # standing pigs,
Barton-Gade et al. (2007)	< 100, 100-200, > 200 (Km)		↑ Mortality
Malena et al. (2007)	< 50, 51-100, 101-200, 201-300, > 300 (Km)		↑ Mortality
Haley et al. (2008a)	0 – 720 Km		↓ Mortality
Sutherland et al. (2009)	30min – 4h		↓ Mortality,
Chai et al. (2010)	40min, 3h, 5h	↓ pH, ↑ drip loss,	↓ Lactate, ↓ cortisol
Becerril-Herrera et al. (2010)	8h, 16h		↑ O2 consumption, ↑ body temperature, ↓ pH

1.6.4. Environmental conditions inside the trailer

Pig homeostasis maintenance inside a vehicle may be extremely challenged as behavioural attempts to lose heat are prevented or limited due to low space allowance and/or poor ventilation rates. Variation in temperature and humidity within the vehicle is related to the radiant heat load, to the amount of water and heat produced by pigs during transport and to the ventilation conditions (Lambooij, 1988). Ventilation is thus critical to help keep the temperature and humidity balance inside the compartments, allowing pigs to dissipate some heat through convection.

As mentioned above, pigs, despite having sweat glands, do not sweat and evaporative heat loss occurs mainly through the respiratory tract. Due to this condition, pigs are more vulnerable to the ambient temperature rise than to changes in humidity (Ingram, 1965). However, the combination of high temperatures and high humidity can result in severe heat stress during transportation. Indeed, an increase of 1ºC in body temperature will result in a 10 % higher metabolic rate (Robertshaw, 2004). On the other hand, a decrease in body temperature can lead to hypothermia and even a fall of 7-8ºC may be fatal. Both conditions will result in increased use of energy substrates, depletion of glycogen reserves, muscle damage and altered meat quality, with consequences on pig's behaviour, such as lethargy (Robertshaw, 2004).

Schrama et al. (1996) reported that the heat during transportation is more important than the cold stress, in terms of transport losses. Handling during loading exacerbates the effects of hot ambient conditions on the thermal status of transported pigs, since exercise and the excitement associated with forced activity results in higher core body temperatures than ambient hot conditions alone (Judge et al., 1973; Brundige et al., 1998). It is known that heat stress leads to higher lactate accumulation in the muscle blood flow resulting in higher incidence of PSE pork (Honkavaara, 1989). Hot conditions have been reported to result in higher mortality (Warriss and Brown, 1994; Haley, 2005) as temperatures may exceed 30ºC (Brown et al., 2011), which is the threshold for the air temperature inside the vehicle. It is important, thus, to respect the acceptable range between lower critical (26°C) and upper critical temperatures (31°C) for pigs (20-100 kg liveweight) during transport (Randall, 1993). Indeed, Haley et al. (2008b) found that total losses are expected to increase by a

factor of 1.26 with an increase of 1ºC in the 90th percentile temperatures ranging from 8.6 to 30.5°C.

Brown et al. (2011) showed an effect of the compartment location on the microclimate variation inside the PB trailers, with higher temperatures being recorded in the front compartments of the middle and bottom deck (or "belly") and lower temperatures being recorded in the upper compartments. The higher and lower temperatures have been explained by reduced ventilation and poor insulation, respectively (Brown et al., 2011). As suggested by these authors, in the summer, bottom and front compartments of a stationary PB trailer can be cooled by increasing the ventilation rate using fans, in combination or not, with water sprinkling to increase evaporative cooling.

To ensure appropriate ventilation, the height of the compartment should be 15 cm above the highest point on the animal in vehicles with efficient forced ventilation and 30 cm above the highest point on the animals in vehicles with natural ventilation (EU-SCAHAW, 2002). Indeed, effective ventilation and water sprinkling in a stationary truck are credited with reducing deaths during transport (Nielsen, 1982; Chevillon, 2000).

European transport studies (Christensen and Barton-Gade, 1999) showed that when forced ventilation is combined with an intermittent misting system at temperatures of 25°C, it can decrease transport mortality. More recently, Nannoni et al. (2012) reported improved welfare (lower lactate levels, as related to both, thermal and physical stress) and pork quality (increased pH_1 in longissimus muscle) in hogs showered during waiting for unloading at temperatures above 20ºC. Similarly, Fox et al. (2012) reported a decrease in core body temperature at the arrival at the plant when pigs were sprinkled at temperatures above 23ºC. The showers seemed to alleviate transport-related heat stress, as observed in the pigs' behaviour. These pigs spent more time lying and had fewer drinking bouts compared to unshowered pigs.

1.6.5. Driving conditions

The effects of driving conditions on animal comfort during transport are associated with the fact that pigs do not lie down immediately after departure from the farm, which may result in pigs standing during short journeys (Grandin, 2002). It has been demonstrated that pigs stand

more on rough journeys (Randall et al., 1995; Bradshaw et al., 1996) and with careless driving (Peeters et al., 2008), which is probably due to their need to cope with the level of vibration, noise and sudden truck movements (Lambooij and van Putten, 1993; Perremans et al., 1998; Peeters et al., 2008). Standing seems to alleviate motion sickness, but may also result in a higher level of injuries and bruises (Barton-Gade and Christensen, 1998). There is evidence that rough journeys can result in lower pH at 45 min and higher incidence of PSE meat (Hoffman and Fisher, 2010).

1.7. Unloading

At unloading, the main concern would be to unload pigs as soon as possible to avoid heat stress and its negative consequences on meat quality and animal welfare (AAFC, 1993). The wait time to unload after arrival at the abattoir is variable, ranging from 5 min to several hours (Aalhus et al., 1992; Jones, 1999).

Driessen and Geers (2001) reported an increase in carcass bruises and in the incidence of exudative pork proportion for unloading times longer than 33 min. On arrival, a "booking-in" schedule, that involves a strict coordination of truck arrivals with the predicted number of pigs in lairage, lairage capacity, and speed of operation would help to reduce waiting times (Jones, 1999).

To avoid jamming and panic in the unloading group, the truck should be emptied gradually by unloading pigs by compartment, rather than by deck and in small groups using paddles or boards only (Jones, 1999). Increased mounting, slipping and turning was observed when small groups of pigs (10 pigs) were unloaded using electrical prods compared to boards (Rabaste et al., 2007). The use of electrical prods makes handling at unloading more difficult as it increases the number of mounting, slipping and turning (Rabaste et al., 2007).

Furthermore, handling and the use of electrical goads in the pre-slaughter procedures are responsible for considerable increases in skin damage on the carcasses (Geverink et al., 1996; Rabaste et al., 2007), which was confirmed by Faucitano et al. (1998) who reported that avoiding the use of electrical goads within lairage, resulted in improved carcass quality, reducing skin blemishes by 50% in pigs.

1.8. Lairage

Lairage is important for welfare and meat quality reasons as it provides an opportunity for animals to recover from stress and fatigue with benefits for meat quality (Warriss, 1987).

Lairage time, handling, facility design (pens and alleys), environment, whether pigs are mixed or not are all linked to the recovery rate of pigs, which in turns, is related to carcass and meat quality (Warriss, 2003; Faucitano and Geverinck, 2008).

There is evidence that a lairage time of 2 h is sufficient for recovery as shown by the blood cortisol levels (Warriss et al., 1992; Pèrez et al., 2002b) and resulting ease of moving pigs based on the lower frequency of electric prod use (Milligan et al., 1998). Based on a review of different studies done by Marchant-Forde and Marchant-Forde (2009), short lairage durations (lower than 1 h) resulted in pigs with higher blood level of cortisol (Saco et al., 2003), lactate (Salajpal et al., 2005) and CK (Brown et al., 1999). In contrast, longer lairage time (more than 3 h) resulted in increased blood levels of acute phase proteins (Saco et al., 2003) and greater number of fights (Nanni-Costa et al., 2002). The increased fighting rate has been associated with increased skin damage scores (Guàrdia et al., 2009) and a greater risk of DFD pork (Guàrdia et al., 2005).

The management of group size and stocking density may limit aggressiveness in mixed groups of pigs in the lairage pen (Weeks, 2008). There is evidence that keeping with sufficient space allowance (0.50–0.67 m^2/100 Kg) in the pen will reduce agonistic encounters regardless of the group size (10 to 200 pigs; Grandin, 1990; Warriss, 1996a,b; Chevillon, 2000). However, Rabaste et al. (2007) observed that pigs kept in medium groups (30 pigs; density = 0.59 m^2/pig) in the lairage pen spent more time standing, fighting and were more involved in agonistic interactions (bites and head knocks) than pigs kept in smaller groups (10 pigs; density = 0.59 m^2/pig).

Considering the evidence that most fights occur in the first 30-60 min of lairage, lairage densities should be adjusted according to lairage times. For instance, a stocking density of 0.42 m^2/pig for short lairage (< 3h) and 0.66 m^2/pig for long lairage (> 3h; Weeks, 2008) are recommended.

Santos et al. (1997) studied the effects of ambient conditions in lairage on pig welfare and found that under extreme climatic conditions (> 30ºC and RH > 80%) pigs have great difficulty in losing heat and show signs of stress, such as increased respiration rate. Temperature at lairage would also represent an important risk factor for poor meat quality (Lammens et al., 2007). Santos et al. (1997) reported that lairage relative humidity together with temperature resulted in a greater incidence of PSE meat.

Other than cooling pigs (3ºC decrease in body temperature) and improving pork quality (less PSE; Long and Tarrant, 1990), showering pigs in lairage also reduces aggressive behaviour and facilitates greater ease of handling upon entrance into the stunning chute (Weeding et al., 1993). A third benefit would be the increased electrical stunning efficiency as wetting the skin lowers its impedance, resulting in an easy and rapid achievement of unconsciousness prior to slaughter (Wotton, 1996). However, at temperatures below 5ºC, showering is not recommended as it causes animal shivering and may lead to darker pork (DFD) due to muscle energy depletion to maintain a constant body temperature (Knowles et al., 1998). The effects of different lairage times on pork quality are summarized in Table 5.

Table 5. Summary of lairage time effect on pork quality and animal welfare.

Author	Lairage durations	Measure (short vs. long)	
		Meat Quality	**Animal Welfare**
De Smet et al. (1996)	1h, 2-3h, 4-5h	↑ Drip loss, ↓ pH, ↑ L*	
Santos et al. (1997)	0.5h, 2-3h	↓ Normal quality carcass	
Warriss et al. (1998a)	1h, 3h, 24h	↑ PSE %, ↓ DFD %	
Fraqueza et al. (1998)	0.5h, 3h	↑ PSE %	↓ carcass damage
Aaslyng and Barton-Gade (2001)	0.3h, 2.5h	↑ Drip loss, ↑ Reflectance	
Fortin (2002)	0.5h, 3h, 6h	↑ PSE incidence	
Nanni-Costa et al. (2002)	2h, 22h	↑ PSE %	↓ carcass damage
Pèrez et al. (2002b)	0h, 3h, 9h	↑ PSE %, ↓ DFD %	↓ red blood cells, ↓ neutrophils, ↓ lymphocytes, ↓ CK, ↑ cortisol
Fàbrega et al. (2002b)	2h, 12h	↑ L*, a*, b*	
Fàbrega et al. (2004b)	2.5h, 14.5h	↑ Conductivity, ↑ PSE %	↓ CK, ↑ cortisol
Salajpal et al. (2005)	2h, 24h	↑ Drip loss, ↓ pH	↓ CK, ↑ lactate
Carr et al. (2008)	45min – 3h	↑ pHu	↓ cortisol
Van de Perre et al. (2010)	<2h, 2-4h, >4h	↓ PSE% (in winter)	

Adapted from Marchant-Forde and Marchant-Forde, 2009.

1.9. Carcass and meat quality

Carcass quality and meat quality are essential criteria for the market and the consumers of pork meat. Genetics, production system, health, transport, handling, processing and packing, all contribute to the final result. Among these factors, genetics can determine between 30 and 60% of the total carcass and meat quality variation (Chesnais, 1996; Andersen, 2000).

1.9.1. Carcass quality

To estimate carcass quality, variables such as carcass weight, fat depth, muscle depth, length, shape, distribution of joints, sex, muscle, and fat quality can be considered. Carcass shape, or conformation is an index of its composition where the muscle/bone ratio indicates the muscular development. The proportion between the weight of the carcass in relation to the weight of live animal is the killing-out percentage (Warriss, 2010):

$$\text{Killing-out} = (\text{Carcass weight}/\text{live weight}) \times 100.$$

Killing-out percentage or dressing percent is an important measurement of meat yield. It is used for carcass grade, which in pigs is estimated to be around 75% and is associated to the carcass commercial value (Fortin et al., 2003; Pomar et al., 2008; Warriss, 2010). Lean yield, the proportion of tissues of interest of a carcass, is obtained according to a reference method by dividing the weight of the tissues of interest by the overall or partial carcass weight (Pomar et al., 2008):

$$\text{Lean yield} = \text{weight of tissues of interest}/\text{carcass weight}.$$

In commercial conditions, lean yield is predicted by subjective (visual) or objective (caliper and optical probes) carcass measurements (Kauffman and Warner, 1993). In Canada, it is estimated from fat thickness and muscle depth as measured at the ¾ last ribs with specific optical probes (Fortin et al., 2003). Although traditionally used, this measure tends to underestimate leanness of more muscled carcasses (Warriss, 2010). According to Marcoux et al. (2007), the current procedure does not consider the weight and leanness of individual cuts, underestimating the market value of the carcass. Novel methods for grading pork carcasses based on video image analysis techniques, which can be combined or not with

other measurements of fat and muscle depths (e.g. computed tomography (CT), magnetic resonance imaging (MRI), and dual energy X-ray absorptiometry (DXA; Pomar et al., 2008) are now under testing as its use under commercial conditions have not been established yet.

According to Ciobanu et al. (2011), the strong negative genetic correlation between back-fat thickness (as measured by ultrasound in live animals) and carcass lean content has oriented the focus of selection programmes over the past 40 years. This, combined with improved nutritional and management practices, resulted in a reduction of more than 50% in back-fat thickness and a simultaneous increase in lean meat content (Andersen, 2000). The muscular hypertrophy (or double-muscling) of breeds, such as Piétrain and, to a lesser extent, the Belgian Landrace resulted in leaner, shorter and heavier carcasses (Sellier, 1998b). The advantage of the Piétrain breed is the production of carcasses with 9% more lean and a 2.5% higher killing-out percentage, with higher proportion of lean on cuts following European reference method (Gispert et al., 2007) These carcass quality traits have been associated to the higher frequency of the HAL gene, associated ot not to the Pietrain breed (Sellier, 1998b; Gispert et al., 2007).

Nevertheless, higher muscle yield affects carcass and pork quality in opposite directions. Indeed, eating quality traits are negatively related to leaner carcasses (r = -0.18 to -0.48) and positively related to carcass fatness (r = 0.24 to 0.34; Ciobanu et al., 2011).

The presence of bruises on pigs skin represents another valuable carcass quality trait. Caused by fighting or rough handling, skin damages are indicative of poor welfare, and detract from the appearance of the carcass grading. The presence of bruises, depending on its level, may result on carcass downgrading, which can reach up to 6% of its value (MLC, 1985). The reduction of the speed of the dressing line, which is needed to remove blemished tissues, and the increased staff need for carcass inspection, represent additional costs. Further costs are also related to loss of market opportunities as severely bruised carcass and/or joints are rejected by the highly sophisticated international markets and can only be used for making lower value products with lower profit margins. According to US

pork industry estimates (Vansickle, 2002; Schultz-Kaster and Hill, 2006), bruises alone contribute to an $0.08 value loss per carcass or more than $48 million in annual trim losses.

The degree of damage on the skin of the carcass is measured subjectively by using pictorial charts, either providing a general score based on the carcass appearance (1 = none to 5 = severe; MLC, 1985; Figure 4) or counting the bruises by anatomical location (ITP, 1996; Barton-Gade et al., 1996a), and defining their shape to identify the source of infliction (e.g., fighting, handling and overcrowding; ITP, 1996). The colour of the bruises can provide information on the timing of bruise infliction, with red bruises being the most recent ones (Faucitano, 2001).

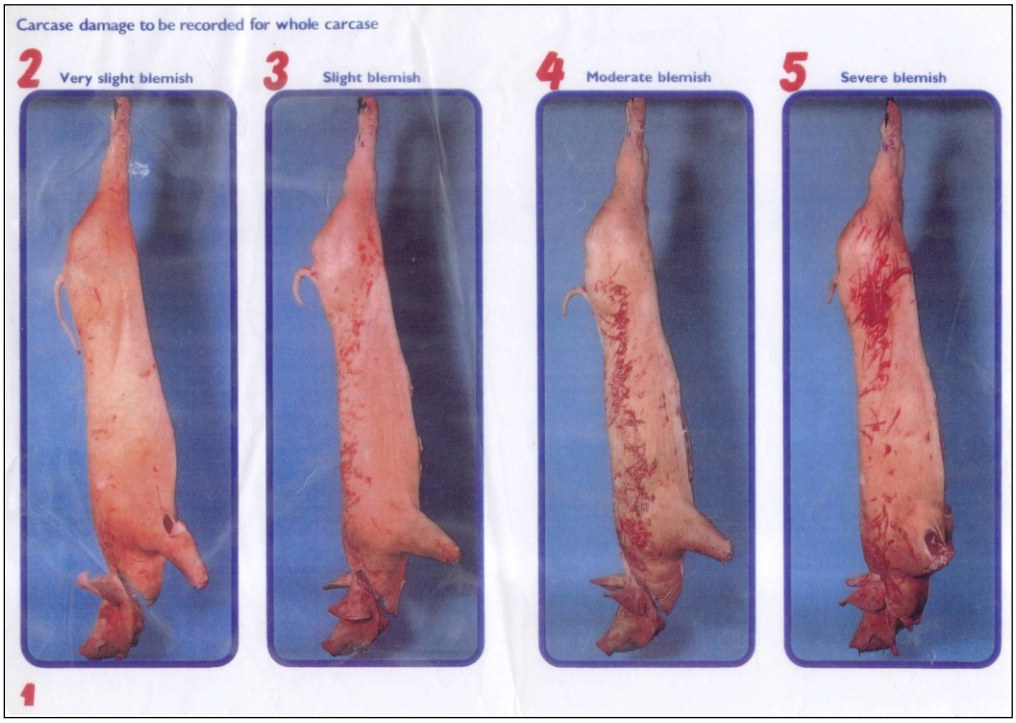

Figure 4. Skin damages scale chart (MLC, 1985).

1.9.2. Meat quality

Meat is the final state of a sequence of biochemical and physical events, which take place in cross-striated muscles of animals after slaughter in a process that may take from 1 day to 2 weeks (Honikel, 2004).

According to Koćwin-Podsiadła et al. (2006), pork quality, regardless of its composition and nutritional value, is determined by a set of animal conditions (healthy status) and the value of its palatability and technological properties. The already mentioned processing yield, meat acidity, colour and water holding capacity are intrinsic pork quality attributes that are affected by feeding regime, genetics and *post-mortem* metabolism (Koćwin-Podsiadła et al., 2006).

Based on pH, colour and drip loss variation, pork meat was initially divided into three quality categories: normal (or RFN: red, firm, non-exudative), PSE and DFD pork. However, for a more reliable quality assessment considering the variation in either colour or drip loss, two new quality categories have been added, namely RSE (reddish-pink, soft, exudative), and PFN (pale, firm, exudative) pork (Warrant et al., 1993, 1997; Koćwin-Podsiadła et al., 2006; Table 6). As the acronyms suggest these classifications address colour and water holding capacity problems, respectively. Cheah et al. (1998) reported that the incidence of RSE meat could be related to the gene halothane and induced by the poor *post-slaughter* management.

As mentioned above, the variation in meat quality classes can be related to muscle fibre type composition (Table 7). Ryu and Kim (2006) reported a higher percentage of type IIb fibres in PSE, followed by RSE pork. Type IIb fibres are characterized by a fast-glycolytic metabolism, have been associated to RSE meat (van Laack and Kauffmann, 1999), and may explain the lower pH_{45min} ($r = -0.33$) and higher drip loss ($r = 0.39$; Ryu and Kim, 2005) in this meat type. Protein denaturation rate is also related to the different pork quality classes. Kazemi et al. (2011) attributed the paler colour found in PFN pork to the higher level of denaturation of sarcoplasmic proteins compared to RFN pork. This is in agreement with previous findings of Joo et al. (1999) who described a negative relationship ($r = -0.72$) between sarcoplasmic protein denaturation and drip loss, both characteristics of PFN meat.

Table 6. Pork quality classification

Quality class	pHu	Colour (L*)	Drip Loss (%)
PSE	< 6	≥50	≥ 5
PFN	< 6	≥ 50	2 - 5
RSE	< 6	42 < 50	≥ 5
RFN	< 6	42 < 50	2 -5
DFD	≥ 6	< 42	≤ 2

Adapted from Warner et al.1993, 1997; Koćwin-Podsiadła et al., 2006.

Other than technological properties, pork from different quality classes can be also characterized by a different shelf life. After 35 days of storage, Faucitano et al. (2010b) reported that total aerobic mesophilic and presumptive lactic acid bacteria counts were higher in DFD pork due to its higher ultimate pH. RSE was the second quality class most susceptible to spoilage, whereas PFN, RFN and PSE pork had similar microbial load.

1.9.2.1. Muscle metabolism

Muscle is made up of fibres, which are held together by interconnecting connective tissues (Gregory, 1998) and is classified according to its anatomy (striated or smooth) and structure (skeletal, smooth or cardiac). Skeletal muscles from which meat is derived are responsible for body movement and are characterized by the high energy consumption and turnover in response to physical exercise (Lefaucheur, 2010).

In general, muscular fibres are categorized according to the metabolic pathway used to produce the energy (ATP) necessary for movement, growing or thermoregulation. They have two main characteristics: contractile (slow or fast speed contraction) and metabolic (oxidative, oxido-glycolytic or glycolytic; Reggiani and Mascarello, 2004; Lefaucheur, 2010). The contractile and metabolic properties of different muscle fibres strongly affect the energy metabolism during exercise in live animals (Hocquette et al., 1998), and the conversion from muscle to meat with consequences on eating quality attributes (Gil et al., 2008).

In pigs, muscle is composed of four different muscular fibres, which are related to the myosin isoforms: I, IIa, IIx and IIb (Lefaucheur et al., 1998). Fibres I and IIa present high oxidative metabolism, which is lower in IIx and IIb fibres, whereas the glycolytic metabolism is low in type I fibres, intermediate in fibres IIa and IIx and high in type IIb fibres (Table 7; Lefaucheur, 2003; Lefaucher et al., 2010).

Table 7. Functional and metabolic characteristics of muscle fibres in pigs

Fibre characteristic	**Type I**	**Type IIa**	**Type IIx**	**Type IIb**
Contraction speed	Slow	Fast/intermediate	Fast/intermediate	Fast
Metabolic oxidative capacity	High	High	Low/intermediate	Low
Metabolic anaerobic capacity	Low	Intermediate	Intermediate	High

Adapted from Lefaucheur et al. (2010).

Fibre composition depends on muscle type and pig genetics. The muscle from modern meat-type pigs presents larger fibres, have lower level of mitochondria and have white, fast-contracting fiber type (type IIb; Reggiani and Mascarello, 2004). To contract, these fibers use mostly the glycolytic pathway producing energy and resulting in a very fast pH decline due to the over production of lactate, which, in turn leads to protein denaturation (Reggiani and Mascarello, 2004). In pigs, fast glycolytic fibres have been associated with the presence of the HAL gene (Fiedler et al., 1999) and Pietrain genetics (Werner et al., 2010).

After death, muscles still metabolise energy, contract and produce heat. However, as there is no blood circulation, oxygen is not delivered to the cells and the metabolic end products are not removed. Under anaerobic conditions, lactic acid is produced from glucose either naturally present or derived from muscle glycogen to produce ATP. This utilisation of energy reserves by glycolysis continues until complete depletion of glycogen, or until a very low pH is reached due to the accumulation of lactic acid, and interferes with the

enzymatic activity. For instance, the accumulation of lactic acid in muscle decreases the pH from a near neutral pH of 6.8-7.2 to about 5.6-5.9. The increased acidity results in reduced water binding ability and calcium release triggering muscle contraction. At this point cells do not have energy left and the metabolism stops. The intracellular pH arrives at its final level. Rigor mortis is achieved when ATP is depleted rendering it impossible to expulse Ca^{2+} from the sarcoplasm and with the establishment of permanent acto-myosin crossbridges (Gregory, 1998; Bowker et. al., 2000). A summary of the evolution of muscular concentrations of glycogen, phosphocreatine (PC), ATP, and lactic acid after slaughter is shown in Figure 5.

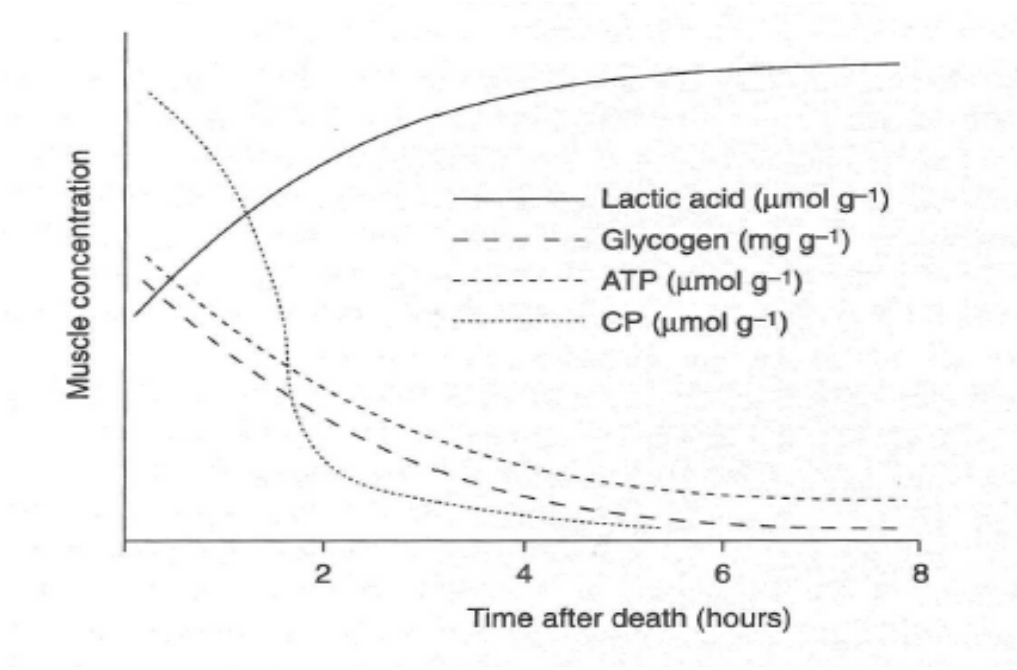

Figure 5. Changes in glycogen, phosphocreatine (PC), ATP, and lactic acid concentrations during *post-mortem* metabolism (Gregory, 1998).

According to Bowker et al. (2000), the following four factors can be associated with the abnormal *post-mortem* metabolism and meat defects, such as PSE: 1) the Ca^{2+} regulation; 2) the muscle ATPase activity; 3) glycogenolytic enzymes; and 4) glycogen regulation. Despite activating different mechanisms, these factors lead to accelerated and extended *post-mortem* glycolysis affecting the pH fall rate. Fibre type composition (Ryu and Kim, 2005), muscle temperature before slaughter (Klont and Lambooy, 1995) and chilling rates

(Bertram et al., 2001) are amongst the factors that will affect *post-mortem* muscle metabolism as well.

1.9.2.2. pH

In pigs, a normal pH decline is determined by a gradual decrease from approximately pH 7.00 in living muscle to a pH around 6.1 in the next 1 h following slaughter. The ideal pH at 24 h *post-mortem* is expected to be between 5.6 and 5.8 (Sellier and Monin, 1994; Figure 6).

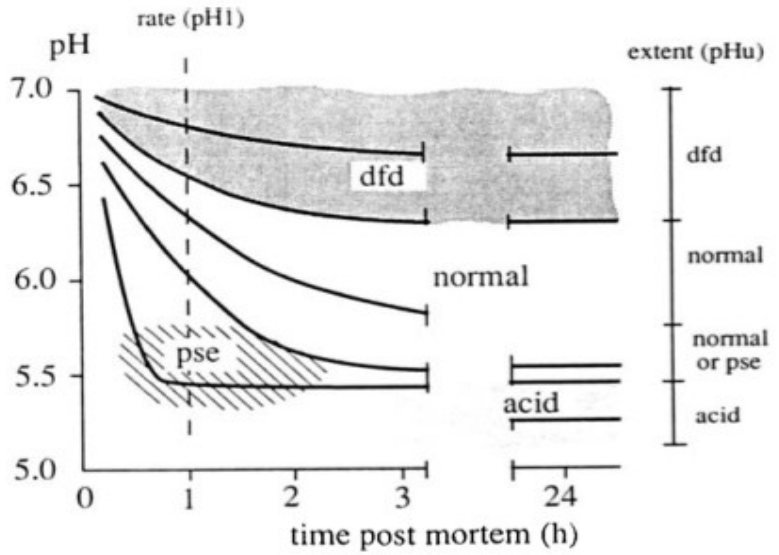

Figure 6. Relationship between *post-mortem* changes in muscle pH and meat quality of pigs (Sellier and Monin, 1994).

However, as previously mentioned, *post-mortem* pH can vary depending on the glycogen content in the muscle at the time of slaughter. If animals are submitted to prolonged stress just before slaughter, their glycogen reserves will be low and the muscle will not have enough substrate to produce lactic acid resulting in an ultimate pH (pHu) higher than 6, which is indicative of the DFD meat quality defect (Tarrant, 1989; Carragher and Matthews, 1996; Gregory, 1998). The darker colour, dry texture, and less pronounced taste, characteristics of DFD meat, makes it less appreciated in fresh cuts (Fisher, 2005). Furthermore, the high pH value, which is conducive to bacterial growth, results in shorter shelf-life (Fischer, 2005; Holmer et al., 2009; Faucitano et al., 2010).

Conversely, high levels of glycogen just prior to slaughter lead to higher lactic acid concentration in the muscle producing a rapid pH decline (lower than 6 at 45 min *post-mortem*; Warriss and Brown, 1987), which if combined with high muscle temperature, may result in the PSE meat quality defect (Gregory, 1998). Consumers will reject PSE meat due to its paler colour, its appearance (presence of exudates) and its poor palatability (reduced tenderness and juiciness after cooking; Fischer, 2005).

Stressful pre-slaughter conditions and/or the presence of the HAL gene in the population are associated with PSE pork (Honkavaara, 1988; Channon et al., 2000; Kuchenmeister et al., 2005). For instance, increased PSE incidence has been reported in halothane carrier (Nn) pigs being negatively handled, transported for a short of time (30 min) and electrically (head-only in V-restrainers) or gas stunned (Channon et al., 2000; Velarde et al., 2001; Fàbrega et al., 2002b). Pommier and Houde (1993) concluded that the PSE condition does not result directly from HAL gene, but is exacerbated by the gene under stressful environmental conditions.

When pre-slaughter conditions are not optimal, it is possible to prevent, or at least to reduce the incidence of PSE pork by controlling carcass chilling rate. A quick decrease in temperatures *post-mortem* will lower the velocity of chemical and biochemical reactions (it slows muscle glycolysis and protein denaturation) lowering the rate of pH decline. In pigs, rapid or blast chilling are reported to be effective in reducing the incidence of PSE pork. However, extreme chilling may result in tougher meat due to cold shortening (Savell et al., 2005). Cold shortening occurs when temperatures decreases too fast, while large amounts of ATP are still available in the muscle, causing maximal muscular contraction with minimal proteolysis, while the opposite is necessary to obtain tender meat (Rosenvold et al., 2010).

1.9.2.3. Colour

Colour is the most important quality factor affecting the consumer's decision "to buy" or "not to buy" pork and hence can affect the profitability in the pork chain. Indeed, some retail studies found that consumers have a tendency to select lean and fresh pork and can discriminate among pork of varying colours (Brewer and McKeith, 1999).

Colour can be adversely affected at all steps of the pork chain, including genetic selection, nutrition, pre-slaughter handling, stunning and bleeding, chilling variables, processing and holding times and temperature before packaging or further use, packaging, distribution and marketing, including lighting and other display conditions (Mancini and Hunt, 2005).

Colour in meat is a perception of its optical properties. Meat is not an opaque (light-permeable), but a translucent material (it lets through light). Therefore, light cast on meat is not entirely reflected, part of it is absorbed and part scattered. Absorption is mainly related to the concentration and state of pigments (Mancini and Hunt, 2005).

The concentration of pigments, mainly myoglobin, naturally present in muscle is the most important contributor to colour perception (Warner, 1994). The concentration of myoglobin in the muscle varies between species, breeds and individual muscles. A higher concentration of myoglobin results in brighter red meat (Young and West, 2001). Myoglobin exists in three-forms: purple-red deoxymyoglobin in the absence of oxygen, bright red oxymyoglobin formed in the presence of air and brown metmyoglobin, resulting from myoglobin oxidation. The oxygenation or "blooming" occurs when freshly cut meat is exposed to oxygen. A blooming time from 10-30 should be sufficient for pork colour measurement when the CIE L*, a* b* system is used (Brewer et al., 2001; Skrlep and Andek-Potokar, 2007).

The light scattering property of meat occurs by the structural elements in the surface layers of the muscle. Scattering in meat may occur both at the boundary between myofibrils and the sarcoplasm, and within the myofilament lattice of the myofibril, and it is caused by the lack of stability of the sarcoplasmic and myofibrillar proteins (myosin). In the *post- mortem* period the proteins are partly denatured due to acidification of meat resulting from the anaerobic glycolysis onset. Rapid rates of muscle glycolysis immediately *post-mortem* results in lower muscle pH while the muscle temperature is still high and causes abnormal denaturation of muscle proteins. This denaturation leads to a higher concentration of particles, which causes more light to be scattered reducing the penetration of light into the material (Mancini and Hunt, 2005). Due to that, there is a lower light absorption and a higher light reflection, which result in a lighter appearance producing PSE meat. The pale

colour of PSE muscles has been reported to result from the "white precipitate" of denatured sarcoplasmic proteins depositing on the myofibrils which mask the red colour and create a semi-opaque background (Joo et al., 1999). There is evidence that the sarcoplasmic protein denaturation in PSE meat causes incident light to be scattered about twice the amount that it is scattered in "normal (reddish-pink)" muscle. In this condition light penetrates to a shallower depth and is adsorbed to a smaller extent by myoglobin. Increased light scattering in PSE muscles arises from reduced gaps between myofibrils causing increases in scattered light. DFD pork, instead, is darker because the tissues are more translucent and scatter or reflect less light (Barton-Gade and Olssen, 1987).

The L*, a*, b* are coordinates of the tristimulus on the CIELAB colour space and specify various colour space by determining the components of lightness and chromaticity (L* for reflectance, red-greenness for a* and yellow-blueness for b*; CIELAB, 1976). L*, a* and b* are usually measured by the Minolta Chromameter which evaluates the reflectance of the myoglobin and haemoglobin after exposure to oxygenation (Brewer et al., 2001). For instance, the greater the concentration of myoglobin, the lower is the reflectance or the L* value. However, reflectance ratios vary according to the state of myoglobin being higher for the oxidated and lower for oxygenated state (Fernandez-Lopez et al., 2000).

1.9.2.4. Water-holding capacity and drip loss

Muscles of live animals contain about 75 % water held by 20 % proteins. Most water (85 %) is contained within the muscle cells, while the remaining 15 % is located around them (Huff-Lonergan and Lonergan, 2005). Half of the water contained in muscle cells is linked to muscle fiber proteins, while the other half is linked to sarcoplasmic proteins. Water in the muscle has various levels of binding to the protein, with only 5 % fully bound (Huff-Lonergan and Lonergan, 2005). In the live animal at normal pH, capillary forces and osmotic pressure restrict water mobility. However, the fall of pH close to the isoelectric point (pH 5.0 to 5.1) of meat proteins results in a considerable reduction in their hydration and in a lower ability to hold water tightly (Toldra, 2003).

The single most important factor for drip loss formation is the lateral shrinkage of muscle fibres (Offer and Cousins, 1992). Shrinkage of myofibrils and denaturation of proteins

results in movement of water within or out of the fiber. During rigor development there will be less space available among the muscle fibers for the retention of immobilised water, which becomes free and flows out the fibrils space around fibres and fibres bundles (Offer and Cousins, 1992).

As already mentioned, water-binding capacity is dependent on the rate of pH drop in early *post-mortem* muscle and thus is influenced by several factors such as genetics, animal and carcass handling, temperature management *post-mortem*, nutrition and processing (Jennen et al., 2007).

Drip is an aqueous solution purged from meat containing sarcoplasmic proteins, such as myoglobin, which, as already mentioned, gives the characteristic red colour to it (Savage et al., 1990). Drip loss has a huge economic importance for the pork industry. Economic losses are related to carcass shrinkage, which affects weight loss and appearance (Kauffman et al., 1986), and nutritional value, as drip loss contains significant amount of proteins, water-soluble vitamins, and amino acids (112 mg of protein/mL of fluid; Savage et al., 1990). For instance, a $0.80 loss was reported for every 1% of drip loss (Rivest et al., 2008).

The term drip loss is an expression's the lack of ability of the meat to hold onto the natural meat juices in the muscle and muscle fibres, even in the absence of external forces (Fisher, 2007). The assessment of drip loss can be done by various methodologies, such as the bag (Honikel, 1998), filter paper (Kauffman et al., 1986) and EZ-Drip loss methods (Christensen, 2003). Christensen (2003) and Otto et al. (2004) reported a high correlation ($r = 0.86$) between the EZ-Drip loss and the bag method, but they recommended it more because of its higher sensitivity and repeatability. Correa et al. (2007) reported a higher accuracy in the distribution of pork quality classes by using a modified EZ-Drip loss method, in which the storage time of the sample was increased from 24 to 48 h and samples were mopped dry before final weighing after storage.

Chapter 2: Hypothesis and objectives

The effects of transportation on behavioural and physiological responses of animals affect their welfare and health status, with negative consequences on meat quality. Depending on the severity of the stress, pigs may become injured and even die during transportation. To minimize stress and avoid extreme conditions, transportation must meet the most important animal welfare criteria for the benefit of animals and producers. The literature available on transport and welfare of pigs suggests that high lean genotypes may be more susceptible to stressors. Pietrain pigs, known for their high performance, lean deposition rate and the high frequency of the HAL gene in the population, can be taken as an example. However, after the eradication of the HAL gene from the population, the extent to which this stress susceptibility is related to the breed or to the gene itself remains unclear. Based on this information the hypotheses of the work presented in this thesis are:

- ✓ Adequate vehicle design reduces the impact of journey distance on pigs welfare and pork quality;
- ✓ Pigs with a higher proportion of Pietrain genetics (50 vs. 25%) but free of the HAL gene (NN) are less susceptible to stress and may produce good pork quality at no detriment to carcass quality.

To validate these hypotheses, the main objectives of this thesis are:

1. Study of the effects of vehicle design and transport distance on behaviour, physiology, and carcass and pork quality;
2. Study of the response to transport stress in Pietrain genotypes – carrying the HAL gene or not - and its effects on carcass and pork quality variation.

An additional study was conducted to validate the efficiency of infrared thermography technology as a tool to assess body temperature and predict the animal welfare condition of pigs at the *pre-mortem* stage and pork quality variation.

Chapter 3: Effects of trailer design on animal welfare parameters and carcass and meat quality of three Pietrain crosses being transported over a long distance

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According to the reviewed literature, transport negatively affects animal welfare and pork quality. It is well known that optimal conditions during transportation, including vehicle design, can minimize animal welfare problems, which in turn positively affects pork quality. In Canada, the most used vehicle type for pig transportation is the PB trailer, an adapted trailer originally designed for cattle transportation. In Canada, pigs can travel from 30 min up to 35 h depending on the farm and slaughterhouse location.

The use of alternative vehicle types with no internal ramps would minimize the above-mentioned problems limiting the stress response of pigs of Pietrain genetics, whose introduction is increasing in North American swine herds. Understanding the behaviour of this breed as well as the quality of its meat will provide important information for the improvement of breeding and transportation programs. This chapter presents the results of the long distance transportation study.

Authors: Angela Vanelli Weschenfelder (Ph.D. candidate; planification and data collection, data analyses and manuscript preparation). Linda Saucier and Luigi Faucitano (research director and co-director: student's supervision, revision and correction of the manuscript). Collaborators in the project, including manuscript revision: Stephanie Torrey and Nicolas Devillers (swine behaviour experts), Anna Bassols, Matilde Piñeiro and Yolanda Saco (animal physiology experts), and Trever Crowe (expert in vehicle aerodynamics).

Effects of trailer design on animal welfare parameters and carcass and meat quality of three Pietrain crosses being transported over a long distance

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ABSTRACT

This study aimed at evaluating the effects of trailer design on stress responses and meat quality traits of 3 different pig crosses: 50% Pietrain breeding with halothane (HAL)^{Nn} (50Nn); 50% Pietrain breeding with HAL^{NN} (50NN); and 25% Pietrain breeding with HAL^{NN} genotype (25NN). Over a 6-wk period, pigs (120 pigs/crossbreed) were transported for 7 h in either a pot-belly (PB) or flat-deck (FD) trailer (10 pigs/crossbreed^{-1}·trailer^{-1}·wk^{-1}). Temperature (T) and relative humidity (RH) were monitored in each trailer. Behaviors during loading and unloading, time to load and unload, and latency to rest in lairage were recorded, whereas a sub-population of pigs (4 pigs/crossbreed^{-1}· trailer^{-1}·wk^{-1}) was equipped with gastro-intestinal tract (GIT) temperature monitors. Blood samples were collected at exsanguination for measurement of cortisol, creatine kinase (CK), lactate, haptoglobin, and Pig-MAP concentrations. Meat quality data were collected at 24 h postmortem from the LM and semimembranosus (SM) and adductor (AD) muscles of all 360 pigs. Greater T were recorded in the PB trailer during transportation ($P = 0.006$) and unloading ($P < 0.001$). Delta GIT temperature was greater ($P = 0.01$) in pigs unloaded from the PB. At loading, pigs tended to move backwards more ($P = 0.06$) when loaded on the FD than the PB trailer. At unloading, an interaction was found between trailer type and crossbreed type, with a greater ($P < 0.01$) frequency of overlaps in 50NN and 25NN pigs and slips/falls in 50Nn and 50NN pigs from the FD than the PB trailer. Cortisol concentrations at slaughter were greater ($P = 0.02$) in pigs transported in the PB than FD trailer. Greater lactate concentrations were found in 50Nn and 50NN pigs ($P = 0.003$) and greater CK concentrations ($P < 0.001$) in 50Nn pigs. As expected, 50Nn pigs produced leaner ($P < 0.001$) carcasses, with greater ($P = 0.01$) dressing percentages, as well as lower ($P < 0.001$) ultimate pH values and greater ($P < 0.001$) drip loss percentages in the LM and greater ($P = 0.002$) drip losses and a paler color (greater L* values, $P = 0.02$) in the SM than 50NN pigs. When used for long distance transportation under controlled conditions, the PB trailer produced no detrimental effects on animal welfare or pork quality. Pigs with 50% Pietrain crossbreeding appear to be more responsive to transport stress, having the potential to produce acceptable carcass and pork quality, provided pigs

are free of the HAL gene.

Key words: animal welfare, genotype, meat quality, pigs, trailer type, transport

INTRODUCTION

The use of pot-belly (**PB**) trailers has been implicated with increases in dead-on-arrivals reported in a recent Canadian swine transport survey (Dewey et al., 2009). The PB trailer is very common in Canada because of its large loading capacity; however, it has been criticized because of the presence of multiple (up to 5) and steep (> 20° slope) internal ramps which make the loading and unloading procedures time-consuming and stressful to pigs (Cormier and Doonan, 2008; Torrey et al., 2008). The PB trailer is now raising additional concerns for some swine producers because of the expectation that the use of Pietrain terminal sires in the selection programs will increase in the years to come (D. Godbout, Genetiporc Inc., St-Bernard, QC, Canada, personal communication). Pigs of Pietrain genetics are known to be leaner and more efficient but also to be more susceptible to stress than other genotypes, resulting in greater mortality rates and inferior pork quality (Fàbrega et al., 2004). These negative effects are related to the greater frequency of the halothane (**HAL**) gene in the population (Leach et al., 1996; Murray and Johnson, 1998; Gispert et al., 2007). Thus, the negative effects of the HAL gene may make the use of these genetics unpopular since there is evidence that the effects of the HAL gene on pork quality may be exacerbated in stressful environmental conditions associated with transportation in PB trailers (Pommier and Houde, 1993; de Vries et al., 2000). It is unclear, however, whether the response of Pietrain pigs to stress is related to the presence of the HAL gene or to the breed itself. The answer to this question is of paramount importance to reduce transport losses and minimize meat quality variation. Therefore, the aim of this study was to investigate the effects of trailer design on the physiological and behavioral responses to transportation stress and on carcass and pork quality traits of Pietrain pigs carrying the HAL gene transported over a long distance.

MATERIAL AND METHODS

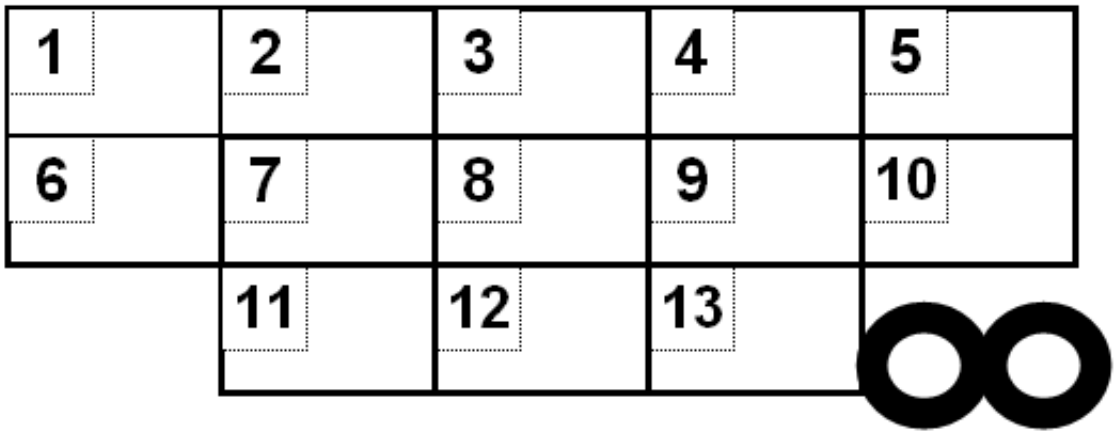
All experimental procedures performed in this study were approved by the institutional animal care committee based on the current guidelines of the Canadian Council on Animal Care (1993).

Animals and Treatments

In a 6-wk trial (6 loads), a total of 360 barrows (BW 115 ± 5kg) were transported for 7 h (450 km) from a commercial growing-finishing farm to a slaughter plant located in eastern Canada by 2 types of trailers [a 3-decked PB trailer equipped with 2 internal ramps vs. a 3-decked flat-deck (**FD**) trailer equipped with middle and upper hydraulic decks and no internal ramps; Figure 7]. Pigs were progenies from crosses of Pietrain homozygous halothane recessive (**HAL^{nn}**), Pietrain homozygous halothane dominant (**HAL^{NN}**), and Duroc × Pietrain (homozygous halothane dominant, HAL^{NN}) sire lines mated to F-20 sows (Genetiporc Inc., St-Bernard, QC, Canada), resulting in 3 swine crossbreds: 50% Pietrain crossbreeding with HAL^{Nn} genotype (**50Nn**); 50% Pietrain crossbreeding with HAL^{NN} genotype (**50NN**); and 25% Pietrain crossbreeding with HAL^{NN} genotype (**25NN**). Transport groups were prepared 4 to 5 d before loading and kept in separate finishing pens by genotype and trailer type and were not mixed within the trailer. Loading started at 2000 h, and the loading order between trailers was rotated every week to avoid the effect of the time of the day (light and temperature) on trailer microclimate and pig response to handling at loading. The PB trailer used in this study transported 222 pigs on the 3 decks distributed in 13 compartments (5 in the upper and middle decks and 3 in the belly; Figure 7a). Pigs were loaded in groups of 3 to 4 pigs through an external ramp and had to climb an internal ramp (22° slope) to reach the upper deck or descend an internal ramp (41° slope) to reach the belly compartments. In the middle deck, pigs only used the external ramp to go to the compartments. The FD trailer had a loading capacity of 235 pigs distributed in 3 decks composed of 8 compartments (3on upper and middle, and 2 in the bottom deck; Figure 7b). However, to respect the loading density applied in the other compartments on both trailers, compartments 3, 4, and 7 were adjusted in size and

subdivided into sub- compartments by a wooden panel (Figure 7b). Transport trials were run in July and October 2009 (3 journeys or replicates each month). Side-slats were used according to the external temperature thresholds following the NPB (2008) recommendations, keeping the porosity settings consistent in each trailer. Feed was withdrawn from all pigs 9 h before loading (19 h from last feed to slaughter), and pigs were loaded on each trailer using paddles and boards. Each trailer was loaded to its full capacity and test pigs were distributed into 3 separate compartments (5, 6, and 11 in the PB trailer and 3, 4, and 7 in the FD trailer), in terms of 1 compartment per crossbreed, at an average loading density of 0.43 m^2/pig. A rotation of the group position in each trailer according to the pig genotype was done at every load to avoid the confounding effect of the truck compartment on the responses of the pigs to transportation. Within each group of 3 to 4 pigs, 1 pig was randomly chosen for the study of the physiological response (4 pigs/ $crossbreed^{-1} \cdot trailer^{-1} \cdot load^{-1}$). This pig, plus another 6 to 7 pigs selected within the same group, was also used for the meat quality assessment (10 pigs/$crossbreed^{-1} \cdot$ trailer $type^{-1} \cdot replicate^{-1}$). The driver of each trailer and the handler at the farm were the same through the 6 wk. On arrival at the plant, pigs were unloaded using a whip only and driven to separate lairage pens on the basis of the transport compartment (no mixing). After a 90-min lairage period, pigs were electrically stunned (head-to-chest electrical stunning) and exsanguinated in the prone position.

a)



b)

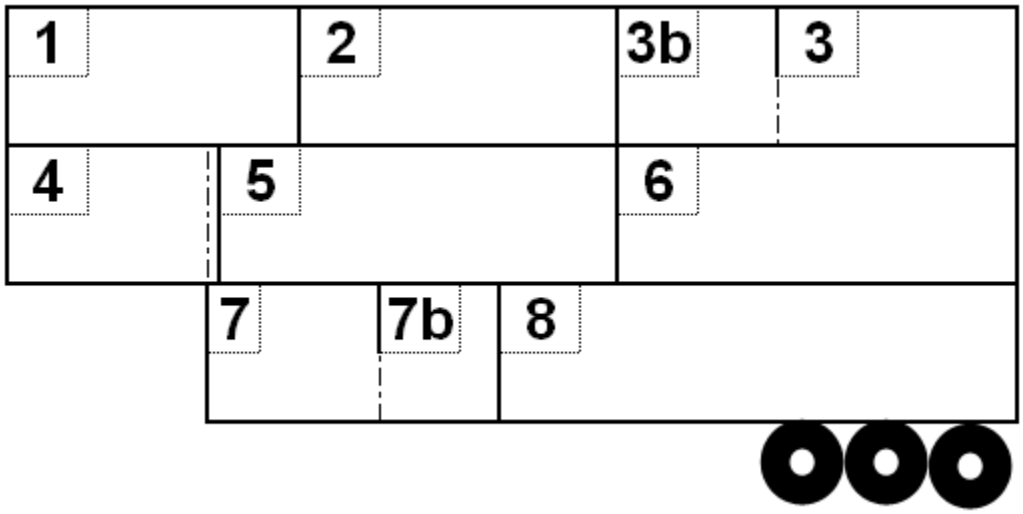


Figure 7. The location of compartments in the a) pot-belly (PB) and b) flat-deck (FD) trailers.

Trailer Temperature and Humidity

The temperature (**T**) and relative humidity (**RH**) of air in each truck compartment were monitored using data loggers (i-Button, Dallas Semiconductor, Maxim Integrated Products,

Sunnyvale, CA) consisting of a 17- × 6-mm stainless steel can containing sensory data and storage equipment. The logger had a T range of −20 to +85°C, with an accuracy of ±0.5°C and RH range of 0 to 100%, with sensitivity of ±0.6%. Five loggers were securely mounted approximately 6 cm below the ceiling of each selected compartment. One logger was positioned in the center of the compartment, and the remaining 4 were placed 15 cm from the midpoint of each wall. The T and RH of air outside of the truck were also recorded by 2 similar loggers mounted on the side-mirrors of each tractor. Data, recorded once each minute, were downloaded from the loggers after each trip and included T and RH from just before loading at the farm until the last pig exited the trailer at the abattoir. Occurrence of key events (start of loading, start of trip, and start, and end of unload) were noted and identified in the string of T and RH data. The data were later imported into Microsoft Excel (version 2002, Microsoft Corp., Redmond, WA) for analysis. The temperature humidity index (**THI**) of each trailer was calculated according to the NRC (1971) formula: THI = {T – [0.55 – (0.0055 × RH)]} × [(1.8 × T) – 26]. Delta temperature (**DT**) and delta humidity ratio (**DHR**) values for each trailer and its compartments were calculated using trailer T and HR data. Using the recorded T and RH data, the humidity ratios (**HR**) were calculated as the mass of water vapor per unit mass of dry air in (g/ kg). Using HR to represent the quantity of water vapor in air was preferred, because these data are independent of T, unlike RH. The DT and DHR values were obtained by calculating the difference in temperature and humidity ratio values between the air inside and the ambient air outside the trailer (Brown et al., 2011). The intra-trailer environmental indicators (T, DT, RH, HR, DHR, and THI) were evaluated during 4 different time periods: before loading (last hour before the start of loading), loading, transportation, and unloading.

Behavioral Observations

Behavior at loading

Behavior of pigs was recorded using 2 digital camcorders (DCR-HC48; Sony, Sony of Canada Ltd., Toronto, ON, Canada) installed using 3 camera mounts overhead at the loading ramp and the alley at the loading ramp. The cameras recorded all occurrences of pig behavior (Table 8) from a predetermined start gate at the farm alley until the trailer

gate. The course was divided into 2 zones; zone 1 was the alley from the start gate until farm door and zone 2 was between the farm door and the end of the external ramp (trailer door). Total frequency of a given behavior represented the sum of zones 1 and 2. Video recordings were analyzed by a trained observer using a handheld Psion Workabout (HC-110, Psion Inc., Mississauga, ON, Canada) computer. The total time taken to move pigs from the starting gate through the door of the trailer was noted as well as all occurrences of manipulator interventions needed to move the pigs (Table 9).

Table 8. Ethogram of pig behavior during loading and unloading (adapted from Brown, 2009).

Pig behavior	Description
Slip/fall	Leg of the pig splits away from the other legs or pig falls down (at least 2 legs buckled under)
Overlap	Pig mounts another pig, with its 2 front legs on the back of the other pig
180° turn	Pig makes a 180° turn, ending with its rear extended in the direction of intended movement
Back up	Pig moves at least 2 steps rearward, opposite the direction of intended motion
Backwards	Pig moves in the intended direction with its body oriented in the opposite direction
Underlap	Head of the pig goes under the body of another pig
Vocalize	Pig vocalizes
Balk	Pig refuses to walk or stops for more than 2 s
Squeeze	Pig is squeezed at the door, corridor, or exit of the ramp (or at the trailer door, when unloading)

Table 9. Handler behavior towards pigs during loading and unloading.

Handler behavior	Description
Vocal sound	Handler uses his voice to encourage forward movement of 1 or a group of pigs
Rattle noise	Handler uses the paddle to produce noise (1 time)
Physical intervention	Handler uses his hands, paddle, or board (or whip at unloading) to push and encourage forward movement of 1 or a group of pigs

Behavior at unloading

Pigs were unloaded by compartment at the abattoir and driven into lairage pens segregated by truck compartment at a density of 0.54 m^2/pig. A video camera (DCR-HC48; Sony, Sony of Canada Ltd.) was placed at the entrance of the lairage room, mounted on the side wall of the corridor, to register behavior during unloading. Total time to unload each compartment was noted using a chronometer, and images were analyzed by a trained observer for all occurrences of slipping/falling, overlapping, turning around, backing up, going backwards, underlapping, vocalization, balks, and squeeze (Table 8).

Behavior in lairage

Behavior during lairage was recorded using video cameras (WV-BP50, Panasonic Canada Inc., Mississauga, ON, Canada) installed overhead in the pens and connected to a digital encoder (Nextiva S5712e, Verint, Melville, NY). Images were captured and registered by the Omnicast system (version 4.0; Genetec Inc., Montréal, QC, Canada) at a frequency of 5 to 7 images/s. Scan sampling was used at 2-min intervals to determine the number of pigs lying (Lehner, 1996). To assess the recovery rate of the pigs after transport, the total time necessary for 75% of the pigs from the same pen to rest (latency to rest) was determined from video recordings. As pigs were showered for the first 10 min of lairage in the pen, the latency to rest was calculated starting from the end of showering to avoid the confounding effect of this practice on pig behavior (Weeding et al., 1993).

Physiological Measurements

Approximately 10 h before loading, a total of 144 pigs (24/wk) were orally administered Thermocron i-Button data loggers (model DS1921H; Dallas Semiconductor, Maxim Integrated Products, Sunnyvale, CA) to monitor gastro-intestinal tract (**GIT**) temperature using a snare, a heavy gauge metal "pig gag", and balling gun. Each data logger was programmed to begin recording from 1 h before loading until slaughter, and to log temperature once per minute. The GIT temperature was measured in 0.125°C increments with ±1°C accuracy (range of 15 to 46°C). Data loggers of selected pigs were recovered after dissection of viscera during the slaughter process. Later, data from 118 pigs (81.9% recovery) were downloaded onto a laptop computer, and GIT temperatures were evaluated for each pig for 5 periods on the basis of determined events, such as loading, transportation, unloading, lairage, and just before slaughter. The delta GIT temperatures values within treatments were obtained by the difference between the measured GIT temperature at any determined event and the GIT temperature measured at rest (basal level).

At exsanguination, 10mL of blood were collected in a tube (BD Vacutainers, VWR International Ltd., Montreal, QC, Canada) to extract serum for creatine kinase (CK), cortisol, and acute phase proteins (haptoglobin and Pig- MAP) analysis. Another 2 mL of blood were collected in a tube containing 3.0 mg of sodium fluoride and 6.0 mg of Na_2EDTA solution to extract plasma for lactate analysis. The 2-mL blood tubes were immediately centrifuged at 4°C for 12 min at 1,400 × *g*, and plasma was transferred into 1.5-mL Eppendorf tubes and stored at −80oC until lactate determination. Serum samples were kept at room temperature (~23°C) for 1 h before refrigeration at 4°C. The next day, serum samples were centrifuged at 4°C for 12 min at 1,400 × *g*, transferred to 1.5-mL Eppendorf tubes, and stored at −80°C until analysis. Lactate concentrations were measured using a commercially available kit (Lactate Assay Kit, Biomedical Research Service Center, University of Buffalo, Buffalo, NY) and CK concentrations with creatine kinase-sl kit (Creatine Kinase-SL Assay of Chemicals Diagnostic Limited, Vancouver, BC, Canada). Plasma lactate concentrations were determined with a microplate reader and serum CK concentrations with a spectrophotometer.The quantitative determination of serum cortisol

was made using a commercial kit (DRG Cortisol Enzyme Immunoassay kit, DRG Instruments GmbH, Marburg, Germany), with a microplate reader and expressed as ng/mL. Haptoglobin concentrations were quantified by a spectrophotometric method (hemoglobin-binding assay) and performed on an automated analyzer (AU400; Olympus, Hamburg, Germany). Serum Pig-MAP concentrations were assessed with an Elisa kit (PigCHAMP ProEuropa, Segovia, Spain) with a microplate reader and expressed as mg/mL. The intra-assay CV was 5.04, 3.99, 4.05, 1.9, and 8.5% for plasma lactate, serum CK, plasma cortisol, haptoglobin and Pig-MAP, respectively.

Carcass Quality Measurements

After slaughter, carcasses were eviscerated, split, and chilled according to standard commercial practices. Hot carcass weight and carcass lean percentage (by Destron probe) were recorded, and HCW was used to calculate dressing percentage. Skin damage was assessed on the day of slaughter in the cooler using the 5-point, photographic scale (1 = none to 5 = severe; MLC, 1985), whereas bruises were classified as fighting type bruises (1 = fewer than 10 bruises; 2 = 11 to 20 bruises; and 3 = more than 20 bruises) or mounting (score 1 = fewer than 5 bruises; 2 = 6 to 10 bruises; and 3 = more than 10 bruises) by visual assessment of shape and size according to the photographic standards of the Institut Technique du Porc (**ITP**, 1996) as described by Faucitano (2001). According to the ITP scale, bruises due to biting during fighting are 5 to 10 cm in length, comma shaped, and concentrated in high number in the anterior (head and shoulders) and posterior (ham) regions of the carcass. Long (10 to 15 cm), thin (0.5- to 1-cm wide), comma shaped bruises densely concentrated on the back of pigs caused by the fore claws were classified as mounting type bruises.

Meat Quality Measurements

Ultimate pH (**pHu**) was measured in the LM, semimembranosus (**SM**), and adductor (**AD**) muscles at 24 h postmortem with a temperature-compensating, spear- type probe (Cole-Palmer Instrument Co., Vernon Hills, IL) attached to a pH meter (pH 100 series; Oakton Instruments, Vernon Hills, IL). In addition, color data were collected on the LM and SM

after a 30-min bloom period. Visual color was evaluated using the Japanese color standards (Nakai et al., 1975), whereas instrumental color (L*, a*, and b* values) was measured with a Minolta Chroma Meter (CR- 300; Minolta Canada Inc., Mississauga, ON, Canada) equipped with a 25-mm aperture, 0° viewing angle, and D65 illuminant. Drip loss was measured in the LM and SM using the modified EZ-driploss method of Correa et al. (2007). Briefly, 3 25-mm-diameter cores were removed from the center of 2.5-cm-thick LM (removed at 3rd/4th last rib) and SM chops, weighed, and placed into plastic drip loss containers (Christensen Aps Industrivaengetand, Hilleroed, Denmark), before being stored for 48 h at 4°C. At the end of the 48-h storage period, muscle cores were removed from their containers, surface moisture was carefully dabbed, cores were reweighed, and drip loss percentage was calculated by dividing the difference between initial and final core weights by the initial core weight. Electrical conductivity was assessed by inserting the pork quality meat probe (PQM-I-INTEK, Classpro GmbH, Sielenbach, Germany) into the same 3 sites where muscle cores were removed from the LM and SM chops.

Statistical Analysis

Data were analyzed using the mixed model proce- dure (SAS Inst. Inc., Cary, NC) for repeated measures with treatments in a 2 × 3 factorial arrangement, with 2 trailer types (PB vs. FD) and 3 crossbreeds (50Nn, 50NN, and 25NN). Trailer type and pig crossbreeds were in- cluded in the model, as well as their interaction, as fixed effects. The means were calculated using the 'lsmeans' option of the mixed model procedure (PROC MIXED) in SAS. Differences in the mean values between the levels of a factor were tested using a Tukey-Kramer correction for multiple comparisons. Behavior data not suitable for ANOVA analysis, such as those registered at unload- ing, were analyzed by the log-normal distribution model (Poisson). A probability level of $P < 0.05$ was chosen as the limit for statistical significance in all tests. Probability levels of $P \leq 0.10$ were considered as a tendency.

RESULTS AND DISCUSSION

Environment Variation Inside the Trailer

During transportation, the variation of heat and moisture produced by the animals combined with ambient temperature and internal air flow can have a major impact on the thermoregulation of the animal, potentially compromising its welfare (Lambooij, 1988; Kettlewell et al., 2001; Lenkaitis et al., 2008). During the trial, the average ambient temperature during transport was 8.1ºC, ranging from −5.1 to 19.4°C. Overall, in both types of trailers, temperatures increased during pig loading and decreased when the vehicle was in motion as a result of increased ventilation (Chevillon, 2005). However, temperatures were greater in the PB than the FD trailer during transport ($P = 0.006$) and at unloading ($P < 0.001$), suggesting less ventilation in the PB trailer as it was in motion or stationary (Table 10). Ritter and Ellis (2008) explained that the poor ventilation in the PB trailer was due tothepunch-typepatternofthesideopenings,whereas the slatted-openings of the FD trailer afforded greater air-flow. The smaller ($P < 0.0001$) delta T observed at unloading in the FD trailer supports this observation (Table 10).

Ritter et al. (2008) also reported higher internal temperatures in the PB trailer than in the FD trailer during transport and unloading periods. Within the PB trailer, registered temperatures were greater in compartments 6 and 11 at loading ($P = 0.0001$), before loading ($P = 0.002$), and during travel ($P = 0.005$), whereas within the FD trailer, compartment 3 had the greatest ($P = 0.002$) temperature at loading (Table 11). The increased temperature in the front compartments of the PB trailer may be explained by the absence of ventilation outlets in the front part of this trailer. Indeed, the greater ($P \leq 0.002$; Table 12) delta T in compartments 6 and 11 at stationary periods supports the observation that the lack of outlets does not facilitate the removal of heat which is both collected in the rear compartments and transported by the air flowing to the front of the trailer and is generated by the tractor engine in close proximity to these compartments (Lenkaitis et al., 2008).

Table 10. Average and delta temperatures (T and DT)[1], relative humidity, humidity ratio, and delta humidity ratio (RH, HR, and DHR, respectively)[2], and temperature humidity index (THI) inside the pot-belly (PB) and flat-deck (FD) trailers at the time periods of preloading, loading, transport, and unloading (n = 6 replicates).

Item	Preloading	Loading	Transport	Unloading
T				
PB	7.8	12.8	12.0	16.4
FD	7.1	11.4	10.6	15.4
SED	1.17	1.39	0.49	0.25
P-value	0.58	0.33	0.006	<0.001
DT				
PB	0.92	6.27	5.67	4.33
FD	0.50	4.48	5.00	1.47
SED	0.92	1.58	0.78	0.57
P-value	0.66	0.24	0.27	<.0001
RH				
PB	89.87	87.64	74.29	73.12
FD	82.34	83.93	78.42	80.13
SED	2.97	2.08	1.55	2.06
P-value	0.02	0.09	0.01	0.002
HR				
PB	6.60	8.77	6.91	9.03
FD	5.70	7.82	6.75	9.26
SED	0.38	0.81	0.15	0.35
P-value	0.007	0.16	0.21	0.44
DHR				
PB	0.68	2.46	0.88	1.52
FD	-0.8	1.79	0.94	1.50
SED	0.40	0.58	0.08	0.34
P-value	0.001	0.32	0.57	0.93
THI				
PB	6.68	13.03	12.27	16.64
FD	7.83	11.68	11.15	15.44
SED	2.22	2.49	0.89	0.83
P-value	0.36	0.34	0.02	0.02

[1]The difference between the air inside and the ambient air outside the trailer
[2]The difference between humidity ratio inside the trailer and the external environment

Table 11. Average temperature (T), humidity ratio (HR), and temperature humidity index (THI) variation between compartments within the pot-belly and flat-deck trailers during transport (n = 6 replicates).

Item	**Pot belly**					**Flat deck**				
Compartment	**5**	**6**	**11**	**SEM**	***P*-value**	**3**	**4**	**7**	**SEM**	***P*-value**
T										
Preloading	7.2[b]	8.0[a]	8.2[a]	0.14	0.002	6.1[b]	7.5[a]	7.8[a]	0.26	0.002
Loading	8.6[b]	13.7[a]	16.0[a]	0.77	0.0001	15.3[a]	10.4[b]	8.4[b]	1.02	0.002
Transport	10.7[b]	12.2[a]	13.2[a]	0.39	0.005	10.5	10.4	10.8	0.22	0.55
Unloading	16.0	16.4	16.8	0.39	0.43	15.7	15.2	15.2	0.42	0.63
HR										
Preloading	6.48[a]	6.64[b]	6.67[b]	0.03	0.003	5.92	5.35	5.78	0.21	0.19
Loading	7.25[b]	9.14[a]	9.93[a]	0.29	0.0002	10.31[a]	6.91[b]	6.26[b]	0.47	0.0002
Transport	6.84	6.90	7.00	0.10	0.55	6.84	6.67	6.74	0.07	0.26
Unloading	9.71[a]	8.30[b]	9.10[ab]	0.24	0.006	9.79	8.67	9.30	0.30	0.07
THI										
Preloading	7.2	4.6	8.2	2.66	0.25	6.7[b]	8.3[a]	8.5[a]	0.57	0.0034
Loading	8.6[b]	14.7[a]	15.8[a]	1.88	0.0014	15.3[a]	10.8[b]	9.0[b]	1.75	0.0028
Transport	11.2[b]	12.1[a]	13.5[a]	0.65	0.0032	11.1	11.0	11.4	0.35	0.39
Unloading	16.0	17.3	16.6	1.67	0.63	15.8	15.2	15.3	0.67	0.55

[a,b] Within a row and trailer type, least squares means lacking a common superscript letter differ, $P < 0.05$.

Table 12. Delta temperature (DT)[1] and delta humidity ratios (delta DHR)[2] variation between compartments within the pot-belly and flat-deck trailers during transport (n = 6 replicates).

Items	Pot belly					Flat deck				
Compartment	**5**	**6**	**11**	**SEM**	***P*-value**	**3**	**4**	**7**	**SEM**	***P*-value**
DT										
Preloading	0.4[b]	1.1[a]	1.3[a]	0.14	0.002	-0.5[b]	0.8[a]	1.2[a]	0.26	0.002
Loading	2.1[b]	7.2[a]	9.6[a]	0.77	0.0001	8.4[a]	3.5[b]	1.5[b]	1.02	0.002
Transport	4.4[b]	5.8[ab]	6.8[a]	0.39	0.005	5.0	4.8	5.2	0.22	0.55
Unloading	3.9	4.3	4.7	0.39	0.43	1.8	1.3	1.3	0.42	0.63
DHR										
Preloading	0.56[a]	0.72[b]	0.75[b]	0.03	0.003	-0.15	-0.72	-0.28	0.21	0.19
Loading	0.94[b]	2.83[a]	3.61[a]	0.28	0.0002	4.27[a]	0.87[b]	0.22[b]	0.47	0.0002
Transport	0.80	0.87	0.96	0.09	0.55	1.02	0.86	0.93	0.07	0.26
Unloading	2.1[a]	0.81[b]	1.56[ab]	0.24	0.006	2.03	0.91	1.54	0.30	0.07

[a,b] Within a row and trailer type, least squares means lacking a common superscript letter differ, $P < 0.05$.
[1]The difference between the air inside and the ambient air outside the trailer
[2]The difference between humidity ratio inside the trailer and the external environment

The lower temperatures in the upper compartments of the PB trailer can be explained by the presence of more exposed surfaces and increased thermal radiation from the upper deck cooling these compartments (Lenkaitis et al., 2008; Brown et al., 2011). The lack of insulation in the roofs of both trailers allowed heat to be lost through this surface. The temperature increase in the rear upper compartment of the FD trailer at loading may be attributed to the effect of the loading order. This compartment was always the first to be loaded and the heat generated by pigs combined with the lack of air flow (as the vehicle was stationary) may have contributed to the temperature rise at this location. It is also worth noting that the lower deck height and the proximity of the data loggers to the pigs may have contributed to the increase in T and HR values in the FD trailer. Indeed, when

compared with the PB trailer, the FD trailer had lower ceilings for the middle (91.4 vs. 111.8 cm), bottom (96.5 vs. 111.8 cm), and upper (96.5 vs. 106.7 cm) decks, resulting in reduced room between the backs of the pigs and the deck ceiling, which prevents an adequate ventilation rate around the pigs. This was particularly the case in the lowest compartments which did not comply to the 30-cm minimum head room between the ceiling and the greatest point on the pig recommended by the European Commission to ensure adequate airflow in vehicles with natural ventilation (EU- SCAHAW, 2002). The lower deck height also reduced the distance between the data loggers and the pigs, which may be responsible for the increase of T and HR values. The DHR only varied between trailers before loading with greater (P = 0.007) values in the PB trailer (Tables 10). At loading, greater (P = 0.0002) HR and DHR were observed in the rear upper compartment in the FD trailer, whereas greater (P = 0.0002) HR and DHR were found in compartments 6 and 11 inside the PB trailer indicating less airflow at those locations (Table 11 and 12).

The THI was greater ($P \geq 0.003$) in the PB trailer during transport and at unloading (Table 11). Higher ($P \geq 0.003$) THI were recorded in compartments 6 and 11 of the PB trailer at loading and during transport and in compartment 3 of the FD trailer at loading (Table 11). These results would reflect the aforementioned variation in T and HR among compartments within each trailer. However, the THI recorded in this study was below the THI threshold (23.9°C) indicator of potential risk for heat stress in pigs (NWSCR, 1976; Lucas et al., 2000).

Behavioral Response

Loading

Loading and unloading are important sources of stress for pigs because of the reaction of pigs to environmental change and close human contact (Bradshaw et al., 1996; Chevillon, 2001; Edwards et al., 2010b). Aside from the tendency of pigs to move backwards (0.047 vs. 0.094 ± 0.017 occurrences/pig; P = 0.06; results not presented) during loading on the FD trailer, no differences in behavior were observed between the 2 trailers at loading. The greater occurrence of pigs loading backwards may be explained by the presence of a step at the entrance into the trailer resulting from the middle and upper moving decks being piled

up on the bottom deck floor during loading because these decks were the first to be loaded and then lifted up to allow the loading of the bottom deck. Height differences between the truck deck and loading ramp have been implicated as cause in the refusal of pigs to move forward (EU- SCAHAW, 2002). Crossbreed type had no effect on behavioral responses at loading, and load time did not differ between trailer types (4.2 vs. 4.3 ± 0.4 s/pig; $P = 0.90$; results not presented) or among the 50Nn, 50NN, and 25NN pigs (4.4, 4.4, and 4.0 ± 0.4 s/pig, respectively; $P = 0.53$; results not presented).

Unloading and lairage

Accounting for the number of pigs, the time taken to unload pigs from the PB trailer was longer ($P = 0.007$) than from the FD trailer (1.01 vs. 0.8 ± 0.01 s/pig; results not presented). Torrey et al. (2008) reported that unloading pigs from the PB trailer took greater time compared with unloading from a double-decked truck equipped with a moving upper deck. The longer unloading time was the result of greater difficulty in handling pigs exiting the truck because of the presence of internal ramps and a step between the trailer floor and the slaughter plant dock.

The greater difficulty in handling was reflected by the greater ($P = 0.007$) number of interventions (hand taps) on pigs unloaded from the PB trailer compared with those unloaded from the FD trailer (0.65 vs. 0.37 occurrences/pig; results not presented). There were interactive effects of trailer type and crossbreed type for overlaps ($P < 0.001$; Figure 8) and slips and falls ($P < 0.01$; Figure 9), with 50NN and 25NN pigs overlapping more than 50Nn pigs and 50Nn and 50 NN pigs slipping and falling more than 25NN pigs at unloading from the FD trailer compared with the same crossbreeds in the PB trailer. The greater frequency of overlaps and slips and falls was observed when unloading compartment 3, which was located on the top floor of the FD trailer, which confirms the effect of the presence of the step on the bottom deck floor previously observed at loading. No difference in other behaviors was observed among crossbreeds or trailer types at unloading.

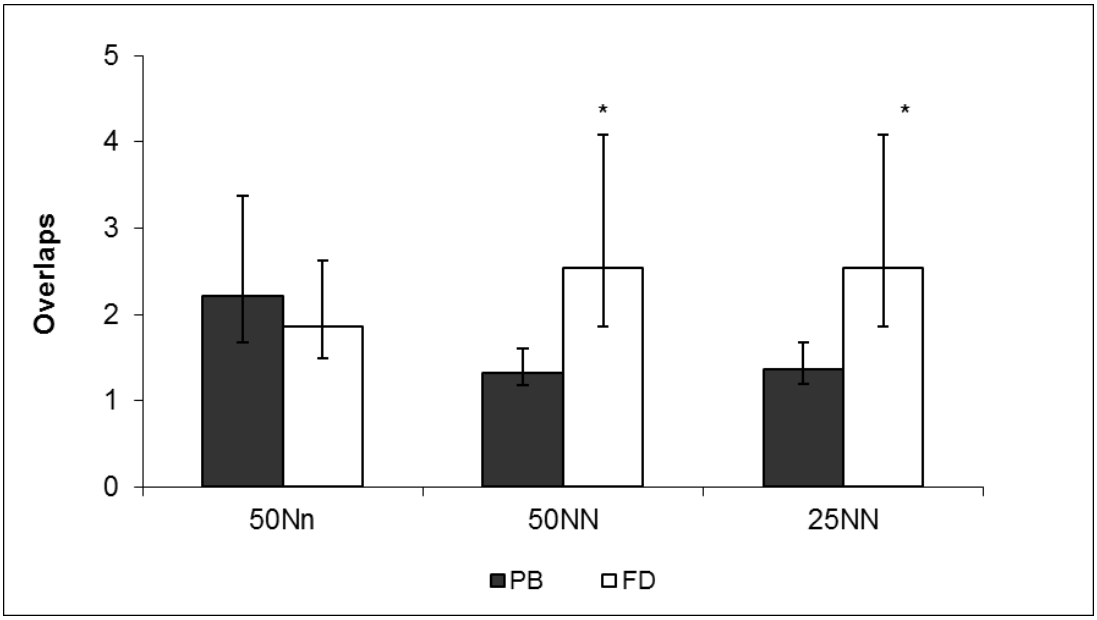


Figure 8. Overlaps (back-transformed least square means with confidence interval) during unloading among 50% Pietrain-cross pigs with the halothane $(HAL)^{Nn}$ genotype (50Nn), 50% Pietrain-cross pigs with the HAL^{NN} genotype (50NN), and 25% Pietrain-cross pigs with the HAL^{NN} genotype (25NN) after transportation in either a pot-belly (PB) or flat-deck (FD) trailer. Within a crossbreed, bars with an asterisk (*) indicate greater ($P < 0.001$) overlaps.

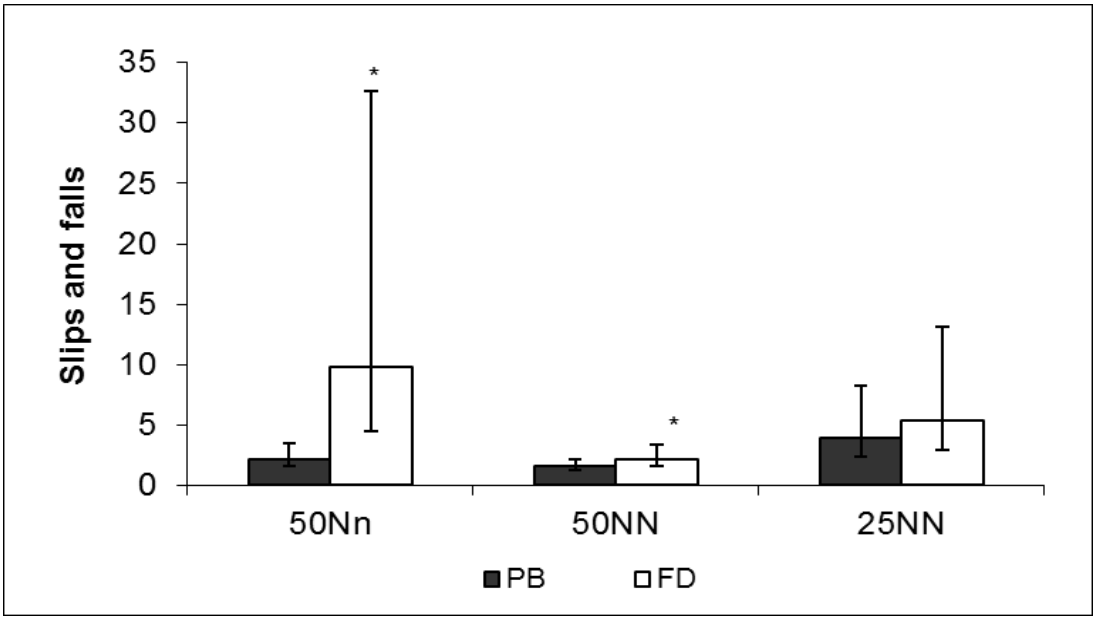


Figure 9. Slips and falls (back-transformed least square means with confidence interval) during unloading among 50% Pietrain-cross pigs with the halothane $(HAL)^{Nn}$ genotype (50Nn), 50% Pietrain-cross pigs with the HAL^{NN} genotype (50NN), and 25% Pietrain-cross pigs with the HAL^{NN} genotype (25NN) after transportation in either a pot-belly (PB) or flat-deck (FD) trailer. Within a crossbreed, bars with an asterisk (*) indicate greater ($P < 0.01$) slips and falls.

During lairage, latency to rest was shorter ($P = 0.04$) for pigs unloaded from the PB trailer than the FD trailer (30 vs. 38 ± 3 min; results not presented). The difference in the time taken to lie down in the lairage pen between pigs transported in the PB and FD trailers may indicate either that 1) PB-transported pigs were more fatigued than the FD-transported pigs because of the physical exertion needed to negotiate the ramps, or 2) FD- transported pigs were more excited than PB-transported pigs because of the greater difficulty of handling (more overlaps and falls) at the exit of the FD trailer. Latency to rest did not differ between crossbreeds ($P = 0.68$).

Physiological Measurements

In response to heat stress, the sympathetic vasodilator system can increase blood flow in the skin, which, in turn, increases convective heat transfer from the core to the periphery (Charkoudian, 2003). For this reason, GIT temperature is considered an indirect indicator of the variation in metabolic rate of pigs in response to heat stress (Webb, 1995), which has an impact on pig physiology and pork quality (Hambrecht et al., 2005; Ritter et al., 2009). Similar to previous studies which reported increased GIT temperatures of pigs in response to loading and transportation (Carr et al., 2008; Tamminga et al., 2008), delta GIT temperature tended to be greater ($P = 0.06$) in PB-transported pigs compared with FD-transported ones at loading (13). This result is in agreement with previous findings (Tamminga et al., 2008) where different GIT temperatures were reported between pigs loaded on a PB trailer equipped with less steep (up to 19° lower slope) ramps and pigs loaded on a double-decked truck using hydraulic devices.

Overall, delta GIT temperatures progressively decreased from loading to unloading, with the most evident decline being recorded during transportation because of the effect of the ventilation. Pigs unloaded from the PB trailer presented greater ($P = 0.01$) delta GIT temperatures, probably because of the warmer and more humid environment inside this trailer type (Table 10). After unloading, GIT temperatures increased until prestunning and tended to be greater ($P = 0.06$) in pigs unloaded from the PB trailer. This result would

indicate that PB pigs were still under the effects of transport and unloading stress at the time of slaughter. Delta GIT temperatures did not differ between crossbreed types. However, when compared with 50Nn and 50NN pigs, the decrease in GIT temperature tended to be pronounced in 25NN pigs during transport (P = 0.09). The greater GIT in crossbreeds with the 50% Pietrain crossbreeding may reflect the difficulty of this breed to dissipate heat under humid (greater RH values) conditions.

Lactate, CK, and cortisol concentrations are often used as stress indicators and predictors of meat quality variation in pigs (Doizé et al., 1989; Fàbrega et al., 2004; Edwards et al., 2010a). Blood CK and lactate concentrations are indicators of physical stress (Bickhardt and Wirtz, 1987; Broom, 1996), whereas the variation in cortisol concentrations in blood generally indicates a fear/arousal response caused by psychological stressors (Knowles and Warriss, 2000). There were no ($P \geq 0.10$) interactive effects of trailer type and crossbreed on any blood constituent measured; therefore, data were pooled across treatments and only the effects of trailer type and genotype are presented in Table 14. There was a trend for plasma lactate concentrations to be greater (P = 0.07) in pigs transported in the FD trailer, whereas serum cortisol concentrations were increased (P=0.02) in PB-transported pigs. On the basis of the relatively rapid speed of plasma lactate to return to basal concentrations after physical exercise (2 h; Benjamin et al., 2001), the greater lactate in blood at slaughter may reflect the increased behavioral response of pigs from the FD trailer compared with those from the PB trailer. Because blood cortisol concentrations can be an indicator of long-term stress response (Nyberg et al., 1988; Bradshaw et al., 1996), greater serum cortisol concentrations at slaughter in PB-transported pigs may indicate their incomplete recovery from the additive effect of the handling and transportation stressors.

Table 13. Delta gastro-intestinal tract temperature (Delta GIT)[1] observed during each period of transportation in each pig crossbreed[2] within the pot-belly (PB) and flat-deck (FD) trailers.

	Trailer			Crossbreed					
	PB	FD		50Nn	50NN	25NN		P-value	
No.	58	60	SEM	38	42	38	SEM	Trailer	Crossbreed
Loading	0.18	0.06	0.04	0.11	0.17	0.08	0.05	0.06	0.48
Transport	-0.26	-0.33	0.06	-0.25	-0.21	-0.42	0.07	0.38	0.09
Unloading	-0.24[a]	-0.50[b]	0.09	-0.29	-0.33	-0.48	0.11	0.01	0.24
Lairage	-0.15	-0.28	0.08	-0.24	-0.17	-0.25	0.09	0.15	0.67
Prestunning	-0.11	-0.28	0.08	-0.23	-0.10	-0.25	0.09	0.06	0.34

[a,b] Within a row and main effect, least squares means lacking a common superscript letter differ, $P < 0.05$.

[1]The difference in GIT temperatures values within treatments (GIT temperature at each determined event – GIT temperature at the rest level)

[2]50Nn = 50% Pietrain inheritance with HAL^{Nn} genotype; 50NN = 50% Pietrain inheritance with HAL^{NN} genotype, and 25NN = 25% Pietrain inheritance with HAL^{NN} genotype.

Similar to Fàbrega et al. (2002), the presence of the HAL gene affected serum CK concentrations at slaughter, with concentrations being greater ($P < 0.001$) in 50Nn pigs compared with 50NN and 25NN pigs (Table 14). Additionally, plasma lactate concentrations were greater ($P = 0.003$) in 50Nn and 50NN than in 25NN pigs. These results would indicate that pigs with a greater percentage of Pietrain crossbreeding were generally more fatigued at slaughter, whereas the increased serum CK concentrations, typically associated with muscle damage and fatigue, were more pronounced in pigs carrying the HAL gene. Similar to a number of previous studies (Geers et al., 1994, Yoshioka et al., 2004; Breinekova et al., 2007), no effect of the HAL gene was found on serum cortisol concentrations at slaughter in this study.

Previous studies (Saco et al., 2003; Salamano et al., 2008; Averós et al., 2009) showed an increase in concentrations of acute phase proteins (haptoglobin and Pig-MAP) in blood, reflecting the occurrence of an inflammatory response caused by tissue injury, damage, or trauma (Baumann and Gauldie, 1994; Lampreave et al., 1994; Heegard et al., 2011) in response to transport stress. In the present study, neither trailer design ($P = 0.29$) nor crossbreed ($P = 0.18$) affected the serum concentrations of either acute phase protein at exsanguination. The explanation for this lack of effect can be 2-fold. First, the length of the preslaughter period may not have been sufficiently long enough to cause a significant variation of blood acute phase proteins. In fact, Piñeiro et al. (2007) reported only a moderate increase in acute phase proteins after 12 h of transportation and 6 h of lairage. Second, similar to previous studies (Piñeiro et al., 2007; Salamano et al., 2008), the effects of trailer type and crossbreed on the variations in serum acute phase proteins in blood at slaughter may have been confounded by other stressful preslaughter conditions which were not controlled in the present study.

Table 14. Concentrations of plasma lactate and serum creatine kinase (CK), cortisol, and the acute phase proteins (Pig-MAP and haptoglobin) of 3 crossbreeds of pigs transported in pot-belly (PB) and flat-deck (FD) trailers[1].

	Trailer			Crossbreed					
	PB	FD		50Nn	50NN	25NN		*P*-value	
No.	72	72	SEM	48	48	48	SEM	Trailer	Crossbreed
Plasma lactate, mmol/L	21.25	23.75	0.95	23.84[a]	24.74[a]	18.94[b]	1.16	0.07	0.003
Serum CK, log	3.40	3.43	0.05	3.73[a]	3.30[b]	3.22[b]	0.06	0.50	<0.001
Serum cortisol, ng/mL	29.34 [a]	25.40 [b]	2.93	26.97	29.42	25.73	3.05	0.02	0.20
Serum Pig-MAP, mg/mL	1.46	1.35	0.08	1.48	1.35	1.40	0.09	0.63	0.33
Serum haptoglobin, mg/mL	1.96	2.04	0.25	2.07	1.72	1.96	0.20	0.29	0.18

[a,b] Within a row and main effect, least squares means lacking a common superscript letter differ, $P < 0.05$.
[1]50Nn = 50% Pietrain inheritance with HAL^{Nn} genotype; 50NN = 50% Pietrain inheritance with HAL^{NN} genotype, and 25NN = 25% Pietrain inheritance with HAL^{NN} genotype.

Carcass and Meat Quality Traits

Dressing percentages ($P = 0.01$) and carcass lean yields ($P < 0.001$) were greater in 50Nn pigs than 50NN and 25NN pigs (Table 15). The effect of the HAL gene on these carcass traits has been reported in previous studies (Monin et al., 1981; Zhang et al., 1992; Gispert et al., 2007). An interaction was found between trailer and crossbreed type for skin damage and fighting-type bruise scores, with 50Nn pigs transported on the PB and 50NN pigs transported on the FD trailer showing a greater skin damage score ($P = 0.05$). Bruises observed on the carcasses of these pigs were mostly of fighting type ($P = 0.006$). Since no difference in the activity rate (latency to rest) was found between crossbreeds in lairage, it may be assumed that, in the case of 50Nn pigs transported on the PB trailer, the greater number of bites may have originated from fighting occurring in the PB trailer during the wait at the farm before departure. As groups were kept intact (no mixing) in the trailer, the greater aggressiveness of 50Nn pigs transported on the PB trailer may be related to the excitement caused by the stress of negotiating ramps. Although the greater skin damage score in 50NN carcasses may be explained by the greater frequency of overlaps and slips and falls at unloading, the greater number of fighting-type bruises in FD-transported pigs is hard to explain.

Except for drip loss in the LM muscle, which tended to be greater ($P = 0.08$) in pigs transported in the PB trailer, meat quality was not affected by trailer type (Table 16). The greater drip loss in pigs transported on the PB trailer may result from the physical effort of live pigs to negotiate internal ramps. In agreement with previous studies (Leach et al., 1996; Pommier et al., 1998; Salmi et al., 2010), the HAL gene had a detrimental effect on pork quality as the LM from 50Nn pigs had lower ($P < 0.001$) pHu and greater electrical conductivity and drip loss percentages ($P < 0.001$) than the LM from 50NN and 25NN pigs. Furthermore, the SM from 50Nn pigs was paler (greater L* values; $P = 0.02$) than the SM of 50NN and had greater electrical conductivity ($P = 0.009$) and drip losses ($P = 0.002$) than the SM from 50NN and 25NN pigs. The pHu value was greater ($P < 0.001$) in the AD muscle of 50Nn and 50NN pigs, but did not exceed the pHu threshold of 6.3, which, when exceeded in red muscles such as the AD, is indicative of DFD pork (Barton-Gade et al., 1996a).

Table 15. Carcass quality traits and skin bruises of 3 pig crossbreeds transported in pot-belly (PB) and flat-deck (FD) trailers[1].

	PB			FD					
Crossbreed	**50Nn**	**50NN**	**25NN**	**50Nn**	**50NN**	**25NN**		**P -value**	
								Trailer	**Crossbreed**	**Tr × Cb**
No.	61	60	60	59	60	60	**SEM**	**(Tr)**	**(Cb)**	
HCW, kg	96.39	96.63	95.80	96.85	96.48	96.26	0.66	0.34	0.15	0.58
Dressing percent	82.29	82.20	81.60	82.66	82.10	82.13	0.23	0.10	0.01	0.27
Fat thickness, mm	16.06	17.64	17.71	16.30	17.73	17.45	0.45	0.95	0.004	0.85
Muscle depth, mm	69.78	67.82	63.73	69.95	68.22	65.18	0.88	0.32	<0.0001	0.71
Lean yield, %	62.23	61.39	61.17	62.14	61.40	61.35	0.22	0.85	<0.001	0.83
Skin damage score [2]	2.16	1.89	1.98	1.79	2.09	1.87	0.12	0.31	0.79	0.05
Fighting-type bruise score [3]	1.10	0.79	1.03	0.72	1.07	0.85	0.10	0.21	0.94	0.006
Mounting-type bruise score [4]	0.54	0.32	0.35	0.33	0.48	0.38	0.89	0.98	0.74	0.13

[1] 50Nn = 50% Pietrain inheritance with HAL^{Nn} genotype; 50NN = 50% Pietrain inheritance with HAL^{NN} genotype, and 25NN = 25% Pietrain inheritance with HAL^{NN} genotype.
[2] 1 = none to 5 = severe (MLC, 1985)
[3] 1 = less than 10 bruises to 3 = greater than 21 bruises (ITP, 1996)
[4] 1 = less than 5 bruises to 3 = greater than 11 bruises (ITP, 1996)

Table 16. Quality characteristics of the LM, semimembranosus (SM), and adductor (AD) muscles of 3 pig crossbreeds transported in either a pot-belly (PB) or flat-deck (FD) trailers[1].

	Trailer			Crossbreed				*P*-value	
	PB	FD		50Nn	50NN	25NN			
No.	181	179	SEM	120	120	120	SEM	Trailer	Crossbreed
LM									
pHu	5.69	5.70	0.02	5.66[b]	5.71[a]	5.72[a]	0.02	0.47	< 0.001
L*	51.24	50.97	0.24	51.24	51.11	50.97	0.27	0.28	0.66
a*	8.21	7.97	0.10	8.39[a]	7.94[b]	7.95[b]	0.13	0.11	0.03
b*	5.40	5.26	0.08	5.52	5.25	5.23	0.09	0.19	0.06
EC[2]	5.49	5.45	0.28	6.33[a]	5.05[b]	5.03[b]	0.29	0.83	<0.001
Drip loss, %	4.20	3.80	0.15	5.10[a]	3.34[b]	3.57[b]	0.18	0.08	<0.001
SM									
pHu	5.76	5.76	0.03	5.77	5.79	5.73	0.03	0.86	0.10
L*	47.40	47.80	0.25	48.15[a]	47.20[b]	47.91[a]	0.28	0.72	0.02
a*	9.78	9.56	0.19	9.84[a]	9.74[a]	9.42[b]	0.21	0.09	0.02
b*	5.49	5.43	0.07	5.74[a]	5.30 [b]	5.35[b]	0.08	0.56	0.002
EC[2]	9.18	9.20	0.48	9.96[a]	9.02[b]	8.6[b]	0.50	0.96	0.009
Drip loss, %	2.83	2.97	0.13	3.25[a]	2.74[b]	2.71[b]	0.15	0.26	0.002
AD									
pHu	6.03	6.00	0.04	6.04[a]	6.09[a]	5.92[b]	0.04	0.49	<0.001

[a,b] Within a row and main effect, least squares means lacking a common superscript letter differ, $P < 0.05$.

[1]50Nn = 50% Pietrain inheritance with halothane $(HAL)^{Nn}$ genotype; 50NN = 50% Pietrain inheritance with HAL^{NN} genotype, and 25NN = 25% Pietrain inheritance with HAL^{NN} genotype.

[2]pHu = ultimate pH.

[3]EC = Electrical conductivity measured by the pork quality meter (PQM-I-INTEK, Classpro GmbH, Sielenbach, Germany).

CONCLUSIONS

Results of the present study indicated that genetics has a larger impact on animal welfare variables and pork quality traits than the trailer type used for long- distance transportation. The long transportation time may have masked the effect of trailer type, because pigs transported long distances with sufficient space allowance have time to acclimate and recover from the stress of pretransit loading (Pérez et al., 2002). However, results of this study also suggest that the design of both trailer types should be modified to improve the comfort of pigs during transportation, especially ventilation, and ease of pig handling when exiting the trailer (presence of steps and ramps). Increasing the proportion of Pietrain inheritance from 25 to 50% in crossbred pigs appears to cause pigs to be more responsive to stress, regardless of HAL genotype. However, the greater stress susceptibility associated with increasing the percentage of Pietrain does not appear to detrimentally affect pork quality unless the Pietrain-crossbred pig carries the HAL gene.

Chapter 4: Stress responses and carcass and meat quality variation in pigs of three Pietrain crosses transported by two different trailer types over a short distance. **(Published in the Livestock Science, 2013. 157: 234-244).**

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Effects of trailer design on animal welfare parameters and carcass and meat quality of three Pietrain crosses being transported over a short distance[1]

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ABSTRACT

The objective of this study was to evaluate the effects of trailer design on the stress responses and meat quality traits of 3 different pig crosses: 50% Pietrain breeding with HAL^{Nn} (50Nn), 50% Pietrain breeding with HAL^{NN} (50NN) and 25% Pietrain breeding with HAL^{NN} genotype (25NN). Market barrows (n=360), as a subset of 12 trailer loads of pigs, were transported from farm to slaughter on 6 dates in 2009 for 45 min in either a pot-belly (PB) or flat-deck (FD) trailer, with 120 pigs/genetic group being represented. Temperature (T) and relative humidity (RH) were recorded by data loggers mounted in both trailers. Behaviours and handler interventions were video-taped at loading, unload- ing and in lairage. At exsanguination, blood samples were collected for the assessment of lactate, cortisol, creatine kinase (CK), haptoglobin and Pig-MAP concentrations. Meat quality was measured in the longissimus dorsi (LD), semimembranosus (SM) and adductor (AD) muscles of all pigs. Temperatures were warmer in compartments 6 and 11 at loading ($P < 0.001$), compartment 11 during travelling ($P = 0.05$), and in compartment 5 at unloading ($P = 0.01$) of the PB trailer. Pigs unloaded from the FD trailer overlapped more ($P < 0.001$), whereas ($P < 0.001$) the frequency of jamming was noted for pigs unloaded from the PB trailer. Pigs with 50% Pietrain genetics overlapped and jammed more ($P < 0.001$) than pigs with 25% Pietrain genetics, regardless of HAL status. Greater ($P = 0.03$) CK levels were found in 50Nn pigs transported in the PB trailer, while 50Nn and 50NN pigs had greater ($P = 0.028$) lactate levels than 25NN pigs. Carcasses from 50Nn and 50NN pigs were leaner ($P = 0.04$), and the skin damage score was lower ($P = 0.04$) in 25NN carcasses. Overlap-type bruises were greater ($P = 0.02$) in pigs transported in the FD trailer. Pigs transported in the PB trailer had greater ($P = 0.05$) pHu in the SM and AD muscles ($P = 0.013$). Except for pHu in the SM muscle, all meat quality parameters were affected by the Hal gene ($P = 0.04$). The use of a PB trailer for short distance transportation of pigs to slaughter negatively affected stress responses and meat quality. The greater proportion of Pietrain genetics in the selection resulted in leaner carcasses, but also in pigs being more difficult to handle. Crossbreeding appeared to have a greater impact on animal welfare and meat quality than vehicle type, but trailer type may emphasize these negative genotype-related defects.

Key words: animal welfare, genotype, meat quality, pigs, transport, trailer type

INTRODUCTION

The pot-belly (PB) trailer is the most commonly used trailer for swine transportation in Canada, in large part because of its large load capacity (up to 220 animals per load) resulting in decreased transportation cost per animal. How- ever, this trailer design is raising some concern because of the increased difficulty of handling pigs at loading and unloading, resulting in longer load and unload times and higher incidence of animal losses and meat quality defects (Correa et al., 2013; Ritter et al., 2008; Torrey et al., 2013). These effects appear mostly due to the presence of multiple steep ramps and 1801 turns inside this trailer (Torrey et al., 2013). Considering the greater stress responsiveness of leaner and stress-susceptible pigs (Busse and Shea-Moore, 1999), these truck features may have a large impact when the PB trailer is used for the transport of Pietrain pigs with carrier Halothane (HAL) genotypes (Fàbrega et al., 2002, 2004b). According to Fàbrega et al. (2002, 2004b), HAL-free Pietrain crossbreds were less reactive to transport stress than the HAL carriers when using trucks equipped with hydraulic devices for loading and unloading. Given the expected increasing introduction of HAL-free Pietrain terminal sire lines into the North American pig population (Godbout, Genetiporc Inc., personal communication), there is a need to understand their responsiveness to the use of the most common trailers to help stakeholders to adapt the current management practices to the production of these crossbreds. Based on the results of a previous study (Weschenfelder et al., 2012), Pietrain genetics had a larger impact on animal welfare parameters and pork quality traits than the trailer type when used for long-distance transportation. It appeared that long transportation time had masked the effect of trailer type, because pigs transported long distances under controlled transport conditions (sufficient space allowance) have time to acclimate and recover from the stress of pre-transit loading. However, the effects of PB trailer design on the response to transport stress and meat quality of these genotypes can be emphasized under short distance conditions. Short transpor- tation is generally considered to be more critical than longer durations as pigs have insufficient time to recover from loading stress, resulting in increased risk of animal losses and PSE (pale, soft, exudative) pork (Haley et al., 2008; Pérez et al., 2002). The objective of this study was to determine the effects of trailer design on the physiological and behavioural responses to transport stress, and on the carcass and meat quality variation in pigs of 3 Pietrain crosses.

MATERIAL AND METHODS

All experimental procedures performed in this study were approved by the AAFC Animal Care Committee in Sherbrooke (Quebec, Canada) based on the current guide- lines of the Canadian Council on Animal Care (2009).

Animal management

A total of 360 barrows (BW=115±5 kg) were evalu- ated as a subset of 12 trailer loads (6 loads/trailer and each load being a replicate) of market pigs transported on 6 dates in June and September (3 dates/month) of 2009. All pigs were transported on the same day for 45 min (44 km) from the same commercial growing–finishing farm to the same slaughter plant located in Eastern Canada using 2 types of trailers, a 3-decked pot-belly trailer equipped with 2 internal ramps or a 3-decked flat-deck trailer with hydraulic decks and no internal ramps (Fig. 10). Pigs were progenies from crosses of Pietrain homozygous halothane recessive (HAL^{nn}), Pietrain homozygous halothane domi- nant (HAL^{NN}) and Duroc Pietrain (HAL^{NN}) sire lines mated to homozygous halothane dominant F-20 sows (Genetiporc Inc., St.-Bernard, Canada) resulting in 3 genetic groups: 50% Pietrain crossbreeding with HAL^{Nn} genotype (50Nn), 50% Pietrain crossbreeding with HAL^{NN} genotype (50NN) and 25% Pietrain crossbreeding with HAL^{NN} genotype (25NN).

Transportation treatments

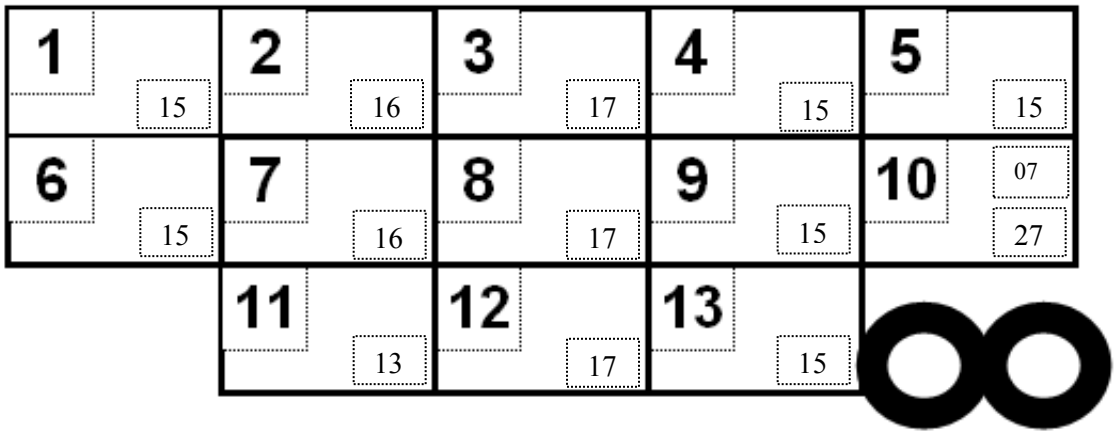
Transport groups were prepared 5 days before loading and kept in separate finishing pens (20 pigs/pen) by crossbred and trailer type. In order to avoid mixing of pigs from different pens inside the trailer, the group size in each pen was the same as that of the truck compartment. The PB trailer used in this study transported 220 pigs on the 3 decks distributed in 13 compartments (5 in the upper and middle decks and 3 in the belly; Fig. 10a). Pigs were loaded in groups of 3–4 pigs through an external ramp and had to climb an internal ramp (22º slope) to reach the upper deck or to descend an internal ramp (41º slope) to reach the belly compartments. Pigs only used the external ramp to go to the compartments in the middle deck. The FD trailer had a loading capacity of 235 pigs distributed in 3 decks composed of 8 compartments (3 on upper and middle, and 2 in the bottom deck; Fig. 10b). Each trailer was loaded to its full capacity and test pigs were distributed into 3 separate compartments (5, 6, and 11 in the PB trailer and 3, 4, and 7 in the

FD trailer), in terms of 1 compartment per crossbreed, at an average loading density of 0.43 m^2/pig. Within the PB trailer, the height of compartments was 106.7 cm for com- partment 5 and 111.8 cm for compartments 6 and 11. Inside the FD trailer, the height of compartments was 91.5 cm for compartment 4 and 96.5 cm for compartments 3 and 7. The test compartments in the PB trailer were chosen as they showed to be the most detrimental to meat quality and animal welfare in previous studies using a similar PB trailer model (Correa et al., 2013 Tamminga et al., 2009; Torrey et al., 2013).

Within the FD trailer, test compartment locations were selected to ensure a reliable comparison between genotypes in the response to transport stress in the 2 trailers. To replicate the same loading density applied to the PB trailer, compartments 3, 4, and 7 of the FD trailer were adjusted in size and subdivided into sub-compartments by a wooden panel (Fig. 10b). Side-slats were used in both trailers according to the external temperature thresholds following the NPB (2008) recommendations keeping the porosity settings consistent in each trailer.

Both trailer types were loaded early in the morning, starting at 0145 h. As the same loading quay was used for loading at the farm, the loading order between vehicles was rotated every week to avoid the effect of the time of the day (light and temperature) on truck microclimate and pig responses to handling at loading. A rotation of the group position in each trailer according to the pig crossbred was done at every load to avoid the confounding effect of the truck compartment on the pig responses to transportation. This compartment rotation allowed each genetic group to be transported twice in each test com- partment in each trailer. Due to logistical constrains, a rotation of time of departure from the farm was also applied, in that the first trailer loaded each week had to wait at the farm for 1 h before leaving to the slaughter- house, so as to respect a 30 min delay between arrival at the plant and avoid waits before unloading for both trailers. Feed was withdrawn from all pigs 9 h before loading (19 h from last feed to slaughter) and was loaded on each trailer using paddles and boards.

Within each genetic group, trailer type and load, 4 pigs were randomly chosen for the study of the physiological response and these pigs plus another 6 pigs were selected for the meat quality assessment (10 pigs/crossbreed/trailer type/load). The driver of each trailer and the handler at the farm were the same for each of the 6 transportation dates.



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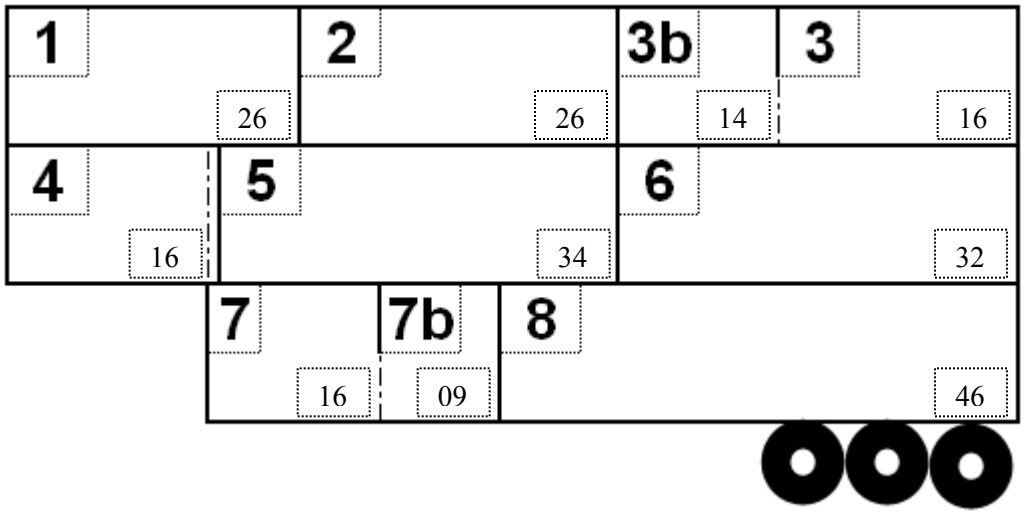

Figure 10. The location of compartments and distribution of pigs by compartment in the (a) pot-belly (PB) and (b) flat-deck (FD) trailers.

On arrival at the plant, pigs were unloaded using a whip only and driven to separate lairage pens based on the transport compartment (no mixing). After a 90 min lairage period, pigs were electrically stunned (head-to-chest electrical stunning) and exsanguinated in the prone position.

Trailer Temperature and Humidity

The temperature (T) and relative humidity (RH) in each trailer compartment was monitored using data loggers (i-Button, Dallas Semiconductor, Maxim Integrated Products,

Sunnyvale, CA) consisting of a 17 6 mm^2 stainless steel can containing sensory and storage equipment. The logger had a temperature range of -20 to +85 ºC, with an accuracy of 70.5 ºC and RH range of 0–100%, with sensitivity of ±0.6%. Five loggers were securely mounted approximately 6 cm below the ceiling of each selected compartment. One logger was positioned in the center of the compartment, and the remaining 4 were placed 15 cm from the midpoint of each wall. The T and RH of air outside of the truck were also recorded using 2 similar loggers mounted on the side-mirrors of each tractor. Data, recorded once each minute, were downloaded from the loggers after each trip and included T and RH from just before loading at the farm until the last pig exited the trailer at the abattoir. Occurrence of key events (start of loading, waiting at the farm, start of trip, and start and end of unload) were noted and identified in the string of T and RH data. The data were later imported into Microsoft Excel (version 2002) for analysis. The temperature humidity index (THI) of each trailer was calculated according to the NRC (1971) formula: THI = [1.8 x T+32] - [0.55 - (0.0055 x RH)] x [1.8 x T - 26] as the results are given in degrees Fahrenheit. The data were then transformed in degrees Celsius by the formula (ºF - 32) x 5/9 = ºC. Delta temperature (DT) and delta humidity ratio (DHR) values for each trailer and its compartments were calculated using trailer T and RH data. Using the recorded T and RH data, the humidity ratios (HR) were calculated as the mass of water vapour per unit mass of dry air in g/kg. Using HR to represent the quantity of water vapour in air was preferred, because these data are independent of T, unlike RH. The DT and DHR values were obtained by calculating the difference in temperature and humidity ratio values between the air inside and the ambient air outside the trailer (Brown et al., 2011). The intra-trailer environmental indicators (T, DT, RH, DHR and THI) were evaluated during 5 different time periods: before loading (last hour before the start of loading), loading, waiting at the farm, trans- portation and unloading.

Behavioral Observations

Behavior at loading

Behaviour of pigs was recorded using 2 digital camcorders (DCR-HC48; Sony, Sony of Canada Ltd., Toronto, Canada) installed using 3 camera mounts overhead the loading ramp and the alley at the loading ramp. The cameras recorded all occurrences of pig behaviour

(Table 17) from a predetermined start gate at the farm alley until the trailer gate. The course was divided into 2 zones; zone 1 was the alley from the start gate until farm door and zone 2 was between the farm door and the end of the external ramp (trailer door). Total frequency of a given behaviour represented the sum of the behaviour frequencies in zones 1 and 2. Video recordings were analyzed by a trained observer using a handheld Psion Workabout (HC-110, Psion Inc., Mississauga, Canada) computer. The total time taken to move pigs from the starting gate through the door of the trailer was noted, as well as all occurrences of manipulator interventions needed to move the pigs (Table 18).

Behavior at unloading

Pigs were unloaded by compartment at the abattoir and driven into lairage pens segregated by truck compartment at a density of 0.54 m^2/pig. A digital camcorder (DCR- HC48; Sony, Sony of Canada Ltd., Toronto, Canada) was placed at the entrance of the lairage room, mounted on the side wall of the corridor, to record pig behaviour during unloading. The total time to unload each compartment was noted using a chronometer. The video recordings were analyzed by a trained observer who counted all occur- rences of slipping/falling, overlapping, turning around, backing up, going backwards, underlapping, vocalization, balks and jamming (Table 17). The total time taken to unload pigs from the trailer until the lairage pen was noted as well as handler interventions (Table 18).

Table 17. Ethogram of pig behavior during loading and unloading (adapted from Brown, 2009).

Pig behavior	Description
Slip/fall	Pig's leg splits away from the other legs or pig falls down (at least 2 legs buckled under)
Overlap	Pig mounts another pig, with its 2 front legs on the back of the other pig
180° turn	Pig makes a 1801 turn, ending with its rear extended in the direction of intended movement
Back up	Pig moves at least 2 steps rearward, opposite the direction of intended motion
Backwards	Pig moves in the intended direction with its body oriented in the opposite direction
Underlap	Pig's head goes under the body of another pig
Vocalize	Pig vocalizes
Balk	Pig refuses to walk or stops for greater than 2 s
Jamming	Pig is squeezed at the door, corridor, or at the exit of the ramp (or at the trailer door, when unloading)

Table 18. Handler behavior towards pigs during loading and unloading.

Handler behavior	Description
Vocal sound	Handler uses his voice to encourage forward movement of 1 or a group of pigs
Rattle noise	Handler uses the paddle to produce noise
Physical intervention	Handler uses his hands, paddle, or board (or whip at unloading) to encourage the forward movement of 1 or a group of pigs

Behavior at lairage

Behaviour during lairage was recorded using video cameras (WV-BP50, Panasonic Canada Inc., Mississauga, Canada) installed overhead the pens and connected to a digital encoder (Nextiva S5712e, Verint, Melville, NY). Videos were captured and recorded using the Omnicast system (version 4.0; Genetec Inc., Montréal, Canada) at a frequency of 5–7 images/s. Scan sampling was used at 2-min intervals to determine the number of pigs lying (Lehner, 1996). To assess the recovery rate of the pigs after transport, the total time necessary for 60% of the pigs within the same pen to lie down (latency to rest) was determined from video recordings. As pigs were showered for the first 10 min of lairage in the pen, the latency to rest was calculated starting from the end of showering to avoid the confounding effect of this practice on pig behaviour (Weeding et al., 1993).

Physiological Measurements

At exsanguination, 10 mL of blood was collected in a tube (BD Vacutainers, VWR International Ltd., Montreal, Canada) to extract serum for creatine kinase (CK), cortisol, and acute phase proteins (haptoglobin and Pig-MAP) analysis. Another 2 mL of blood was collected in a tube containing 3.0 mg of sodium fluoride and 6.0 mg of Na2EDTA solution to extract plasma for lactate analysis. The 2 mL blood tubes were immediately centrifuged at 4 ºC for 12 min at 1400g. Plasma was transferred into 1.5 mL Eppendorf tubes and stored at -80 ºC until lactate determination. Serum samples were kept at room tem- perature (~23 ºC) for 1 h before refrigeration at 4 ºC. The following day, serum samples were centrifuged at 4 ºC for 12 min at 1400g, transferred to 1.5 mL Eppendorf tubes, and stored at -80 ºC until analysis. Lactate levels were measured using a commercially available kit (Lactate Assay Kit, Biomedical Research Service Center, University of Buffalo, Buffalo, NY) and CK levels were determined with creatine kinase-sl kit (Creatine Kinase-SL Assay

of Chemi- cals Diagnostic Limited, Vancouver, Canada). Plasma lactate concentrations were determined with a microplate reader and serum CK concentrations were determined with a spectrophotometer. The quantitative determination of serum cortisol was made using a commercial kit (DRG Cortisol Enzyme Immunoassay kit, DRG Instruments GmbH, Marburg, Germany), with a microplate reader and expressed as ng/mL. Serum haptoglobin levels were quanti- fied by a spectrophotometric method (hemoglobin-binding assay) and performed on an automated analyzer (AU400; Olympus, Hamburg, Germany). Serum Pig-MAP levels were assessed with an ELISA kit (PigCHAMP ProEuropa, Segovia, Spain) with a microplate reader and expressed as mg/mL. The intra-assay CV was 5.04%, 3.99%, 4.05%, 1.9%, and 8.5% for plasma lactate, serum CK, plasma cortisol, haptoglobin and Pig-MAP, respectively.

Carcass Quality Measurements

After slaughter, carcasses were eviscerated, split, and chilled according to standard commercial practices. Hot carcass weight (HCW) and carcass lean percentage (by Destron optical probe) were recorded, and HCW was used to calculate the dressing yield percentage. Skin damage was assessed on the day of slaughter in the cooler using a photographic scale ranging from 1 (none) to 5 (severe; MLC, 1985). Bruises were also classified as fighting-type bruises (1 = less than 10 bruises; 2 = 10–20 bruises; and 3 = greater than 20 bruises) or overlap-type bruises (score 1 = less than 5 bruises; 2 = 5–10 bruises; and 3 = greater than 10 bruises) by visual assessment of shape and size according to the photographic standards of the Institut Technique du Porc (ITP, 1996) as described by Faucitano (2001). According to the ITP scale, bruises due to biting during fighting are recognized as being 5–10 cm in length, comma shaped, and concentrated in high number in the anterior (head and shoulders) and posterior (ham) regions of the carcass. Long (10–15 cm), thin (0.5–1 cm wide), and comma shaped bruises are assumed to be caused by the fore claws (due to climbing of a pig over the back of another pig) in high density situations and were classified as overlap-type bruises. In order to study the bruises inflicted in the ante-mortem period, only red or “fresh” bruises were considered in these evaluations.

Meat Quality Measurements

Ultimate pH (pHu) was measured in the longissimus dorsi (LD) muscle at the 3rd/4th last

rib level, and in the semimembranosus (SM; middle region) and adductor (AD) muscles at 24 h post-mortem. Measures were taken on the left side of the carcass using a pH metre (Oakton Instru- ments Model pH 100 Series, Vernon Hills, IL) fitted with a Cole Palmer spear type electrode (Cole Palmer Instrument Company, Vernon Hills, IL) and an automatic temperature compensation (ATC) probe. After 30 min of blooming time, LD and SM muscles had visual colour evaluated at the same anatomical locations as for pH measurement using the Japanese colour standards (Nakai et al., 1975), whereas instrumental colour (L^n, a^n, and b^n values) was measured with a Minolta Chromameter (CR-300; Minolta Canada Inc., Mississauga, Canada) equipped with a 25-mm aperture, 01 viewing angle and D65 illuminant. Electrical conductivity was assessed by inserting the pork quality meat probe (PQM-I-INTEK, Classpro GmbH, Sielenbach, Germany) into the same 3 sites where muscle cores were removed from the LM and SM chops. Drip loss was measured in the LD and SM muscles using the modified EZ-driploss method as described by Correa et al. (2007). Briefly, 3 25-mm- diameter cores were removed from the center of 2.5-cm- thick LM (removed at 3rd/4th last rib level) and SM chops, weighed, and placed into plastic drip loss containers (Christensen Aps Industrivaengetand, Hilleroed, Denmark), before being stored for 48 h at 4 ºC. At the end of the 48-h storage period, muscle cores were removed from their container, surface moisture was carefully dabbed, cores were reweighed, and drip loss percentage was calculated by dividing the difference between initial and final core weights by the initial core weight.

Statistical Analysis

Data were analyzed by the mixed model procedure of SAS (version 2.0.3; SAS Inst., Inc., Cary, NC) for repeated measures with treatments in a 2 3 factorial arrangement, with 2 trailer types (PB vs. FD) and 3 crossbreeds (50Nn, 50NN, and 25NN). Trailer type and pig crossbreeds were included in the model, as well as their interaction, as fixed effects. The means were calculated using the “lsmeans” option of the mixed model procedure (PROC MIXED) in SAS. Differences in the mean values between the levels of a factor were tested using a Tukey–Kramer correction for multiple comparisons. Behaviour data not suitable for ANOVA analysis, such as those registered at loading and unloading, were analyzed using the log-normal distribution model (Poisson). A probability level of $P < 0.05$ was chosen as the limit for statistical significance in all tests. Whereas, means with probability levels of $P \leq 0.10$ were considered a tendency.

RESULTS AND DISCUSSION

Environment temperature and humidity variation inside the trailer

Ambient temperature during transport ranged from 3.3 to 24.6ºC and the average was 11.2ºC. The T did not differ between trailer types in any observed period (Table 19). The DT was greater ($P < 0.008$) in the PB trailer before travelling indicating a warmer interior environment in this vehicle. Before loading, RH was greater ($P = 0.01$) in the FD trailer, whereas the DHR was greater ($P = 0.03$) in the PB trailer during unloading. No difference in THI was found between trailer types in any period. However, the overall THI values recorded in this study were below the threshold (23.9ºC) considered as conducive to heat stress in pigs (Lucas et al., 2000; NWSCR, 1976).

In the PB trailer, T was greater ($P < 0.001$) in compartments 6 and 11 at loading, in compartment 6 before departure from the farm and in compartment 11 during travelling ($P = 0.03$ and $P = 0.05$, respectively; Table 20), and in compartment 5 during unloading ($P = 0.01$). Similarly, DT values were greater ($P < 0.001$) in compartments 6 and 11 at loading, and in compartment 11 during travelling ($P < 0.05$; Table 21). The higher temperature values at these locations reflect the poor air flow in the front middle and belly compartments of the PB trailer which was also previously reported by Brown et al. (2011) and Lenkaitis et al. (2008). RH was greater ($P < 0.03$) in compartments 6 and 11 at loading, in compartment 11 during transport and in compartment 5 at unloading (Table 20).

Table 19. Actual (T) and delta temperatures (DT)[a], relative humidity (RH) and delta humidity ratios (DHR)[b], and temperature humidity index (THI)[c] inside the pot-belly (PB) and flat-deck (FD) trailer at the time periods of preloading, loading, pre-transport, transport, and unloading (n = replicates).

	Preloading	Loading	Pre-transport	Transport	Unloading
T					
PB	10.7	13.3	19.2	19.3	15.0
FD	11.9	13.1	15.0	18.6	14.9
SED	0.58	0.99	3.34	0.65	0.58
P	0.06	0.88	0.24	0.21	0.93
DT					
PB	-0.03	2.5	6.1	4.7	5.2
FD	0.8	2.1	2.4	2.6	5.0
SED	0.47	0.79	0.73	1.19	1.17
P	0.22	0.48	**0.008**	0.23	0.74
RH					
PB	78.76	75.69	65.14	71.50	71.90
FD	80.56	80.80	74.23	74.10	73.98
SED	1.93	2.18	8.54	1.62	2.71
P	0.35	**0.027**	0.31	0.11	0.45
HR					
PB	6.28	7.22	4.76	7.57	10.70
FD	6.91	7.57	3.90	7.85	10.20
SED	0.29	0.47	2.07	0.33	0.63
P	**0.011**	0.37	0.61	0.32	0.32
DHR					
PB	-0.15	0.76	1.46	1.14	2.12
FD	0.04	0.81	0.77	1.34	0.98
SED	0.27	0.28	0.45	0.30	0.48
P	0.38	0.89	0.23	0.47	**0.03**
THI					
PB	10.9	12.9	18.4	14.8	18.6
FD	12.4	13.0	14.7	14.7	18.3
SED	1.35	1.80	4.98	0.98	1.0
P	0.053	0.94	0.21	0.92	0.55

[a] The difference between variables measured inside the trailer and the ambient air outside the trailer.

[b] The difference between humidity ratio inside the trailer and the external environment.

[c] THI1⁄4[1.8 T+32]_[0.55_(0.0055 RH)] [1.8 T_26].

Calculated DT and DHR followed the same pattern of variation (Table 21). The higher HR and DHR values in compartments 6 and 11 in the PB trailer may also be the result of the afore-mentioned poor air flow in these locations, combined with the absence of air outlets in the front part of the trailer. The higher T, RH, DT and DHR in compartment 5 at unloading may be explained by the effect of the unloading order as this compartment was the last to be unloaded. The lack of ventilation in the rear of this trailer, combined with the longer waiting time to unload would have increased evaporative heat loss by the pigs leading to an increased RH. THI was greater ($P < 0.05$) in compartments 6 and 11 at loading, in compartment 6 before travelling and in compartment 11 during traveling (Table 20), These results reflect the afore-mentioned variation in T and RH between compartments. Higher RH values in the front of the FD trailer were reported by Lenkaitis et al. (2008). However, in this study no difference in any internal ambient variable between compartments was found at any observed period ($P > 0.05$) within the FD trailer (Tables 20 and 21).

Table 20. Actual temperature (T), humidity ratio (HR)[1] and temperature humidity index (THI)[2] variation between compartments within the pot-belly (PB) and flat-deck (FD) trailers during transport (n = 6 replicates)

	Pot-Belly					Flat-Deck				
Compartment n = 6	**5**	**6**	**11**	**SEM**	***P***	**3**	**4**	**7**	**SEM**	***P***
T										
Pre-loading	10.3	11.1	10.7	0.23	0.10	11.0	13.1	11.6	0.97	0.31
Loading	10.4[b]	14.7[a]	14.8[a]	0.61	<0.001	13.7	14.2	11.5	1.16	0.25
Pre-transport	18.3[b]	20.9[a]	18.5[b]	0.47	0.03	15.9	13.9	15.0	1.51	0.66
Transport	14.2[b]	14.5[b]	16.2[a]	0.53	0.05	14.1	14.9	15.7	1.02	0.56
Unloading	20.8[a]	19.3[b]	20.0[ab]	0.31	0.01	19.4	18.8	17.6	0.92	0.40
HR										
Pre-loading	6.10	6.47	6.26	0.16	0.28	6.71	6.99	7.02	0.42	0.84
Loading	6.36[b]	7.76[a]	7.53[a]	0.25	0.006	7.54	8.02	7.15	0.55	0.54
Pretransport	4.91	4.98	4.38	0.17	0.06	4.10	3.71	3.84	0.28	0.62
Transport	7.64[ab]	7.26[b]	7.80[a]	0.13	0.03	7.65	7.92	7.97	0.52	0.89
Unloading	12.02[a]	9.78[b]	10.30[b]	0.27	<0.001	11.36	9.37	9.79	0.67	0.13
THI										
Preloading	10.5	11.1	11.0	0.45	0.25	11.1	14.5	11.6	2.09	0.12
Loading	10.5[b]	13.5[a]	14.7[a]	1.55	0.01	13.7	14.0	11.2	2.47	0.33
Pretransport	17.8[b]	19.7[a]	17.7[b]	0.63	0.02	15.6	13.8	14.8	2.38	0.67
Transport	14.2[b]	14.4[b]	15.8[a]	0.80	0.04	14.1	14.7	15.5	1.52	0.53
Unloading	19.1	18.1	18.5	0.62	0.18	19.0	18.5	17.3	1.42	0.33

[a,b] Within a row and trailer type, least squares means lacking a common superscript letter differ ($P < 0.05$).
[1]The difference between humidity ratio inside the trailer and the external environment
[2] THI = [1.8 × T + 32] – [0.55 – (0.0055 × RH)] × [1.8 × T – 26]

Table 21. Delta temperature (DT)[1], and delta humidity ratios (delta DHR)[2] variation between compartments within the pot-belly (PB) and flat-deck (FD) trailers during transport (n = 6 replicates).

	Pot-Belly					Flat-Deck				
Compartment n = 6	**5**	**6**	**11**	**SEM**	***P***	**3**	**4**	**7**	**SEM**	***P***
DT										
Preloading	-0.4	0.4	-0.07	0.23	0.10	-0.2	2.0	0.5	0.97	0.31
Loading	-0.1[b]	4.2[a]	4.4[a]	0.61	<0.001	2.7	3.2	0.5	1.15	0.25
Pretransport	8.4[b]	11.0[a]	8.6[b]	0.47	0.03	6.3	4.2	5.4	1.51	0.65
Transport	4.5[b]	4.7[b]	6.4[a]	0.54	0.05	4.2	5.0	5.8	1.02	0.56
Unloading	6.0[a]	4.5[b]	5.2[ab]	0.31	0.01	2.5	1.9	0.7	0.92	0.40
DHR										
Preloading	-0.33	0.04	-0.17	0.15	0.28	-0.16	0.13	0.16	0.42	0.84
Loading	-0.1[b]	1.30[a]	1.07[a]	0.25	0.006	0.78	1.26	0.38	0.55	0.54
Pretransport	1.61	1.68	1.08	0.17	0.06	0.98	0.59	0.72	0.28	0.62
Transport	1.21[ab]	0.83[b]	1.37[a]	0.13	0.03	1.14	1.41	1.46	0.52	0.89
Unloading	3.44[a]	1.20[b]	1.73[b]	0.27	<0.001	2.17	0.19	0.60	0.67	0.13

[a,b] Within a row and trailer type, means lacking a common superscript letter differ (P<0.05).

[1] The difference between variables measured inside the trailer and the ambient air outside the trailer.

[2] The difference between humidity ratio inside the trailer and the external environment.

Behavioral Response

No difference in turning around, backing up, going backwards, underlapping, vocalization and balks between truck loads or genetic groups were found at any pre- slaughter event in this study.

Loading

An interaction was found between trailer type and crossbreed on the frequency of overlaps, with 50Nn pigs overlapping more than 50NN and 25NN pigs when loaded on the FD trailer (P = 0.038; Fig. 11), while there was no difference between pigs loaded on the PB trailer. The effect of the FD trailer type on pigs' welfare was also observed by a higher (P = 0.023) frequency of handler interventions using the voice (FD: 19.78 versus PB: 10.83±3.42; results not presented) to drive pigs forward, indicating a reduced ease of handling these pigs at loading.

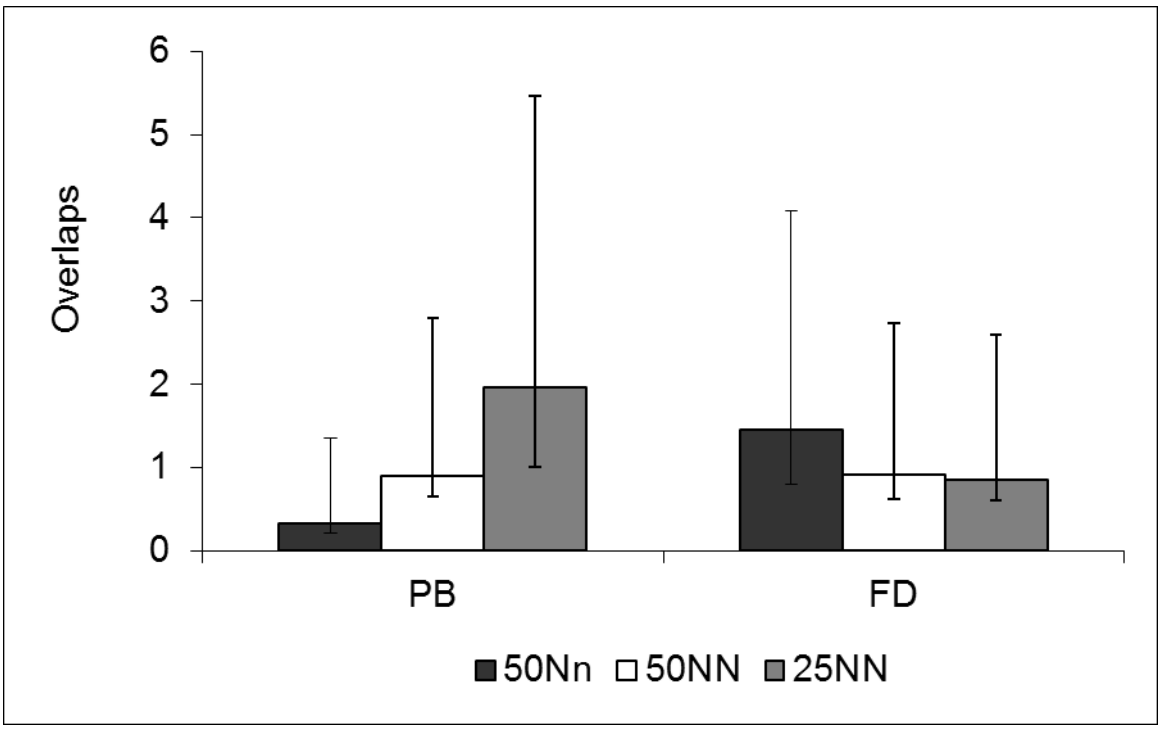

Figure 11. Frequency (%) of overlaps (back transformed least square means with confidence interval) during loading among 50% Pietrain-cross pigs with the HAL^{Nn} genotype (50Nn), 50% Pietrain-cross pigs with the HAL^{NN} genotype (50NN), and 25% Pietrain-cross pigs with the HAL^{NN} genotype (25NN) before transportation in either a pot-belly (PB) or flat-deck (FD) trailer. There was an interaction between trailer type and crossbred (P = 0.038).

The presence of a ramp to compensate for the height difference between the loading platform and the FD trailer bottom floor and the existence of a step right after the entrance into the trailer may explain the greater reluctance to move of Hal gene carriers resulting in

more overlaps. A similar behavioural response was observed in pigs being loaded on the same FD trailer model in a previous study (Weschenfelder et al., 2012). A greater (P = 0.027) use of rattle noise to load pigs on the PB trailer (FD: 0.63 [0.35-0.70] versus PB: 1.00 [0.52-1.01]/pig; results not presented) may have been caused by the difficulty of these pigs to manage going up and down the internal ramps to access or exit the upper and lower compartments. Ramps and steps are considered potential causes of fear and reluctance to move in pigs (Goumon et al., in press; SCAHAW, 2002; Warriss et al., 1991). As for the genotype, the negative effect of the HAL gene to handling was previously reported by Fàbrega et al. (2004a) who observed a lower level of fear, expressed as greater activity level, in HAL-free pigs.

Despite trailer and genotype effects on pigs' behaviour, no difference in loading duration was found between trailer types (PB: 1.0±0.1 versus FD: 0.9±0.1 s/pig; P = 0.19; results not presented) or among crossbreds (50Nn: 1.4±0.2; 50NN: 1.3±0.2; and 25NN: 1.6±0.2 s/pig; P = 0.26; results not presented).

Unloading and lairage

Pigs unloaded from the FD trailer overlapped more com- pared with pigs unloaded from the PB trailer (P < 0.001; Fig. 12), while jamming was greater (P o 0.001) in pigs unloaded from the PB than from the FD trailer (Fig. 13). The overlapping may have been caused by the step which also caused handling problems during loading into the FD trailer, while the jamming was most likely the result of PB pigs being forced to move from a group situation into single file through a laterally closed external unloading ramp. Crossbreeds con- taining a greater proportion of Pietrain genetics, with or without the HAL gene (50Nn and 50NN pigs), overlapped and jammed more compared with 25NN pigs (P < 0.001; Figs. 12 and 13). Both overlap and jamming incidences show thehigher responsiveness to stressful stimuli in crossbreeds with a higher percentage of Pietrain, regardless of the presence of the HAL gene.

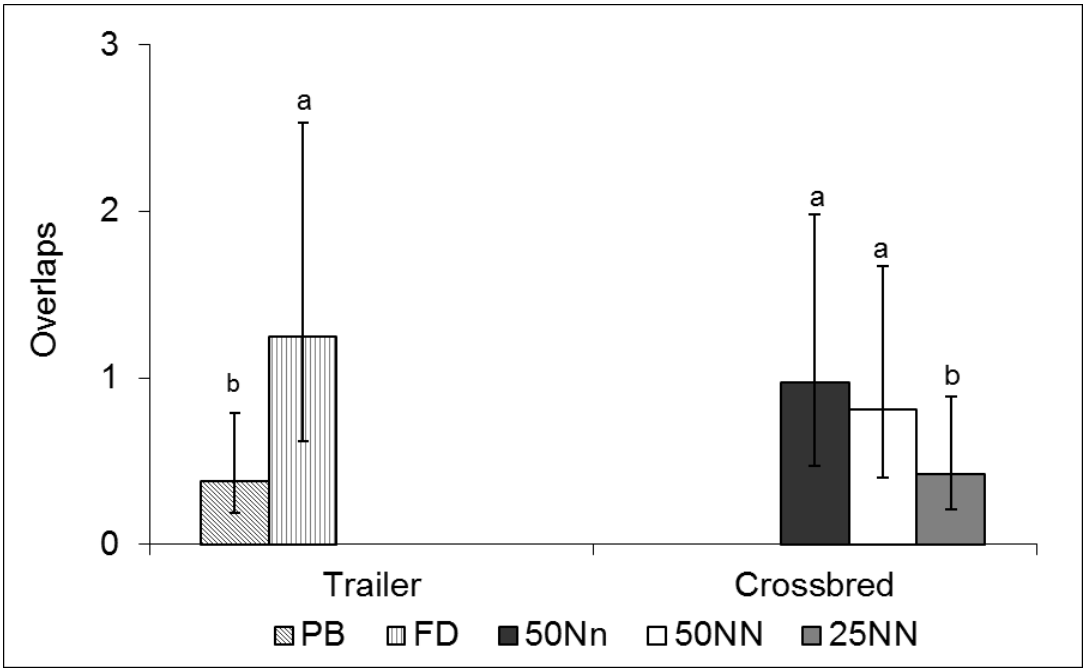

Figure 12. Frequency (%) of overlaps (back transformed least square means with confidence interval) during unloading among 50% Pietrain-cross pigs with the HAL^{Nn} genotype (50Nn), 50% Pietrain-cross pigs with the HAL^{NN} genotype (50NN), and 25% Pietrain-cross pigs with the HAL^{NN} genotype (25NN) after transportation in either a pot-belly (PB) or flat- deck (FD) trailer (within a trailer type and crossbred, bars lacking a common superscript letter differ ($P < 0.001$).

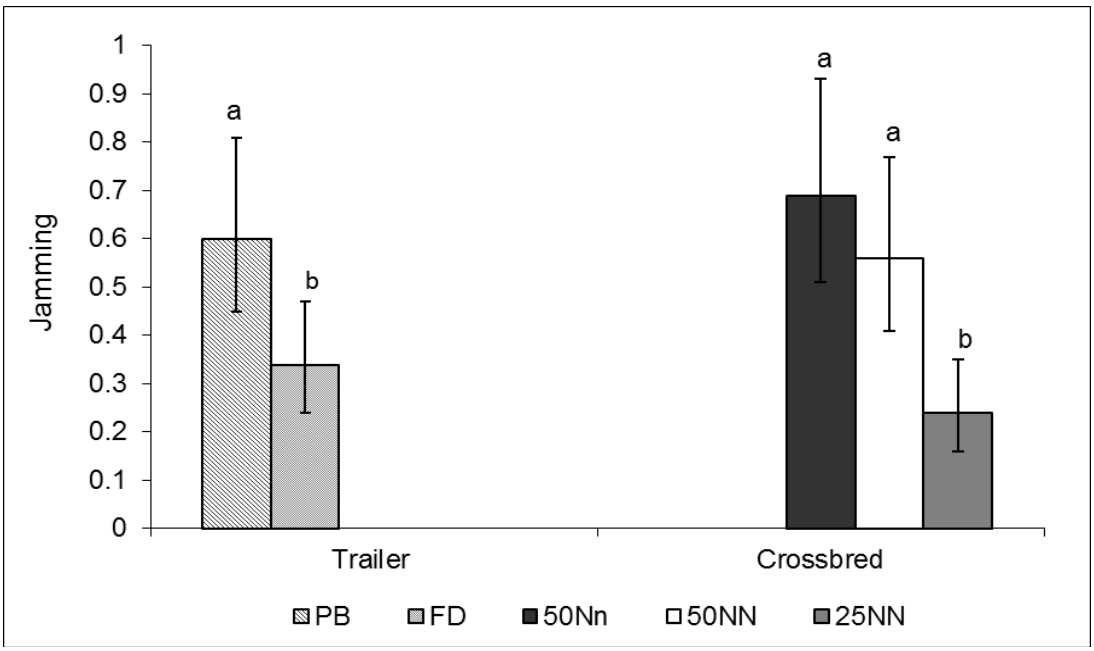

Figure 13. Frequency (%) of jamming (back transformed least square means with confidence interval) during unloading among 50% Pietrain-cross pigs with the HAL^{Nn} genotype (50Nn), 50% Pietrain-cross pigs with the HAL^{NN} genotype (50NN), and 25% Pietrain-cross pigs with the HAL^{NN} genotype (25NN) after transportation in either a pot-belly (PB) or flat- deck (FD) trailer (within a trailer type and crossbred, bars lacking a common superscript letter differ ($P < 0.001$).

Unloading duration was longer for the PB compared to the FD trailer (1.45±0.05 s/pig versus 0.9±0.05 s/pig; P < 0.001; results not presented). In agreement with Torrey et al. (2013), this effect can be attributed to the presence of steep internal ramps and 1801 turns inside the PB trailer which may have reduced the ease of handling and consequently delayed the unloading process.

Similar to Weschenfelder et al. (2012) in long distance transport trials using the same PB trailer model, the greater physical exertion of pigs to negotiate the internal ramps while exiting the trailer appears to result in more fatigued pigs as shown by the shorter latency to rest in lairage for PB pigs compared to FD ones (6.5±2.7min versus 20.4±2.4 min; P = 0.002; results not presented). Latency to rest in lairage was not affected (P = 0.90) by crossbreed type.

Physiological Measurements

Higher lactate and CK levels are usually observed in blood of pigs subjected to physical exertion as a result of muscle tissue damage (Knowles and Warriss, 2000). As shown in Table 22, 50Nn and 50NN pigs had greater (P = 0.028) exsanguination lactate levels compared to 25NN pigs. The higher lactate levels in these Pietrain crossbreeds can be explained by the higher glycolytic potential in these leaner pigs, resulting in higher glycogen utilization in response to pre-slaughter stress (Edwards et al., 2010; Hambrecht et al., 2005; Werner et al., 2010). Based on the speed of lactate to return to the basal level after physical exercise (2 h; Anderson, 2010), the higher lactate in blood at slaughter may reflect the response to unloading stress in combination with the increase in overlaps and jamming.

A significant interaction between trailer type and cross- breed was found for blood CK levels at exsanguination (P = 0.03), with levels being greater in 50Nn pigs trans- ported on the PB trailer compared to those located in the FD trailer. Higher levels of CK in HAL- carriers were previously described as being related to their natural hypermetabolic state, with physical exercise predisposing to a non-ambulatory status (Bickhardt and Wirtz, 1987; Fàbrega et al., 2002). The need to negotiate the PB internal ramps at loading and unloading was the triggering factor for the higher fatigue, as shown by the higher release of CK into

the blood flow of 50Nn pigs at slaughter. Higher CK levels at slaughter have been also reported in these pigs after long distance transportation on the same PB trailer model (Weschenfelder et al., 2012). However, differently from Weschenfelder et al. (2012), neither trailer type nor crossbreed influenced the blood cortisol levels at slaughter in the present study. This result confirms that this hor- mone can be more considered an indicator of long-term stress response (Bradshaw et al., 1996; Nyberg et al., 1988). The lack of effect of the HAL gene on blood cortisol variation is in contrast with the findings of Geers et al. (1994) in HAL-carrier pigs.

Acute phase proteins (APPs), such as haptoglobin and Pig-MAP, have been reported to be reliable indicators of transport stress in pigs (Saco et al., 2003). However, in this study, haptoglobin and Pig-MAP concentrations in exsan- guination blood, despite being on average greater than the reference values previously reported for finishing pigs (Piñeiro et al., 2009), did not vary significantly between treatments (Table 22). As the aforementioned effects of transport stress on blood APPs were only reported after 18 h transportation (Piñeiro et al., 2007; Salamano et al., 2008), the lack of effects on APPs in this study may mean that they are not valid indicators of transport stress under short transport conditions. Averós et al. (2009) and Weschenfelder et al. (2012) did not report any variation in blood APPs at slaughter after transport times ranging from 0.6 to 8 h either.

Table 22. Concentrations of plasma lactate and serum creatine kinase (CK), cortisol, and the acute phase proteins (Pig-MAP and haptoglobin) of 3 crossbreds[1] of pigs transported in pot-belly (PB) and flat-deck (FD) trailers.

n	PB	FD	SEM	50Nn	50NN	25NN	SEM	*P* value		
	72	72		48	48	48		Trailer (Tr)	Crossbred (Cb)	Tr × Cb
Lactate (mmol/L)	24.06	25.41	2.87	26.30[a]	27.32[a]	20.59[b]	2.97	0.38	0.028	0.85
CK (log)	3.40[a]	3.26[b]	0.04	3.55[a]	3.20[b]	3.23[b]	0.05	0.006	<0.001	0.03
Cortisol (ng/mL)	28.26	24.73	2.67	25.58	27.52	26.19	2.97	0.19	0.79	0.30
Pig-MAP (mg/mL)	1.53	1.37	0.14	1.50	1.48	1.38	0.15	0.17	0.65	0.42
Haptoglobin (mg/mL)	1.81	1.77	0.19	1.83	1.78	1.78	0.20	0.81	0.94	0.35

[a,b] Within a row and main effect, least squares means lacking a common superscript letter differ, $P < 0.05$

[1]50Nn = 50% Pietrain inheritance with HAL^{Nn} genotype; 50NN = 50% Pietrain inheritance with HAL^{NN} genotype, and 25NN = 25% Pietrain inheritance with HAL^{NN} genotype.

Carcass Quality Traits and Skin Bruises

As there was no interactive effect of trailer type and crossbreed on carcass quality traits and skin bruises, data were pooled across treatments and only the single effects of these factors are presented (Table 23).

In general, carcass quality traits were not affected by trailer type or crossbreed, except for the greater (P = 0.04) lean yield of 50Nn and 50NN carcasses compared to those of 25NN pigs (Table 23). This result indicates that increasing the percentage of Pietrain genetics in the cross favours lean deposition, independently from the presence of the HAL gene, as previously reported by Gispert et al. (2007) and Kaić et al. (2009).

Skin damage was greater (P 1⁄4 0.04) on 50Nn carcasses than 25NN carcasses (Table 23). Transporting pigs on the FD trailer produced a greater (P = 0.02) number of overlap-type bruises on the carcass compared to the PB trailer. Overall, the increased number of bruises on the carcass of these pigs would reflect their aforementioned behavioural response while being handled at loading and unloading.

Table 23. Carcass quality traits and skin bruises of 3 pig crossbreds[1] transported in pot-belly (PB) and flat-deck (FD) trailers.

	Trailer			Crossbred				*P* values	
	PB	**FD**	**SEM**	**50Nn**	**50NN**	**25NN**	**SEM**	**Trailer**	**Crossbred**
N	180	180		120	120	120			
HCW (kg)[2]	94.09	93.83	0.45	93.76	94.28	93.84	0.5	0.55	0.59
Carcass yield (%)	82.38	82.53	0.33	82.57	82.52	0.37	0.73	0.65	0.73
Lean yield (%)	62.10	61.80	0.13	62.23[ε]	62.02[a]	61.61[b]	0.16	0.13	0.04
Skin damage score [3]	1.77	1.91	0.18	1.99[a]	1.83[ab]	1.70[b]	0.18	0.13	0.04
Fighting-type bruise score [4]	1.15	1.25	0.13	1.26	1.24	1.10	0.13	0.17	0.11
Overlap-type bruise score [5]	0.59	0.76	0.12	0.73	0.61	0.68	0.13	0.02	0.35

[a,b] Within a row and main effect, least squares means lacking a common superscript letter differ ($P < 0.05$).
[1]50Nn = 50% Pietrain inheritance with HAL^{Nn} genotype; 50NN = 50% Pietrain inheritance with HAL^{NN} genotype, and 25NN = 25% Pietrain inheritance with HAL^{NN} genotype.
[2]HCW = hot carcass weight
[3]1 = none to 5 = severe (MLC, 1985)
[4]1 = less than 10 bruises to 3 = greater than 21 bruises (ITP, 1996)
[5]1 = less than 5 bruises to 3 = greater than 11 bruises (ITP, 1996)

Meat Quality Traits

As with carcass traits, there was no interactive effect of trailer type and crossbreed on meat quality parameters, so data were pooled across treatments and only the single effects of these factors are presented (Table 24).

Trailer type more consistently influenced meat quality traits in the ham than in the loin, with pHu values being greater in the SM ($P = 0.05$) and AD muscles ($P = 0.013$) of PB pigs (Table 24). It is not surprising that more significant effects of the treatment were observed in locomotor muscles, such as the SM and AD muscles rather than in postural muscles, such as the LD, as they are more susceptible to physical stress. Other studies reported no effect of physical stress on pork quality as measured in the LD muscle (Channon et al., 2000; Hambrecht et al., 2005). These results confirm that the effects of a specific stress, either physical or psychological, on meat quality are muscle-dependent (Correa et al., 2010). The higher pHu in the ham muscles in combination with the higher blood CK levels at slaughter are indicative of a potential risk of muscle fatigue in pigs being transported with the PB trailer.

Similar to a previous study (Weschenfelder et al., 2012) with the same genotypes, crossbred type affected all meat quality parameters, except for the pHu in the SM muscle (P = 0.24). In this study, loins and hams from 50Nn pigs were paler (higher L^* value; $P < 0.001$ and P = 0.02, respectively) and more exudative as shown by the greater EC ($P < 0.001$ and $P = 0.001$, respectively) and greater ($P < 0.001$) drip loss values in the LD and SM muscles (Table 24). The detrimental effect of the HAL gene on pork quality resulting in increased incidence of PSE pork has been already reported by a number of other studies in the past (Fernandez et al., 2002; Leach et al., 1996; Oliver et al., 1993).

Table 24. Quality characteristics of the Longissimus dorsi (LD), semimembranosus (SM) and adductor (AD) muscles of 3 pig crossbreeds[1] transported in either a pot-belly (PB) or flat-deck (FD) trailer.

n	Trailer			Crossbred				*P* value	
	PB	FD	SEM	50Nn	50NN	25NN	SEM	Trailer	Crossbred
	180	180		120	120	120			
LD muscle									
pHu	5.69	5.69	0.02	5.64[a]	5.70[b]	5.73[b]	0.03	0.93	0.001
L*	51.20	51.23	0.55	52.10[a]	51.21[b]	50.33[b]	0.57	0.94	<0.001
a*	7.96	8.03	0.13	8.28[a]	8.14[a]	7.64[b]	0.14	0.34	<0.001
b*	5.20	5.30	0.18	5.55[a]	5.30[a]	4.90[b]	0.19	0.44	0.002
EC[2]	5.93	5.54	0.23	6.53[a]	5.80[b]	4.86[c]	0.24	0.05	<0.001
Drip loss (%)	4.46	4.14	0.39	5.90[a]	3.81[b]	3.20[b]	0.41	0.22	<0.001
SM muscle									
pHu	5.77a	5.74	0.02	5.74	5.77	5.75	0.02	0.05	0.24
L*	47.78	47.57	0.39	47.99[a]	47.41[b]	47.18[b]	0.41	0.69	0.020
a*	9.13	9.60	0.12	9.61[a]	9.31[b]	9.16[b]	0.13	<0.001	0.013
b*	4.97	5.24	0.09	5.40[a]	4.98[b]	4.93[b]	0.11	0.013	0.001
EC[2]	9.47	8.84	0.25	10.09[a]	9.21[b]	8.16[c]	0.29	0.064	0.001
Drip loss (%)	3.31	3.34	0.31	4.07[a]	3.20[b]	2.70[c]	0.32	0.84	<0.001
pHu AD	6.04	5.97	0.03	6.02[a]	6.04[a]	5.96[b]	0.03	0.013	0.045

[a,b,c] Within a row and main effect, least squares means lacking a common superscript letter differ ($P < 0.05$).

[1]50Nn = 50% Pietrain inheritance with HAL^{Nn} genotype; 50NN = 50% Pietrain inheritance with HAL^{NN} genotype, and 25NN = 25% Pietrain inheritance with HAL^{NN} genotype.

[2]EC: Electrical conductivity measured by the pork quality meter (PQM-I-INTEK, Classpro GmbH, Sielenbach, Germany)

CONCLUSIONS

This study showed that the PB trailer does not appear to be a suitable trailer for pig transportation over a short distance as it resulted in fatigued pigs at slaughter. The potential causes of fatigue are the presence of multiple internal steep ramps and turns that impose a vigorous physical exercise on pigs at loading and unloading. The FD trailer appears to be a better trailer type than the PB trailer, although some modifications in its design should be made to provide a better microclimate control within the trailer and greater ease of handling of pigs at loading and unloading. Increasing the proportion of Pietrain genetics (25–50%) resulted in leaner carcasses, but also in higher responsiveness to handling stress, regardless of the presence of the HAL gene. However, in this study, pork quality was only detrimentally affected when the HAL gene was present in the Pietrain cross. In general, the crossbred type showed to be more detrimental to animal welfare and meat quality than the trailer type; however trailer type may emphasize genotype related defects.

CONFLICT OF INTEREST STATEMENT

None.

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Chapter 5: The use of infra-red thermography for the measurement of the pigs' body temperature just prior to slaughter and the prediction of the variation in stress indicators levels in blood and pork quality traits. **(A manuscript based on the information presented in this chapter was Published in Meat Science, 2013. 95: 616-620).**

Infrared thermography (IRT), a modern, non-invasive measure of animals' body temperature, has been used to assess animal welfare and health status under different conditions. Its use under commercial conditions would be valuable because of its non-invasive and practical characteristics. However, despite its potential, only a few studies used infrared cameras to measure welfare or pork quality. Data-loggers (e.g., i-button), which are invasive in nature, have been employed to measure body temperature and to assess metabolic changes due to heat stress in pigs. Given the importance of such measurements and the possible benefits IRT can bring to the producers and the animals, it is important to evaluate how this technology would correlate with other animal welfare variables (including body temperature as assessed with i-Buttons data loggers) and pork quality traits.

Authors: Angela Vanelli Weschenfelder (Ph.D. candidate; planification and data collection, data analyses and manuscript preparation). Linda Saucier and Luigi Faucitano (research director and co-director: student's supervision, revision and correction of the manuscript). Collaborators in the project, including manuscript revision: Xavier Maldague and Allan L. Schaefer (infrared thermography and stress physiology experts).

The use of infra-red thermography for the measurement of the pigs' body temperature just prior to slaughter and the prediction of the variation in stress indicators levels in blood and pork quality traits.

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Short title: Infra-red thermography for animal welfare and pork quality applications

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ABSTRACT

Infra-red thermography (IRT) body temperature readings were taken on the animal's back (experiment 1; N=133 pigs) or in the ocular region (experiment 2; N=258 pigs). Levels of cortisol, creatine kinase and lactate were evaluated in exsanguination blood. Meat quality was assessed in the longissimus dorsi (LD), semimembranosus (SM) and adductor (AD) muscles. Ocular IRT temperature was positively correlated with blood lactate levels measured after lairage (r = 0.23; P = 0.0002) and in the restrainer (r = 0.20; P = 0.001). IRT recordings may also help explain the variation in some pork quality traits in the LD muscle, such as pH_1 (r = - 0.18; P = 0.03) and drip loss (r = 0.20; P = 0.02) and pH_1 in the SM muscle (r = - 0.19; P = 0.02). The measurement of IRT body temperature in the ocular region appears to be a potential useful tool to assess the physiological condition of pigs at slaughter and predict the variation of important meat quality traits.

Key words: Infra-red thermography, heat stress, animal welfare, meat quality, pigs

INTRODUCTION

The stimulation of the sympatho-adrenal axis due to physical stress response increases metabolic activity and heat production, resulting in higher core body temperature (Sawka, Wenger, & Pandolf, 1996; Terrien, Perret, & Aujard, 2011). Pigs are particularly sensitive to heat stress and are often near their upper threshold of thermal tolerance during handling, transport and lairage (Brown et al., 2007; Asala, Ayo, Rekwot, Minka, & Adenkola, 2010), with clear consequences on body metabolism and meat quality variation (Hambrecht et al., 2004; Ritter et al., 2009). For this reason, body temperature is considered a sensitive measure of short- and long-term effect of thermal stress in pigs subjected to handling (de Jong, Lambooij, Mechiel Korte, Blokhuis, & Koolhaas, 1999; Carr, Newman, Rentfrow, Keisler, & Berg, 2008) and transport and lairage conditions (Knowles, Brown, Edwards, & Warriss, 1998; Carr et al., 2008; Weschenfelder et al., 2012). However, in most studies this measurement was taken using invasive equipment, such as gastro-intestinal, ear, and rectal data loggers or telemetric transmitters, whose installation implies animal restraint or surgery and thus the application of stressful and time consuming procedures requiring trained manpower.

Infra-red thermography (IRT) represents a non-invasive technique allowing the recording of body temperature without touching the animal (Warriss, Pope, Brown, Wilkins, & Knowles, 2006; Stewart, Webster, Schaefer, Cook, & Scott, 2005). By converting the infra-red radiation emitted by a heat source into pixel intensity, the IRT provides an image map of temperatures or thermogram of the skin surface (Griffith, Türler, & Goudey, 2002). In past studies, based on the close association between muscle temperature rise and early post-mortem pH fall rate (Klont & Lambooij, 1995), IRT has been applied to detect the temperature rise in pigs in response to pre-slaughter handling with the ultimate objective to predict meat quality variation (Gariépy, Amiot, & Naday, 1989; Schaefer, Jones, Murray, Sather, & Tong, 1989). IRT has also been used to assess the welfare status of livestock under routine management practices, i.e. dehorning or health status control in calves (Schaefer et al., 2012; Stewart, Stafford, Dowling, Schaefer, & Webster, 2008). However, the findings of these studies were not conclusive and showed a lack of consistency and accuracy in this technique. A possible explanation for the results may be that in the afore-mentioned studies the IRT thermograms were obtained on the pigs' back skin. According to

Banhazi, Kitchen and Tivey (2009), when the IRT measurement is taken at this location, its sensitivity to detect temperature differences may be reduced by the presence of dirt, hair or water on the skin which may influence the level of emitted radiation and bias the values.

The brain is the major source of metabolic-produced heat and houses the central nervous system regulating temperature of the body, i.e. temperature of the brain is the core temperature (McCafferty, 2007). Ocular temperature, due its close proximity to the brain, is considered a good indicator of core temperature (Kessel, Johnson, Arvidsson, & Larsen, 2010; Johnson, Rao, Hussey, Morley, & Traub-Dargatz, 2011). As the eye blood flow is tightly related to the sympathetic activity (Stewart, Stafford, Dowling, Schaefer, & Webster, 2008), even mild stress responses can be detected as changes in the ocular temperature. Thus, the detection of superficial IR body temperature in the orbital (eye) region can be an alternative increasing the efficiency and reliability of this measure.

Several studies in humans and other mammals have demonstrated a good correlation – $r \geq 0.80$ – between core body temperature and the IR thermograms near the ocular region (Tan, Ng, Acharya, & Chee, 2009; Kessel et al., 2010; Johnson et al., 2011). Changes in IROT have been reported in response to acute stress, such as physical pain and inflammatory process in cattle (Johnson et al., 2011; Stewart et al., 2008; Schaefer et al., 2012). However, the reliability of IROT for the monitoring the physiological conditions at slaughter and early prediction of meat quality variation in pigs was never assessed as yet.

The results of two different experiments using IRT to estimate core body temperature in pigs in the *pre-mortem* phase (just before stunning) are presented in this chapter. In experiment 1, body temperatures were estimated from the back skin thermograms, while in experiment 2 they were estimated from thermograms of the ocular surface. The objective of both experiments was to determine whether the IRT temperature estimates were correlated with other physiological indicators of animal stresses and with pork quality parameters.

MATERIAL AND METHODS

All experimental procedures performed in experiments 1 and 2 were approved by the institutional animal care committee based on the current guidelines of the Canadian Council on Animal Care (2009).

Animals and Treatments

Experiment 1

Animals

A total of 133 crossbred barrows (BW of 115 ± 5kg) were used. Pigs were progenies from crosses of Pietrain homozygous halothane recessive (HAL^{nn}), Pietrain homozygous halothane dominant (HAL^{NN}) and Duroc × Pietrain (homozygous halothane dominant, HAL^{NN}) sire lines mated to F-20 sows (Genetiporc, St. Bernard, Canada) resulting in 3 swine crossbreds: 50% Pietrain crossbreeding with HAL^{Nn} genotype (50Nn); 50% Pietrain crossbreeding with HAL^{NN} genotype (50NN); and 25% Pietrain crossbreeding with HAL^{NN} genotype (25NN). Pigs were originated from two commercial growing-finishing farms, located either at 450 km (7 h; 60 pigs) or at 44 km (45 min; 60 pigs) far from the slaughterhouse. Pigs were transported using two types of trailers, a three-decked pot-belly (PB) trailer equipped with two internal ramps vs. a three-decked flat-deck (FD) trailer equipped with middle and upper hydraulic decks and no internal ramps. The PB trailer used in this study transported 222 pigs on the three decks distributed in 13 compartments (five in the upper and middle decks and three in the belly). The FD trailer had a loading capacity of 235 pigs distributed in three decks composed of eight compartments (three on upper and middle, and two in the bottom deck). All compartments in both trailers had their size adjusted to respect the same loading density (0.43 m^2/pig). Transport trials were run in September (3 journeys or replicates) and October (4 journeys or replicates) 2009. Side-slats were used according to the external temperature thresholds following the NPB (2008) recommendations keeping the porosity settings consistent in each trailer. Feed was withdrawn from all pigs 9 h before loading (19 h from last feed to slaughter) and pigs were loaded on each trailer using paddles and boards. Within each group of 3 to 4 pigs, 1 pig was randomly chosen for the study of the physiological response (4 pigs/crossbreed^{-} · trailer^{-1} ·

load^{-1}). This pig, plus another 6 to 7 pigs selected within the same group, was also used for the meat quality assessment (10 pigs/crossbreed · trailer type^{-1} · replicate^{-1}). The driver of each trailer and the handler at the farm were the same throughout the 6 wk. On arrival at the plant, pigs were unloaded using a whip only and driven to separate lairage pens based on the transport compartment (no mixing). After a 90-min lairage period, pigs were electrically stunned (head-to-chest electrical stunning) and exsanguinated in the prone position.

Experiment 2:

Animals

This study was part of an extend study conducted at a federally inspected swine slaughter plant in Eastern Canada, where 600 pigs were randomly chosen on arrival at the slaughter plant over 6 slaughter days (100 pigs per day) with the objective to validate the efficiency of real-time blood lactate measurement at different lairage events (unloading, lairage, restrainer and slaughter) in terms of prediction of pork quality traits variation. Pigs were kept in lairage for a duration ranging from 3 to 8 h (overnight). At the end of lairage, pigs were electrically stunned (head-to-chest electrical stunning) and exsanguinated in the prone position. At the entrance into the restrainer, a sub-sample of 258 pigs was randomly selected over a 3 weeks period for the infra-red thermography assessment of body temperature just prior to slaughter.

Physiological Measurements

Experiment 1

Approximately 10 h prior to loading, pigs were orally administered Thermocron i-Button data loggers (model DS1921H; Dallas Semiconductor, Maxim Integrated Products, Sunnyvale, CA) to monitor gastro-intestinal tract (GIT) temperature using a snare, a heavy gauge metal "pig gag", and balling gun. Each data logger was programmed to begin recording from 1 h prior to loading until slaughter, and to log temperature once per minute. The GIT temperature was measured in 0.125ºC increments with ± 1ºC accuracy (range of 15 to 46ºC). Data loggers of selected pigs were recovered after dissection of viscera during

the slaughter process. Later, data from 82 pigs (68.3% recovery) were downloaded onto a laptop computer and GIT temperatures were evaluated for each pig for the determined event at the restrainer (GITR).

At exsanguination, 10 mL of blood were collected in a tube (BD Vacutainers, VWR International Ltd., Montreal, Canada) to extract serum for creatine kinase (CK) and cortisol analysis (Correa et al., 2010). Another 2 mL of blood were collected in a tube containing 3.0 mg of sodium fluoride and 6.0 mg of Na_2EDTA solution to extract plasma for lactate analysis (Correa et al., 2010). The 2-mL blood tubes were immediately centrifuged at 4°C for 12 min at 1,400 × g, plasma was transferred into 1.5-mL Eppendorf tubes and stored at -80ºC until lactate determination. Serum samples were kept at room temperature (~23°C) for 1 h before refrigeration at 4°C. The following day, serum samples were centrifuged at 4°C for 12 min at 1,400 × g, transferred to 1.5-mL Eppendorf tubes, and stored at -80°C until analysis. Lactate levels were measured using a commercial kit (Lactate Assay Kit, Biomedical Research Service Center, University of Buffalo, Buffalo, NY) and CK levels with creatine kinase-sl kit (Creatine Kinase-SL Assay of Chemicals Diagnostic Limited, Vancouver, Canada). Plasma lactate concentrations were determined with a microplate reader (SpectraMax Plus 384, Molecular Devices, Sunnyvale, CA) and serum CK concentrations with a spectrophotometer (Lambda 35 Spectrophotometer UV/VIS, Perkin Elmer Canada INC, Woodbridge, ON). The quantitative determination of serum cortisol was made using a commercial kit (DRG Cortisol Enzyme Immunoassay kit, DRG Instruments GmbH, Marburg, Germany), with a microplate reader and expressed as ng/mL. The intra-assay CV were 5.04, 4.05 and 3.99 for plasma lactate and cortisol, and serum CK, respectively.

Experiment 2

Blood samples were collected from each pig by picking one of the animal's distal ear veins with a retractable gauge needle. A drop of blood from the animal's ear was immediately dripped onto a sample strip (two strips or replicate/animal) and inserted into a hand-held Lactate Scout Analyzer (LSA; EKF Diagnostic GmbH, Magdeburg, Germany), and the results were obtained in approximately 15 s. Pigs were sampled for lactate analysis at five different sampling points; unloading (UN), after lairage at the exit of the resting pen (LA)

and in the restrainer before stunning (RE). The blood collection in the restrainer was carried out by stopping the restrainer for a few seconds right after the entrance of the animal into it. After electrical stunning, exsanguination blood was collected from the bleeding wound (EX) in a plastic cup and lactate level was immediately assessed in duplicate with the LSA by dipping the test strips in the collected blood sample in order to collect 0.5 µl of blood in each strip.

Meat Quality Measurements

Experiments 1 and 2

Muscle pH was measured in the *longissimus dorsi* (LD at the 3re/4th last rib level) and *semimembranosus* (SM; in the middle region) muscles at 1 h (experiment 2 only) and 24 h *post-mortem* (pH1 and pH24, respectively) with a temperature-compensating, spear-type probe (Cole-Palmer Instrument Co., Vernon Hills, IL) attached to a pH meter (pH 100 series; Oakton Instruments, Vernon Hills, IL). In addition, colour data were collected in the LD and SM muscles at the same anatomical locations after a 30 min blooming period. Colour of the meat (L*, a* and b* values) was measured with a Minolta Chromameter (CR-300; Minolta Canada Inc., Mississauga, Canada) equipped with a 25 mm aperture, 0º viewing angle, and D65 illuminant. Drip loss was measured in the LD and SM muscle using the modified EZ-driploss method of Correa, Méthot and Faucitano (2007). Briefly, three 25 mm-diameter cores were removed from the centre of 2.5 cm thick LD (removed at 3rd/4th last rib level) and SM cuts weighed, and placed into plastic drip loss containers (Christensen Aps Industrivaengetand, Hilleroed, Denmark), before being stored for 48 h at 4°C. At the end of the 48 h storage period, muscle cores where removed from their containers, surface moisture was carefully dabbed, cores were reweighed, and drip loss percentage was calculated by dividing the difference between initial and final core weights by the initial core weight.

Infrared thermographic images

Experiment 1

IRT images were collected in 133 pigs lining up in the stunning chute. The infra-red thermal camera (ThermaCan, iR5, Flir Systems USA, Boston, MA) was placed above the stunning chute in order to capture the fore-back skin surface images for each pig while they were moving along the chute. A typical fore-back thermograph is presented in Figure 14. Camera was set for the emissivity of pig's skin (0.98), the reflected air temperature (20ºC) and distance between the camera and the skin surface (1.0 m). Infra-red skin temperatures (IRST) data were recovered by processing the thermographic images with the Flir Quickreport program (version 1.2, Flir Systems 2008, Boston, MA) for the determination of the average temperature (ºC) in a squared area in the fore-back (loin-neck) region.

Experiment 2

An infrared camera (ThermaCan i60, Flir Systems USA, Boston, MA) was used to collect eye images of each pig at the entrance into the restrainer. A typical ocular thermography image is shown in Figure 15. The images were taken while the pig flow in the restrainer was stopped to allow the LSA blood collection. The camera was set by emissivity of pig ocular surface (0.98), the reflected air temperature (18ºC) and distance between camera and ocular surface (0.25 m). All pigs were scanned on the right side. IRT ocular temperatures (IROT) images were analysed with the Flir Quickreport program (version 1.2, Flir systems 2008, Boston, MA) for the determination of the maximum temperature (ºC) within the area of the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle.

Figure 14. Infra-red thermographic image of a pig fore-back (loin-neck) in the stunning chute (Experiment 1). By converting the infra-red radiation emitted by a heat source into pixel intensity, the IRT provides an image map of temperatures or thermogram of the skin surface. The thermogram shows temperature in a scale from white to black. The scale at the right side of the picture shows the higher attributed temperature (34.3ºC), which is associated to the whiter pixel in the image.

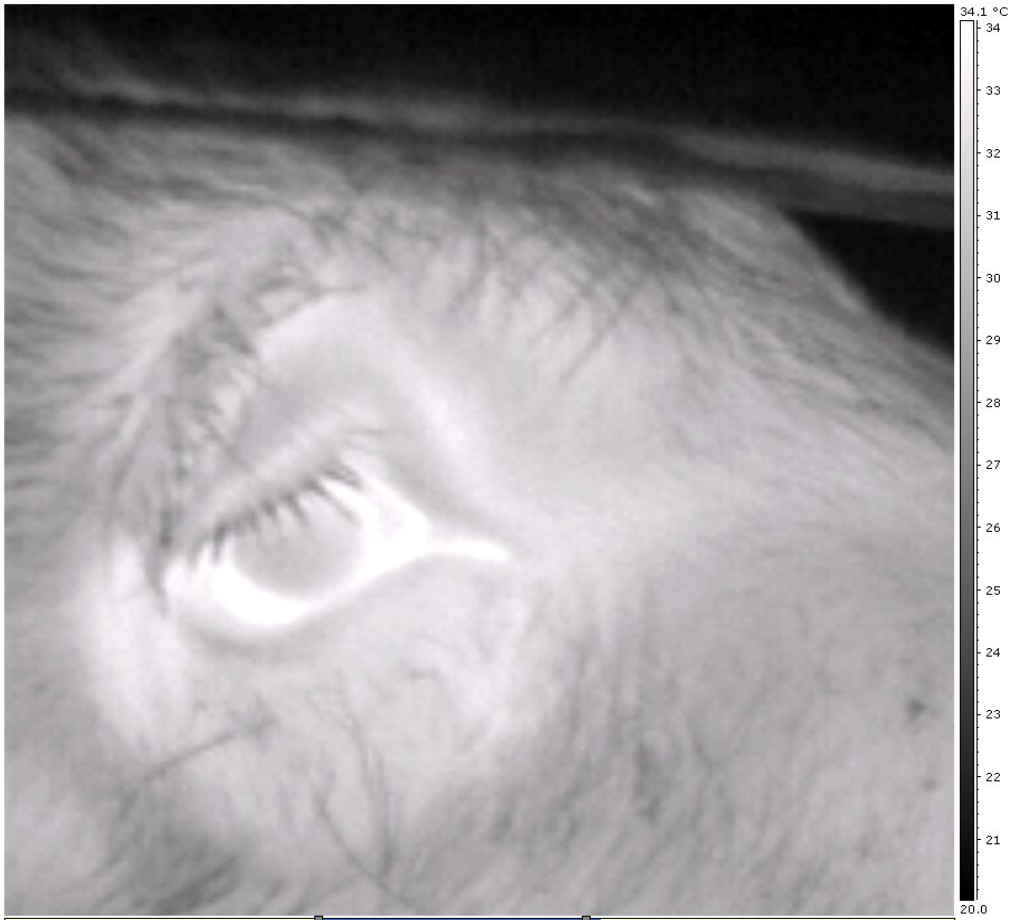

Figure 15. Infra-red thermographic ocular image of a pig in the restrainer (Experiment 2). The thermogram shows temperature in a scale from white to black. The scale at the right side of the picture shows the higher attributed temperature (34.1ºC), which is associated to the whiter pixel in the image.

Statistical Analysis

Data were analysed using the mixed model procedure of SAS (version 2.0.3; SAS Institute, Inc., Cary, NC). The CORR procedure of SAS was used for calculating the Pearson correlation coefficients to relate IRT either to physiological (GIT temperature, CK, lactate and cortisol) or meat quality parameters. The individual animal was the experimental unit. A probability level of $P < 0.05$ was chosen as the limit for statistical significance in all tests, whereas probability levels of $P \leq 0.10$ were considered as a tendency.

RESULTS

Experiment 1

Descriptive statistics of physiological and meat quality data obtained in experiment 1 are presented in Table 25.

Pearson coefficients of correlation between IRT skin (IRST) temperature value and pork quality and physiological stress parameters are shown in Table 26. IRST was negatively correlated with L* as measured on the SM muscle (r = - 0.19; P = 0.02). There was a tendency for negative correlation between skin IRST value and L* value of LD muscle (r = - 0.14; P = 0.09). No correlations were found between skin IRST and GITR, plasma lactate, serum CK and cortisol.

Table 25. Descriptive statistics of pork quality, physiological and body temperature measures.

Parameter	Mean	Standard deviation	Minimum	Maximum
		Pork quality		
Longissimus dorsi (LD)				
pH24	5.73	0.10	5.51	6.05
T24h	-0.11	0.43	-0.90	1.30
Minolta L*	50.65	2.08	43.41	57.92
Drip loss (%)	3.63	2.08	0.69	10.93
Semi-membranosus (SM)				
pH24	5.79	0.13	5.55	6.23
T24h	1.87	0.59	0.20	3.50
Minolta L*	47.38	2.05	43.04	54.04
Drip loss (%)	2.90	1.29	0.67	8.55
Adductor (AD)				
pH24	6.04	0.23	5.53	6.69
T24h	2.01	0.57	0.70	3.80
		Physiological measures		
Blood measures				
Serum Cortisol (ng/mL)	30.71	10.17	11.52	56.28
Plasma lactate (mmol/L)	20.98	7.04	6.24	42.50
Serum CK log	3.43	0.34	2.86	4.56
Temperature measures, ºC				
GITR[a]	39.24	0.38	38.15	40.45
IRST[b]	25.23	2.67	21.40	34.50

[a]GITR: Gastro-intestinal tract temperatures of pigs measured in the stunning chute
[b]IRST: infra-red skin temperature

Table 26. Pearson correlations exhibiting the relationship between skin infra-red temperature (IRST) images with meat quality traits and physiological variables.

Variable *r*[a] *P-value* n	GITR	CORTISOL	CK	LACTATE	DLLD	L*LD	PH24LD	DLSM	L*SM	PH24SM
	-0.14	0.07	0.11	-0.09	-0.06	-0.14	0.03	-0.06	-0.19	0.04
IRST[b]	0.14	0.40	0.22	0.28	0.46	0.09	0.67	0.45	**0.02**	0.62
	106	128	132	132	133	133	133	131	131	131
	1		-0.09	0.09	0.03	-0.04	-0.04	-0.10	-0.20	0.08
GITR[c]		0.11	0.31	0.31	0.72	0.57	0.57	0.22	0.02	0.32
	129	0.18	128	128	128	129	129	127	127	127
		125	0.06	0.31	0.16	0.05	-0.03	0.12	0.05	-0.01
CORTISOL		1	0.44	<.0001	0.03	0.48	0.69	0.14	0.49	0.87
			163	163	161	162	162	160	160	160
		163	1	0.11	0.35	0.08	-0.06	0.10	-0.01	0.22
CK[d]				0.12	<.0001	0.27	0.42	0.16	0.87	0.003
			167	167	165	166	166	164	164	164
				1	0.16	0.13	-0.06	0.19	0.10	0.33
LACTATE					0.03	0.07	0.43	0.01	0.19	<.0001
				167	165	166	166	164	164	164
					1	0.58	-0.51	0.39	0.28	-0.09
DLLD[e]						<.0001	<.0001	<.0001	0.0002	0.20
					166	166	166	164	164	164
						1	-0.57	0.39	0.51	-0.21
L*LD							<.0001	<.0001	<.0001	0.006
						167	167	165	165	165
							1	-0.39	-0.29	0.27
PH24LD								<.0001	0.0001	0.0003
							167	165	165	165
								1	0.52	-0.34
DLSM[f]									<.0001	<.0001
								165	165	165
									1	-0.34
L*SM										<.0001
									165	165
										1
PH24SM										
										165

[a] *r* = coefficient of correlation
[b] IRST = skin infrared thermography
[c] GITR: Gastro-intestinal tract temperatures of pigs measured in the stunning chute
[d] CK = creatine kinase
[e] DLLD = drip loss as measured in the longissimus dorsi muscle
[f] DLSM = drip loss as measured in the semimembranosus muscle

Experiment 2

Descriptive statistics of physiological and meat quality data obtained in experiment 2 are presented in Table 27.

Table 27. Descriptive statistics of pork quality, physiological measures, and body temperature measures.

Variable	Mean	Standard deviation	Minimum	Maximum
		Pork quality		
Longissimus dorsi (LD)				
pH1	6.62	0.20	6.10	7.06
T1h	38.40	1.80	25.80	42.60
pH24	5.69	0.14	5.50	6.54
T24h	2.17	0.46	1.20	4.10
Minolta L*	52.02	2.84	42.39	58.27
Drip loss (%)	2.94	1.77	0.34	10.02
Semi-membranosus (SM)				
pH1	6.82	0.17	6.08	7.13
T1h	40.3	1.00	34.9	42.30
pH24	5.69	0.14	5.50	6.54
T24h	2.20	0.46	1.20	4.10
Minolta L*	49.45	2.88	41.78	57.51
Drip loss (%)	2.13	1.28	0.38	6.58
Adductor (AD)				
pH24	6.07	0.26	5.38	6.70
T24h	2.96	0.52	1.60	4.40
		Physiological measures		
Temperature measures, ºC				
IROT[a]	35.7	0.87	34.00	38.10
LACR[b]	5.66	2.74	1.40	15.60

[a]IROT = Infra-red ocular temperature
[b]LACR = Lactate concentration measured in the restrainer

Pearson correlation coefficients between IROT and pork quality traits and physiological parameters are shown in Table 28. IROT was positively correlated with blood lactate level in the restrainer (r = 0.20; P = 0.001). As expected, based on the afore-mentioned correlations between IROT and blood lactate levels, negative correlations were found between IROT and pH_1 in the LD (r = - 0.18; P = 0.03) and SM (r = - 0.19; P = 0.02) muscles, while positive correlations were observed with muscle temperature (r = 0.33; $P <$ 0.001) and drip loss (r = 0.20; P = 0.02) in the LD muscle.

Table 28. Pearson correlations exhibiting the relationship between infra-red ocular temperature (IROT) and meat quality traits and physiological parameters.

Parameter	PH1LD	T1hLD	DLLD	PH1SM	LACR[b]
r[a]	-0.18	0.33	0.20	-0.19	0.20
P-value	**0.03**	**0.0002**	**0.02**	**0.02**	**0.001**
Number	129	129	129	129	258

IROT[c]

[a]*r* = coefficient of correlation
[b]LACR = Lactate concentration measured in the restrainer
[c]IROT = Infra-red ocular temperature

DISCUSSION

Experiment 1

As part of the physiological response to heat stress, the superficial vessels of pigs undergo vasodilation, reducing the animal's thermal insulation and allowing better cooling of the body (Terrien, Perret, & Aujard, 2011). In previous studies, skin temperature has been reported to change in parallel with rectal temperature depending on the health status (Loughmiller et al., 2001), the change in the feeding regime (Loughmiller et al., 2005) and environmental temperature changes inside the truck during transportation (Nanni Costa et al., 2012).

In this study, the range of variation of IRST corresponds to that reported by Gariépy et al. (1989). Nevertheless, except for the weak negative correlation with the L* value, no significant correlations between IRST and other pork quality varibales were found. These results are consistent with the results reported by Schaefer et al. (1989). However, they are neither consistent with the previous findings from Gariépy et al. (1989) who found higher values of IRST to be associated with a higher incidence of meat quality defects, such as palid, soft and exudative (PSE) pork, or with those from Warriss et al. (2006) who found cortisol to be positively correlated ($r = 0.55$) with IRST (ear thermograms).

The reason for this lack of correlation can result from the limited sensitivity of the IRST due to the wetness of the skin at the time of measurement as pigs were sprinkled before leaving the lairage pen. This is a standard practice recommended to improve meat quality by dropping body temperature at the time of slaughter and to improve the electrical stunning efficiency (Long & Tarrant, 1990; Wotton, 1996).

Experiment 2

The positive correlation between IROT and blood lactate in the *pre-mortem* phase (a few seconds prior to slaughter) indicates how high is the level of muscle activity and stress as the animals are handled from a free-moving group situation to a single line of aligned and restrained individuals as described in previous studies (Troeger, 1989; Griot, Boulard, Chevillon, & Kérisit, 2000; Edwards et al., 2010). Higher lactate concentrations were already found in pigs presenting higher body temperatures as measured by rectal temperature (Klont & Lambooij, 1995). Contrary to what reported by Schaefer et al. (1989), the correlations between IRST and meat quality traits confirm the contribution of the elevated body and muscle temperature at the time of slaughter on the speed of early post-mortem meat acidification rate, eventually resulting in pale and /or exudative pork. As mentioned abouve, Gariépy et al. (1989) also reported higher IRT of the skin surface in association with PSE pork (Gariépy, Amiot, & Naday, 1989). However, when compared to the results reported by Gariépy et al. (1989), a smaller range of variation in the IROT was found in this study (26.5–32.2ºC, SD = 2.67 *vs.* 34.0–38.1ºC, SD = 0.87), which would indicate a better precision of the IROT compared the IRT measured on the skin. No significant correlations were found between IROT and the other meat quality traits assessed

in this study (**SM muscle**: T1h (r = 0.09; P = 0.28); pH24h (r = - 0.02; P = 0.82); T24h (r = - 0.11; P = 0.19); L* value (r = 0.06; P = 0.50); DL (r = - 0.14; P = 0.12); **LD muscle**: pH24h (r = 0.04; P = 0.66); L* value (r = 0.05; P = 0.55); and, **AD muscle**: pH24h (r = 0.01; P = 0.84); T24h (r = - 0.12; P = 0.15).

In conclusion, our hypothesis about a better reliability and precision of infra-red body temperature as measured in the orbital region compared to that on the skin assessed in previous studies has been validated in this study. However, besides the different anatomical location for the IR scans, the difference in the results between this study and the previous ones can be also explained by the advances in the infra-red technology resulting in the development of more accurate cameras over the last years.

Overall, our results suggest that IROT is capable of detecting temperature changes associated with the physiological condition of pigs preslaughter and may represent a potential tool to predict pork quality variation under commercial conditions now that the cameras are more affordable. However, although significant, the magnitude of the correlations found in this study is still low. Thus, for a more reliable monitoring of the preslaughter conditions and control of pork quality variation, further development in the accuracy of IR cameras is needed.

Chapter 6: General conclusions, implications, and future work

This thesis presents the examination of the impact of vehicle design on pig's behavioral and physiological responses, as well as on pork quality. The results are analysed separated by short (45 min) and long distance (7 h) transportation. This thesis also presents important considerations regarding the use of infrared thermography as a tool to predict animal welfare and meat quality. In meat production, transportation is a mandatory step and appropriate choice vehicle design is important. Along with vehicle design, genetic background plays a major role on animal response to stress with important consequences on meat quality. It is also important to properly identify, prior to slaughter, animals at risk of being neglected or becoming nonambulatory, regarding its fatigue and health status, notably, because of the impact on meat quality. The increasing introduction of Pietrain genetics has raised the question whether the stress response of these leaner pigs are related to the breed itself or to the presence of the HAL gene. In a context of increasing attention towards animal welfare, along with the need to optimize production, this thesis presents relevant information providing new perspectives for the improvement of breeding programs; the design of more appropriate and more effective transportation systems; and also the development of a promising and noninvasive technique to measure stress.

One of the main hypothesis presented by this thesis stated that the journey distance reduces the impact of vehicle design on pig welfare and meat quality. Differently from what was originally intended, short and long distance transportation where not compared as main effects, but evaluated separately due to main differences between farms origin. However, this condition did not avert to confirm our hypothesis. Indeed, the results presented in this thesis allow the conclusion that the negative effects of transportation on animal welfare and pork quality are alleviated by trailer design. The higher level of challenge imposed to pigs transported by the PB trailer are emphasized over short distance transportation. This conclusion is supported by our findings where higher CK levels were found in pigs transported by PB over the short distance transportation. We also found that it took longer to unload these pigs and, at the lairage, pigs unloaded from PB also took shorter time

periode before they lay down. We also concluded that the incidence of PSE meat was higher in pigs with a higher proportion of Pietrain genetics, carrier for the HAL gene, particularly when transported over a short distance (Figure 17, appendix A). However, another main conclusion was that, despite resulting in lower stress levels, some design features (step at the entrance as a result of the pilling up floors) of the FD trailer should be modified or adjusted to the loading quay to improve handling conditions at loading and unloading.

The importance of knowing environmental conditions during transportation has to do with the need of providing optimal comfort for animals. In therms of microclimate, previous research had indicated that compartments located on the bottom, front and rear of the trailer are the critical ones (Guise and Penny, 1989; Barton-Gade et al., 1996b; Brown et al., 2011). As already mentioned, pigs loaded on those compartment would be more prone to present hotter temperatures and thus poorer meat quality. In order to examine possible differences between the two studied vehicle types, compartments 5, 6 and 11 in the PB and, as a mirror, compartments 3, 4 and 7 inside the FD trailer were chosen (Figs. 7 and 10). As expected, compartments closer to the driver cabin and engine, and on the botton (belly) presented the warmest environmental conditions inside the PB trailer. However, this did not resulted in higher temperatures on pigs probably because the values were under the upper critical temperatures, thus not affecting animals thermoregulation. It is important to note that this study did not evaluate microclimate under stationary periods or extreme weather. Inside the FD, despite not presenting differences in temperature between compartments, internal temperatures were keept similar throughout the periods analysed (mainly pre-transport-transport). As a conclusion, the temperature variation inside the vehicles indicates that ventilation pattern is not optimal in either trailer. In the case of the FD trailer, this condition seems to be the result of too low deck ceilings, while in the PB trailer it may be related to the air openings position and the lower height of compartments in the bottom deck. These ambient conditions may jeopardize the animal welfare of pigs under extreme conditions (summer and winter) and during stationary periods.

The assessment of core body temperature during transportation and just prior to slaughter is critical to understanding how pigs maintain homeostasis. The use of i-Buttons to measure gastro-intestinal temperature has been used for the measurment of pigs body temperature and how it adapts over time and under known situations. One of the benefits of using this tool is its "non-invasive-*in-use*" feature, meaning that once swallowed by pigs, it registers temperature over time without the need of any intervention. The main negative aspects is that it is aversive for pigs to swallow this device; it demands trained man power; and despite its acceptable recovery rate (in order of 75-85%; one i-Button cost $20), it demands hours of workload on animals guts at the evisceration. Another main issue is that at the time data is recovered from the i-Buttons, pigs are already dead. This condition does not allow to preventive measures in the case pigs suffer from hyper or hypothermia, for example. Furthermore, the data obtained may not be recovered if the device is damaged (e.g. bitten) during the process, which will impede the access to the registered information. Also, and may be the most important problem related to the use of i-Button, is that it cannot be calibrated and its level of accuracy may introduce a bias in the results. To overcome this difficulty, we used the variation of temperare instead of the actual temperature in the data presented in this thesis.

All those challenges, along with the relevance of such measure, make the need to develop real time and consistent tools to evaluate thermal response of pigs during pre-slaughter management, primordial. Another tool already in use to evaluate different aspects of animal welfare on livestock is the infrared thermography, which we tested in this thesis. The cameras used in this study provided acceptable data. In experiment 1, the goal was to compare skin temperature as measured by infra-red thermography with physiological responses, primarily, the gastro-intestinal tract temperature as measured by the i-Button. Unfortunately, technical impediments in obtaining the proper images with the minimal environmental influence did not allow validating the new methodology. In experiment 2, it was possible to obtain a scan from the eye of the pigs in a more controlled environment thus allowing to correlate the eye temperature, as measured by the infra-red thermography, with lactate levels in the blood. The results obtained indicate that the infra-red thermography is a promising technology to assess stress in live animals.

With respect to the genetic background of the pig, the main hypothesis was that pigs with higher proportion of Pietrain genetics (50 %) free of the HAL gene could produce acceptable carcass and meat quality with no detriment to animal welfare. This hypothesis was partially proven to be true. It is known that pig carrier of the HAL gene will present impaired muscular metabolism resulting in higher muscle damage, thus poorer meat quality. The presence of the HAL gene increased concentrations of lactate and CK levels in pig's blood, which is related to lower final pH, paler color and higher drip loss. As expected, pigs with a higher proportion of the Pietrain genetics (50%), free from the HAL gene, produced leaner carcasses with no negative effect on meat quality compared to pigs with the lower proportion of Pietrain genetics (25%).

As for the behavioural response, it is known that a higher responsiveness to stress is associated to the genetic selection for improved production. Before the results presented in this thesis, it was not clear whether this higher susceptibility to stress was associated to the presence of the HAL gene, or to the higher percentage of the Pietrain genetics. Our results show that, overall, pigs with a higher proportion of Pietrain genetics were more responsive to stress, which resulted in handling difficulties at loading and unloading. The results allow concluding that the higher susceptibility to stress does not depend on the presence of the HAL gene only, but also the higher percentage of Pietrain genetics.

In general, the results presented in this thesis demonstrate and allow to conclude that genetics possess a greater influence on animal welfare and meat quality parameters than vehicle design. However, vehicle design may amplify the negative effects of distance transportation on animal welfare. It was shown that the proportion of Pietrain genetics up to 50% does not jeopardize meat quality. However, regardless of the presence of the HAL gene or not, it increases pig's responsiveness to stress.

In general, the findings presented in this thesis, were determinant for the elimination of the HAL gene from the herd that is raised for pork production at the company were the experimental phase of this study was performed. The results indicate that the PB trailer should be used to transport pigs over long distances while the FD trailer should rather be used for shorter distances. This doesn't mean that the conditions are optimal to eliminate the stress caused on animals during transportation, however, it minimizes animal losses and

meat quality defects by providing the best possible compromise with regards to the well being of the pigs and industrial efficiency.

Based on the findings of this thesis, further studies should be conducted to improve some design features (ventilation, ramps, turns, flooring, etc.) in both PB and FD trailer types, in terms of their effects on animal welfare and pork quality. Also, further research must be undertaken in terms of proper manipulation of Pietrain pigs free of the HAL gene in order to optimize production without restriction to the well being of animals. And a third research goal should be to study the validation of infrared thermography under commercial conditions and to fix thresholds to identify animals at risk of producing poor meat quality at the early *ante-mortem* stage. To be most effective, infrared images ought to be taken prior to the restrainer allowing segregation of animals at risk

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Appendix A: Pork quality classification

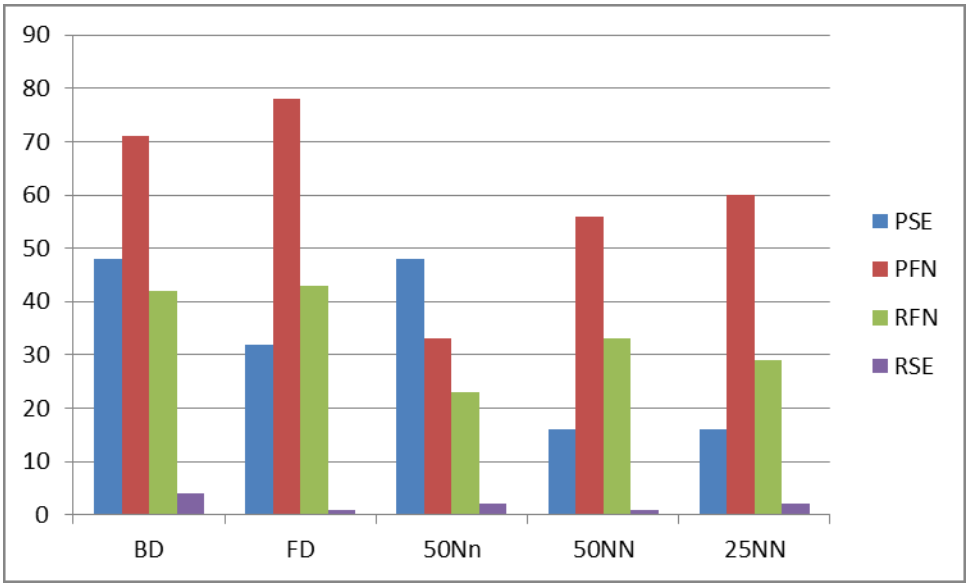

Figure 16. Pork quality distribution based on genotype and trailer type during long distance transportation. There was no significant relationship between pork classes and trailer type or genotype ($P > 0.05$).

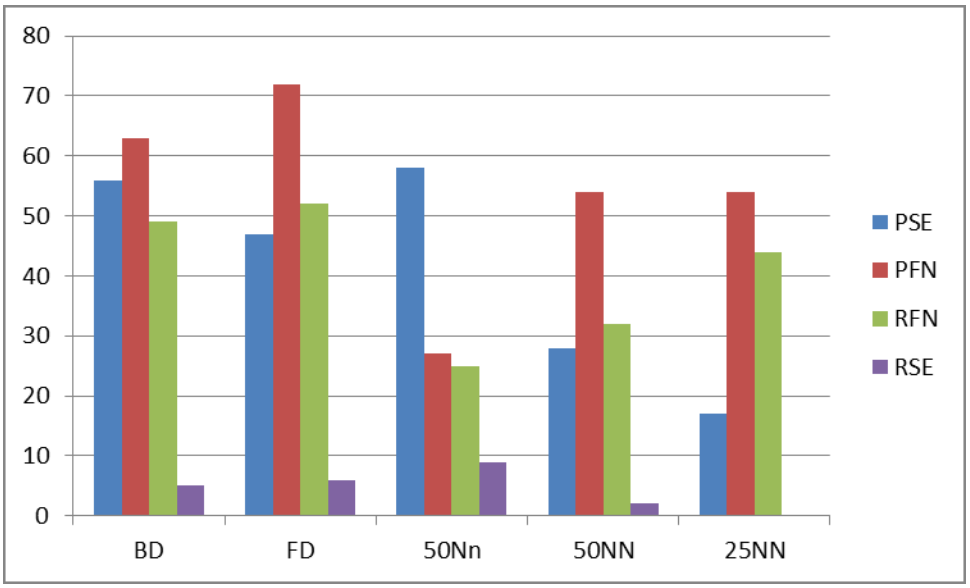

Figure 17. Pork quality distribution based on genotype and trailer type during short distance transportation. There was a significant relationship between genotype (50Nn) and the incidence of PSE meat ($P = 0.05$)