Validation of stress indicators for the assessment of animal welfare and prediction of pork meat quality variation at commercial level

Thèse

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Résumé

Les procédures appliquées avant l'abattage des animaux influencent directement la qualité de la viande en modulant l'état physiologique des porcs; ainsi, l'augmentation de la température corporelle, les taux élevés de lactate sanguin et l'épuisement des réserves de glycogène entre autres, occasionnent la majorité des baisses de qualité. L'objectif de cette thèse était de valider des outils indicateurs de stress porcin pour les fermes et les abattoirs. Ceux-ci seraient appliqués à la surveillance du bien-être animal et à la prédiction de variation de qualité de la viande porcine au niveau commercial. Premierement, les résultats de la thèse ont permis de conclure qu'un des outils développés (analyseur portatif de lactate) mesure la variation du niveau de lactate sanguin associé à l'état physiologique des porcs dans la phase *péri-mortem* et aide à expliquer la variation de la qualité de la viande chez le porc à l'abattoir, en particulier dans les muscles du jambon. Deuxièmement, les résultats des audits du bien-être animal appliqués de la ferme à l'abattoir ont démontré que la qualité du système d'élevage à la ferme d'origine et les compétences du chauffeur de camion sont d'importants critères affectant la réponse comportementale des porcs à la manipulation avant l'abattage. Ces résultats ont également démontré que les conditions de logement à la ferme (la faible densité et l'enrichissement dans les enclos), le comportement des porcs en période pré-abattage (glissade), ainsi que les interventions du manipulateur (utilisation du bâton électrique) dans la zone d'étourdissement de l'abattoir affectent négativement la variation de la qualité de la viande. L'application des protocoles d'audits dans la filière porcine a également démontré que le respect des critères de bien-être animal fixés par un outil de vérification est primordiale et permet de contrôler les conditions de bien-être des porcs à chaque étape de la période pré-abattage, de produire une viande de qualité supérieure et de réduire les pertes. Les audits de bien-être animal sont donc un outil qui apporte des resultats très pertinents pour aider a éviter les variations de la qualité de la viande chez le porc. Troisièmement, la thermographie infrarouge s'est avéré être une technique prometteuse permettant d'évaluer la variation de température corporelle de l'animal pendant et après un stress physique, en particulier lorsque cette mesure est prise derrière les oreilles. En conclusion, les outils validés à travers cette thèse représentent des méthodologies non invasives et potentiellement complémentaires à d'autres approches d'évaluation de l'état physiologique et du bien-être animal par rapport au stress,

permettant de réduire les pertes de qualité de viande (par exemple en utilisation conjointe avec le niveau de lactate sanguin et les indicateurs de stress comportemental, entre autres).

Abstract

The majority of meat quality defects are directly related to the preslaughter procedures as it results from the variation of the physiological state of pigs before slaughter, as indicated by changes in body temperature, lactate levels, glycogen reserves, among others. Therefore, the objective of this thesis was to validate tools that could be useful as stress indicators at the farm and at the slaughter plant for animal welfare monitoring and pork quality variation prediction at the commercial level. The results highlighted that the hand-held scout analyzer is capable of measuring blood lactate levels variation associated with the physiological condition of pigs in the *peri-mortem* phase and may help explain the variation in pork quality, especially in the ham muscles. Likewise, results of the animal welfare audits showed that the quality of the raising system at the farm of origin and the truck driver skills are important sources of variation in the behavioural response of pigs to preslaughter handling. Additionally, on-farm housing conditions (ease of movement), and preslaughter pig behaviour (slips) and handler interventions (electric prod use) during handling in the stunning chute area at the plant as assessed by the audit protocols used in this study, showed to contribute to pork quality variation. Finally, the infrared thermography proved to be a promising technology to assess body temperature variation after physical stress, especially when this measure is taken behind the ears. Overall, the three techniques validated in this thesis represent useful methodologies to complement other techniques in the assessment of animal welfare conditions (*e.g.* animal behaviour, physiological indicators, etc.) in relation to stress. Being non-invasive and rapid these procedures may be applied to monitor animal welfare prio to slaughter and reduce the incidence of pork quality defects.

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List of acronyms and abbreviations

ACTH: Adrenocorticotropic hormone

AD: Adductor

ADP: Adenosine diphosphate

ANS: Autonomic nervous system

ATP: Adenosine triphosphate

BE: Beta-endorphin

CK: Creatine kinase

CNS: Central nervous system

CP: Creatine phosphate

CRH: Corticotrophin-releasing hormone

DA*:* Dopamine

DFD: Dark, firm, dry

DOA: Dead-on-arrival

G-6-P: glucose-6-phosphate

GP: Glycolytic potential

HAR: Human animal relationship

HCW: Hot carcass weight

HPA: Hypothalamic-pituitary-adrenal

HR: Heart rate

IRET: Infrared thermography at the ear location

IR: Infrared thermography

IROT: Infrared thermography at the orbital location

LDH: Lactate dehydrogenase

LL: Longissimus lumborum

LSA: Lactate Scout Analyser

LT: Longissimus thoracis

LVP: Lysine vasopressin

NAD: Nicotinamide adenine dinucleotide

NADH: Nicotinamide adenine dinucleotide (NAD) + hydrogen (H)

pHi: Initial pH

pHu: Ultimate pH

PFN: Pale, firm, non-exudative

PRL: Prolactin

PSE: Pale, soft, exudative

RFN: Red, firm, non-exudative

RR: Respiratory rate

RSE: Red, soft, exudative

SM: Semimembranosus

VIP: Vasoactive intestinal peptide

WHC: Water-holding capacity

WK: Week

WQ®: Welfare Quality

"Don’t be afraid of life and not be afraid of living it.
There is no heaven without storms, or roads without accidents.
It is only worthy of the podium, the one who uses his defeats to reach it.
It is only worthy of wisdom the one who uses his tears to irrigate it.
Fragile people use force; strong people, intelligence.
Be a dreamer, but unite your dreams with discipline,
for dreams without discipline produce frustrated people.
Become a debater of ideas. Fight for what you love."
Augusto Cury

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To my little princess, please forgive mommy for not spending enough time with you during these first months of your life. I know that this is a part of our life that will never come back. Thank you my sweet for giving me reasons to hold on and being the light in my dark days. You are my sunshine, my most precious gift and everything I do, I do it for you.

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Finally, for the pigs, I would like to express my eternal admiration and respect.

Foreword

This thesis is submitted to the "Faculté des études supérieures de l'Université Laval" to obtain the degree of Philosophiae Doctor of Science (Ph.D.). The current thesis consists of seven chapters. Chapter 1 is a literature review of animal welfare and stress concepts, as well as of indicators that are used nowadays to measure and quantify stress response in pigs. Chapter 2 presents the issue, hypotheses and objectives. Chapter 3, 4 and 5 are presented in the form of manuscripts and describe three different sets of experiments with related results and discussion. Chapter 6 is presented in the form of short paper and shows the results of a study conducted as special topic on meat microbiology. Chapter 7 presents a general conclusion, implications and proposed perspectives arising from the results of the studies presented in this thesis.

The first manuscript is entitled “Hand-held lactate analyzer as a tool for the real-time measurement of physical fatigue before slaughter and pork quality prediction” and was published in the Animal journal in 2015 (doi:10.1017/S1751731114002766). Authors: Luiene M. Rocha, Alexandre Dionne, Linda Saucier, Eleonora Nannoni and Luigi Faucitano.

The second manuscript is entitled “Can the monitoring of animal welfare parameters predict pork meat quality variation through the supply chain (from farm to slaughter)?” was published in the Journal of Animal Science in 2016 (doi:10.2527/jas2015-9176). Authors: Luiene M. Rocha, Antonio Velarde, Antoni Dalmau, Linda Saucier and Luigi Faucitano.

Finally, the third manuscript is entitled “Identification of the anatomical location on the pig body ensuring the most efficient use of infrared thermography for the assessment of body temperature variation in response to physical stress” and was presented as poster communication at the 2015 Canadian Society of Animal Science (CSAS)-Canadian Meat Science Association (CMSA) joint meeting in Ottawa (ON). The abstract is being published in the Canadian Journal of Animal Science. Authors: Luiene M. Rocha, Xavier Maldague, Julien Fleuret, Linda Saucier and Luigi Faucitano.

The main objective of this thesis was to validate the efficiency of tools for monitoring stress indicators at the farm and at the slaughter plant in the view of a practical and reliable assessment

of animal welfare prior to slaughter and prediction of pork quality variation at the commercial level. Luiene M. Rocha was in charge of the work planning and data and sample collection for all experiments and also designed the study on body temperature assessment by infrared technology. She was also responsible for the statistical analysis of data and preparation of the three manuscripts. Drs. Linda Saucier and Luigi Faucitano, as tutor and co-tutor of Luiene M. Rocha, oversaw all steps of this work. More specifically, Dr. Luigi Faucitano generated the idea for the experiments 1 and 2. Drs. Antonio Velarde and Antoni Dalmau collaborated in the set-up and work planning of the animal welfare audits study and participated in the interpretation of the results and manuscript revision. Dr. Xavier Maldague collaborated in the analysis of the infrared body temperature images and helped in the results interpretation.

Furthermore, it is worth mentioning that in 2013 the candidate was granted two scholarships. The Canadian Meat Council associate members Scholarship in recognition of the quality of her doctoral research program and its potential impact on the Canadian meat industry, and the Merit Scholarship for Foreign Students (Brazil-Quebec program) granted by the Fonds de recherche du Québec - nature et technologies (FQRNT). Still in 2013, the candidate was awarded with the Canadian Animal Science Association travel award for her participation in the Canadian Society of Animal Science (CSAS)-Canadian Meat Science Association (CMSA) joint meeting in Banff (AB).

During her Ph.D., the candidate co-authored one book chapter: Oliveira, R.L., C.L. Abreu, S.T. Carvalho, T.M. Silva, M.D. Freitas, and L.M., Rocha. 2014. Desafios da produção de bovinos de corte respeitando o conforto e o bem-estar animal. In: R.L. Oliveira, and M.A.F. Barbosa (eds.), Bovinocultura de Corte - Desafios e Tecnologias. Universidade Federal da Bahia, Salvador, Brazil, pp. 673-700.

Finally, the candidate has been invited to include the results of this thesis in a good management practices guide for pigs entitled: Development of an assessment tool to improve production practices (from birth to slaughter), animal welfare and meat quality under the Canadian commercial conditions. This guide is addressed to the pork meat industry and the Canadian pig producers.

Introduction

Pork meat quality variation is directly related to the physiological state of pigs before and at slaughter (Correa et al., 2013; Weschenfelder et al., 2013; Vermeulen et al., 2015a,b). Hence, several studies have been conducted aiming at better understanding the mechanisms of stress resulting in pork quality variation and, thereafter, developing reliable techniques for the assessment of stress. Unfortunately, most assessment techniques are invasive, therefore potentially stressful for the animals, are very laborious and time-consuming and often require trained manpower for their execution. Furthermore, depending on the type of stressor to assess, there may be some limitations on the usefulness of the physiological changes as a measure of stress. These include, for example, difficulties in obtaining true baselines and inadequate sampling time and frequency. Thus, considering the requirements in commercial settings, there is a need to develop non-invasive, rapid and user-friendly tools that can be used to assess stress physiological indicators at the farm and at the slaughter plant in alternative to more traditional analytical procedures.

A first example, in the current thesis, is the real-time analysis of blood lactate content using the the hand-held Lactate Scout Analyzer (LSA), which was originally used to test athletes' performance in sports medicine. In livestock, it has been tested for the quick real-time measurements of blood lactate in live animals and/or at exsanguination at slaughter plants to monitor the quality of the handling system and facilities design with the aim to control pork quality variation (Edwards et al., 2010a). However, few significant, although weak, correlation between lactate as measured with the LSA and pH at 1 h post-mortem (r = -0.32; $P < 0.001$) and drip loss (r = 0.22; P = 0.02) were observed and the results were rather inconclusive (Edwards et al., 2010a). The reason for the weak correlations between lactate and meat quality in the Edwards et al. (2010a) study may be explained due to flaws in the experimental design, such as the small sample (128 pigs), the low-stress marketing conditions (unloading without ramps, limited electric prodding, long lairage, small groups and CO_2 stunning) and the muscle being used (loin) for meat quality evaluation.

An important and landmarking topic on animal welfare is the current shift of consumer confidence, with respect to food safety and animal welfare, from government control to trust branded products that push the pork chain stakeholders to adopt auditing systems, checking on standards through the pork supply chain. However, on-farm audit scores have never been matched with handling audit scores at the slaughter plant. This would help assess the efficiency of animal welfare protocols on the easiness to handle animals at the plant and on meat quality variation. Thus, overall it is unknown whether or not the respect of animal welfare audit criteria set by these audit protocols is contributing to high and uniform quality pork. Therefore, studies are needed to assess the relationship between animal welfare conditions on farm and preslaughter handling as assessed by audit protocols and pork quality variation.

Recently, the infrared thermography (IR) that is capable of detecting changes in radiant body surface temperature has been studied as a potential technology to assess animal welfare. However, the results reported in recent studies are conflicting and no clear indication of the best anatomical location for reliable assessment of body temperature using IR currently exists.

The objective of this thesis was to validate the efficiency of tools for the assessment of stress and animal welfare indicators at the farm and at the slaughter plant under commercial conditions and prediction of pork quality variation.

Chapter 1: Literature Review

1.1. Meat consumption and ethical quality

Some 2.6 million years ago, a remarkable expansion in the human diet started to occur, with some *hominins* starting to incorporate meat and marrow from small to very large animals into their diet (Mann, 2000). The earliest well-accepted evidence for this novel dietary behaviour comes from the discovery of butchery marks on bones at an excavation site in Ethiopia (Domínguez-Rodrigo et al., 2005; Landt, 2007; Pickering et al., 2013).

Eating muscle foods was an important component of our evolution process and the rise in meat consumption improved the nutritional status of our ancestors since meat and marrow are calorie-dense resources with essential aminoacids and micronutrients (Milton, 1999). Increasing consumption of animal proteins allowed *hominins* to increase their body size without losing mobility, agility, or sociality (Milton, 1999), and improved human intelligence (Aiello and Wheeler, 1995; Fish and Lockwood, 2003).

Nowadays, although meat is still a central element in the human eating habits, the pattern of meat consumption is changing based on societal values and concerns (McKendree et al., 2014). The notion of food quality has evolved over time and now extends far beyond nutritional value to include health, safety, environmental impact and even the local economy (Bergeron et al., 2013). The concept of animal welfare has also garnered increasing attention in recent years and is seen as an important attribute of the food quality concept, defined as ethical quality (Kafka and von Alvensleben, 1998; Warriss, 2010; Bergeron et al., 2013).

In Europe, 64% of the consumers have expressed concerns about animal welfare issues in the livestock production (Eurobarometer, 2010). While in Canada, recently the Ontario Farm Animal Care Council has conducted a survey aimed at understanding the preferences of Canadian consumers toward production practices and to identify the main concerns (IPSOS, 2009). According to the results, the care about the treatment of animals raised for their meat was ranked the second most important issue in agriculture (14%), just after food safety (57%; IPSOS, 2009). However, this survey also revealed that the majority of Canadian consumers (52%) demonstrates

complete confidence that animal welfare is respected as fully believes in the ability of producers (55%) and in the efforts of the industry (52%) to protect it (IPSOS, 2009). However, in comparison to the Canadian consumer, the US is showing a growing interest in greater transparency of information on livestock production systems, which include the management techniques and conditions of carriage (Tonsor et al., 2009). However, as the opinion towards the welfare of meat animals plays an increasingly central role in consumer preferences and, consequently, also in trade negotiations, it is important to better understand consumer perceptions and desires with respect to meat products and the latent ethical bases underneath it (McKendree et al., 2014).

In the United States, a study of Tonsor et al. (2009) revealed that 69% of consumers were in favor of the abolition of the cages for sows in gestation since it was associated with a reduction of the health and the meat quality. However, once you know that the removal of such cages would lead to an increase in municipal taxes ($ 230/ year), the percentage of favorable fell to 31% (Tonsor et al., 2009). This change in consumer behaviour shows the importance of taking into account the difference between the intentions and the real desire to purchase, in the interpretation of the survey results.

Thus, the meat market needs to work hard on process differentiation, in which quality attributes could be either translated into profits for producers as into benefits for consumers. To this end, certification labels including clear information about food production and respect of animal welfare standards are required (Schröder and McEachern, 2004).

1.2. Concepts of animal welfare and stress

1.2.1. Animal welfare

The concept of animal welfare was first cited in 1965 by the Brambell Committee (Brambell, 1965), which developed the "The Five Freedoms" (or, more correctly, the Five Needs) of animals, based on our perception of what animals need. Since then, numerous definitions emphasizing different concerns were proposed for animal welfare. However, as animal welfare cannot be defined as a purely scientific concept, it has been studied in different perspectives of animal biology (Duncan and Fraser, 1997).

These perspectives have been grouped under three main approaches: 1) the basic health and functioning, in which the key element is that the difficulty, or the failure, to cope with the environment would cause poor welfare; 2) the ability of animals to live reasonably natural lives by carrying out natural behaviour and having natural elements in their environment, and 3) the affective states of animals; this concept defines the satisfaction and absence of suffering as the fundamental issue for animal welfare (Fraser, 2008). Therefore, animal welfare may be better defined as a multidimensional concept, including both physical and mental health, but also including several aspects, such as physical comfort, biological needs, affective states, absence of hunger and disease and freedom to perform motivated behaviour (Blokhuis et al., 2010).

Animal welfare can vary on a continuum from very poor to very good and it fluctuates during life (Broom, 1991). However, the specific aspects of the welfare concept make its assessment a difficult exercise, particularly because the importance attributed to these perspectives listed above may vary between people and change over time (Broom, 1991; Fraser, 2005; Blokhuis et al., 2010). Overall, poor welfare occurs in situations in which the effects on the animal are adversive as indicated by a reduced performance or signs that it will be reduced (Broom, 1991).

1.2.2. Stress

The concept of stress was developed in the 20th century by the pioneer work of Walter Cannon (1914) and Hans Selye (1936) and should be used to explain the animal's failure to cope (Broom, 2010). Generally, stress does not refer to any single factor or series of body reactions, but to a heterogeneous assortment of phenomena (Fraser, 1975).

Stress can be defined as an environmental effect inducing adaptive behaviour in an individual (Toates, 1995; Jensen and Toates, 1997) involving biological responses to maintain its homeostasis (Moberg and Mench, 2000) with clear consequences on animal performance (Broom and Johnson, 2000), comfort, reproduction and health (Mason, 1975).

1.3. Animal welfare indicators

Animal welfare is a multidimensional concept and it cannot be measured directly or by a single measure (Fraser, 1993). Measures can be divided into two main categories: resource and management-based measures and animal-based measures.

1.3.1. Resource and management-based measures

Traditionally, animal welfare assessment at the farm or at the slaughterhouse has been centred on the measurement of the resources supplied to the animal, such as space allowance, type of floor, vehicle type, and transport duration, among others. These parameters are rather easy to define and measure, but they have low validity as they provide an indirect measure and may interact with other resource and management conditions (Waiblinger et al., 2001). Resource- or management-based measures are, therefore, only used to complement the animal-based measurements or as substitutes when there was no reliable animal-based measure available (Botreau et al., 2007).

According to Whay et al. (2003), the most important resource and management-based measures in pigs production include the assessment of barren conditions, water and food facilities, bedding (cleanliness and thermal comfort), pen space allowance, lighting, ventilation, absence of sharp edges and availability of facilities for group housing. Unfortunely, these resources (*e.g.* housing, stocking density) or management-based measures (*e.g.* feeding strategies, health plans) only provide partial information on the animals' welfare in particular situations, especially because animals differ in genetic background, early experience and temperament, an individual variation in their response to the same environment can be observed (Blokhuis et al., 2010). Therefore, the examination of these input-based measures, *e.g.* specifying the provision of particular resources and practices (*i.e.* prescriptions), only provides information on 'what' or 'how much' different resources are given to the animals (Velarde and Dalmau, 2012) without reliably measuring its health and fitness or overall welfare condition (Whay et al., 2003).

1.3.2. Animal-based measures

Based on the above, since welfare is a condition related to each individual animal, wherever possible, the assessment system should rather focus on animal-based measures, also called "outcome" or "performance" measures (Velarde and Dalmau, 2012). Animal-based parameters

are indicators of the physiological response and health resulting from impaired biological functioning, and of the behavioural response (Whay et al., 2003; Duncan, 2005). Many animal-based measures are currently used, most frequently as tools to assess one specific welfare issue at a time, for example, skin lesion scoring in pigs (Mouttotou et al., 1999).

1.4. Stress physiology

When an animal is confronted with an environmental change, adaptation involves a range of behavioural and physiological responses aiming at maintaining its homeostasis. The term homeostasis was first used in 1926 by the physiologist Walter Cannon who described it as the effort of the physiological systems within the body to actively maintain a level of functioning, within the limits of tolerance of the systems, coping with ever changing conditions (Boyle, 2013).

As Hans Selye used the term "stress" to refer to a "response" it was necessary to employ a term to define the stimulus for the stress response. This term is "stressor". Stressors are divided into two categories: (1) psychosocial stressors and (2) biogenic stressors (Girdano et al., 2009). According to Boyle (2013), psychosocial stressors are either real or imagined environmental events that "set the stage" for the elicitation of the stress response. The psychosocial stressors cannot directly "cause" the stress response, but must work through cognitive appraisal mechanisms. Biogenic stressors actually "cause" the elicitation of the stress response. These stimuli bypass higher cognitive appraisal mechanisms and work directly on affective and neurological triggering nuclei (Boyle, 2013).

The stress response system in vertebrates has both central nervous system (CNS) and peripheral components. The central tissue components of the stress system in mammals are located in the hypothalamus and the *Locus ceruleus* in the brainstem, and include a number of endocrine messengers whose major role is to mediate the neuroendocrine stress response. The corticotrophin-releasing hormone (CRH) is the key peptide in this activation (Tort and Teles, 2011).

The stress response is divided into two types: the active or acute response, originally described by Cannon (1929) as the 'figth-fligth' response, and the long-term or chronic described by Hans

Selye (1946) as a general adaptation response. The acute (short term) response activates behavioural and endocrine responses that prepare the animal for an immediate reaction to any environmental change, whereas the chronic (long term) response involves a substantial adjustment of both autonomic and neuroendocrine systems (Barnett and Hemsworth, 1990).

1.4.1. Short-term stress response

The short term response which is referred to the 'fight-flight response' emphasizes the emergency function of the adrenal medulla during stress. The hypothalamic-adrenal medullary system involves the hypothalamus, the pituitary gland, the sympathetic neural pathways to the adrenal medulla, and the release of epinephrine and norepinephrine from the adrenal gland (Cannon, 1914).

More specifically, when the sympatho-adrenal system is stimulated by the stressors, norepinephrine is released into the blood stream stimulating the adrenal medulla causing release of epinephrine, which prepares the animal for 'fight' or 'flight' (Warriss, 2010) by increasing the blood flow into the muscles and the availability of glucose as a prompt energy reserve (Fig. 1).

1.4.2. Long-term stress response

The general adaptation syndrome is related to the hypothalamic-pituitary-adrenocortical (HPA) stress response system whose activation begins with the release of CRH into the circulation from the paraventricular nucleus of the hypothalamus. The CRH stimulates the pituitary gland to release adrenocorticotropin hormone (ACTH) into the blood stream.

The mammalian adrenal cortex can be divided into three layers or *zona*: *zona glomerulosa*, *zona fasciculata* and *zona reticularis* (Tort and Teles, 2011). However, the action of the ACTH appears to involve *the zona reticularis* and *zona fasciculata* only stimulating the cells to release the glucocorticoids cortisol into the systemic circulation (Boyle, 2013). Among other effects, the release of glucocorticoids increases the urea and glucose production (gluconeogenesis) and the blood free fatty acids levels, and leads to the suppression of immune mechanisms and appetite (Fig. 2.).

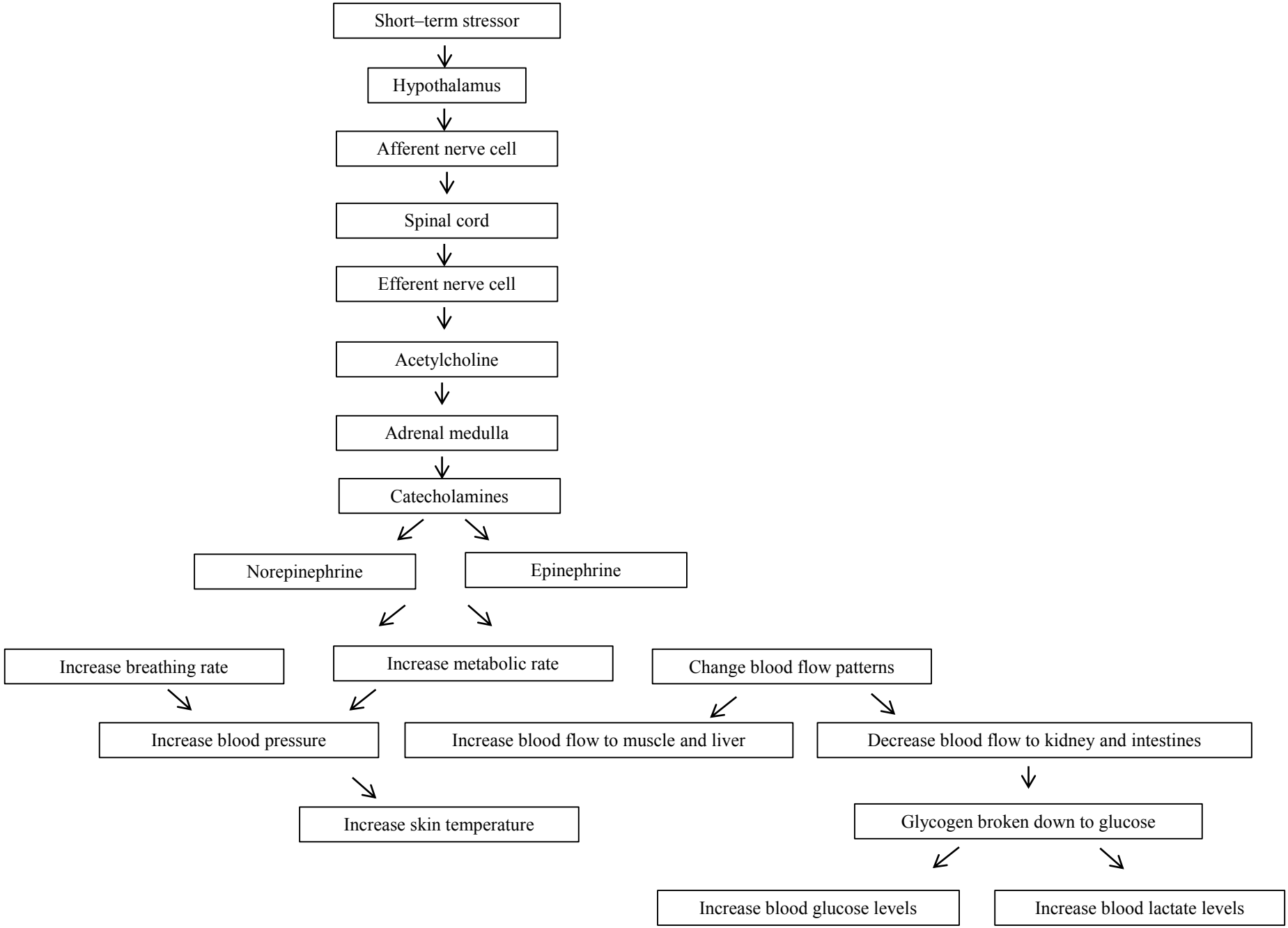

Figure 1. Short-term physiological response to a stressor (adapted from Keeling and Jensen, 2009)

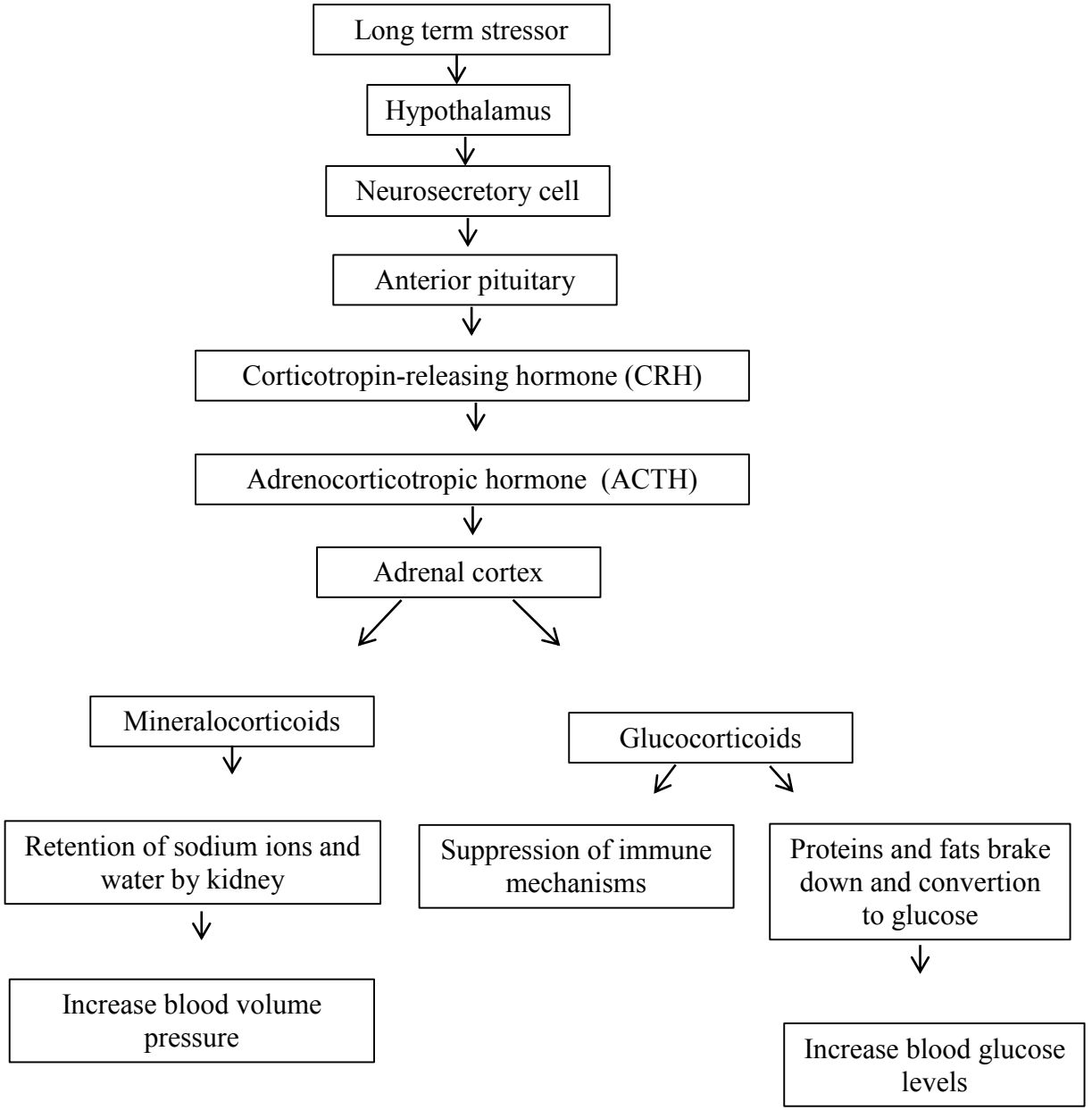

Figure 2. Long-term physiological response to a stressor (adapted from Keeling and Jensen, 2009)

1.5. Stress indicators

As stress is difficult to measure directly, it is often assessed indirectly by measuring its effects on living organisms (Cook et al., 2000). In physiological terms, the stress reaction involves behavioural, metabolic, immunological and neuroendocrine mechanisms. Its effects on the animals can be thus monitored through changes in these mechanisms (Warriss, 2010).

1.5.1. Short-term stress indicators

1.5.1.1. Heart rate

The heart rate (HR) is defined as the number of heart beats per unit time (Mills et al., 2010). According to Warriss (2010), the measurement of HR provides a very rapid response to stress. In livestock, HR, and in particular heart rate variability (Von Borell et al., 2007), has been used regularly to assess their stress levels in different housing systems. Overall, the HR has been largely used as welfare indicator with the assumption that it increases in stressful conditions, such as when eletric prod is used to handle pigs (Rebaste et al., 2007; Correa et al., 2010), during pigs transport (Geverink et al., 1998a) and and when pigs were submitted to wait at loading in the summer (Correa et al., 2013).

Heart rate variability can be described as tachycardia, in terms of acceleration of heart beats induced by the release of epinephrine from the sympathetic nervous system in response to a disturbing situation or physical activity, and bradycardia expressed by deceleration of heart beats in response to an emotional situation, such as fear (Mills et al., 2010). However, there are difficulties to interpreting HR data and their changes as a result of metabolic activity cannot be distinguished from changes related to emotional response. Thereby, measurement of HR may be a useful measure of emotional response of an individual to short-term problems (Broom and Johnson, 2000) whether or not related to stress.

1.5.1.2. Respiratory rate

The normal respiratory rate (RR) of a finishing pig ranges from 8 to 25 breaths/min (Von Borell et al., 2007). Changes in this rate, in terms of intensification or reduction, are signs of stress which are in response to emotional disturbance without body

activity (Mellor and Murray, 1989; Lorschy, 1997), physical exercise and fatigue (Zhang et al., 1992; Ritter et al., 2007) or heat stress (Forrest et al., 1968). As for the latter, studies have shown that RR increases with temperature in pigs in order to maintain homeostasis (Zhang et al., 1992; Brown-Brandl et al., 2001) and pigs pant to increase latent losses.

The RR can be measured non-invasively (Broom and Johnson, 2000) by behaviour observation (McCann et al., 1998; Ritter et al., 2007) or using a respiratory belt (Reefmann et al., 2009).

1.5.1.3. Body temperature

The stimulation of the autonomic nervous system (ANS) during the stress response induces changes in the peripheral vascular tone and blood flow on the animals' body, which increases body temperature (Blessing, 2003; Mitchell, 2013). When an animal becomes stressed, the HPA axis is activated resulting in changes in heat production and dissipation by increasing the blood catecholamines and cortisol levels, and blood flow (Schäefer et al., 2002).

In pigs, for example, stress-induced hyperthermia has been observed after restraint stress (Parrott and Lloyd, 1995). This raise in body temperature has been related to shift in the body temperature set-point of pigs. Thus, it was suggested that this temperatures raises are at least partially mediated by prostaglandins released by the CNS, and thus, involves the same mechanisms as the febrile response (Parrott and Lloyd, 1995). In contrast to raises in body temperature, psychological stress was also related to decreases in core temperature of pigs in response to transport stress (Parrott et al., 1998). Parrott et al. (1998) suggested that vasopressin release may have caused this hypothermic response to transport in pigs.

Additionally, Abrahams et al. (1964) observed changes in surface temperature as an index of muscle vasodilatation occurring during defence reactions. Hence, changes in body surface temperature, which are related to changes in blood flow, have been associated with emotional responses to stress in pigs (Gariépy et al., 1989; Lonardi et al., 2015), horses (Yarnell et al., 2013), rabbits (De Lima et al., 2013), lambs (Pascual-Alonso et al., 2015) and cattle (Stewart et al., 2005).

Measuring body temperature is an index of the thermal balance of the body (Hanneman et al., 2004), while measuring skin surface temperature measure the dynamics of surface temperature distribution governed by two main factors, convection by blood flow in the surface layer and from deeper blood vessels and sweat evaporation from the cutaneous layer (Ludwig, 2013).

Several body temperature measurement techniques have been extensively tested over the years. For the measurement of core body temperature these techniques include the copper-constantan thermocouple for arterial temperature (Eichna et al., 1951) and indwelling thermistor catheter for tympanic, rectal and bladder temperature (Wilson et al., 1971; Moorthy et al., 1985; Shinozaki et al., 1988) and I-button data loggers for gastro-intestinal tract temperature (Davidson et al., 2003; Conte et al., 2015). However, these techniques are invasive and need trained manpower.

For skin surface temperatures measurement the most used techniques are ear tag data loggers (Andersen et al., 2008), radiant thermometers (Huynh et al., 2006) and IR cameras (Gariépy et al., 1989; Stewart et al., 2005; Warriss et al., 2006; Weschenfelder et al., 2013). The IR temperature measurement equipment is gaining popularity, because of its advantages, in terms of farm animals monitoring, since handling and restraint increases stress and can causes an effect on core and surface temperatures (Warriss et al., 2006).

The IR measures changes in radiant body surface temperature caused by alterations in blood flow underlying the skin. Small changes in temperature may result in substantial amounts of emitted photons (or radiated energy) that can be detected very accurately using an IR camera. However, some IR results may be biased by direct sunlight and wind drafts, and dirty or moistened hair coat. Dirt, in fact, alters the emissivity and conductivity and excessive moisture increases local heat loss to the environment (Palmer, 1981).

The IR was successfully used to record surface temperature variation in nutritional studies in young broilers (Ferreira et al., 2011) and ruminants (Montanholi et al., 2008), and in pigs subjected to preslaughter handling (Gariépy et al., 1989; Schäfer et al., 1989; Weschenfelder et al., 2013).

There are a number of anatomical locations, alone or in combination that can be monitored with IR to indicate the impact of a wide range of potential adverse events. In pigs, Warriss et al. (2006) and Weschenfelder et al. (2013) reported significant, although rather weak, correlations between the IR temperature at the orbital location and blood creatine kinase ($r = 0.55$; $P < 0.05$) and lactate at exsanguination ($r = 0.20$; $P = 0.001$). The measurement of the eye IR temperature proved to be a useful tool for measuring stress in horses (Johnson et al., 2011), elks (Cook et al., 2005) and cattle (Stewart et al., 2005).

Johnson et al. (2011) explained the better efficiency of the IR ocular temperature compared to the skin surface by its close proximity to the brain. Additionally, it appears that IR at this location may be capable of detecting acute sympathetic responses based on the significant association with the activation of the HPA axis (Cook et al., 2001; Warren et al., 2001). Schäefer et al. (2004) explain that the temperatures of small areas around the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle, which have rich capillary beds innervated by the sympathetic system, respond to changes in the blood flow. However, although interesting correlations were observed, the results of these studies are rather inconclusive, likely because it is hard to conclude if the greater accuracy observed in each studied body site is due to the anatomical location or to the IR technology advances over the years.

1.5.1.4. Catecholamines and corticosteroids

Epinephrine and norepinephrine

The major metabolites of the adrenal medullary in response to emergency situations are the catecholamines, epinephrine and norepinephrine (Broom and Johnson, 2000). Catecholamines are released from the adrenal medulla into the blood stream within 1 or 2 sec after the perception of the triggering stimulus (McCarty, 1983) and their levels are maintained in blood for about 3 min (for both, epinephrine and norepinephrine; Whitby et al., 1961).

The most important biochemical consequence of the epinephrine release is the increased rate of glycogen breakdown in the muscles to yield lactate, and in the liver to yield blood glucose (Nelson and Cox, 2008). Conversely, an increased plasma noradrenaline levels results in increased heart rate and blood pressure, as well as

increased blood flow to skeletal muscle, and its release is predominantly affected by conditions involving physical exercise (Kågedal and Goldstein, 1988).

Their activity, which can be either measured in plasma or in the urine (Fernández et al., 1995; Hay and Mormède, 1997), is associated with cardiac and packed cell volume responses to stressors (Gregory, 1998).

Dopamine

Dopamine (DA) is the immediate metabolic precursor of norepinephrine and epinephrine and is measured in blood or urine (Hay and Morméde, 1997). The DA system is both activated by acute and chronic stressors **(**Hall and Bradshaw, 1998) and acts as a learning signal for behavioural reinforcement (Schultz, 1998). In pigs, DA responds to stressful situations, for example, it has been shown that stress caused by restraining pigs increases dopamine turnover in certain brain areas (Piekarzewska et al., 2000). Furthermore, the variation of the DA homeostasis has been linked to behavioural illness, such as mood disorders, dysfunctional behaviour (including aggressiveness), escape/avoidance behaviour, and stereotypic and compulsive behaviours (Le Moal and Simon, 1991; Mills et al., 2010).

In animals, dopamine is more likely involved in motivation and aversive or appetitive behaviours (Berridge and Robinson, 1998; Redgrave et al., 1999). Although the link between certain sorts of stereotyped behaviours and the DA systems in the brain have been known for some time (Sharman and Stephens, 1974; Fry et al., 1976), its efficiency as indicator of stress is not clear (Broom, 1988).

Cortisol

Cortisol is a steroid hormone produced by the *zona fasciculata* of the adrenal cortex (Boyle, 2013). Its circulating levels are generally primary biomarkers of stress, since they respond less rapidly to stress and recover slowly compared with other parameters of stress (Warriss et al., 1994; Russell et al., 2012). In pigs, cortisol is cleared from the plasma with a half life of 60 min (Jensen-Waern and Nyberg, 1993), following a metabolic clearance rate calculated to be about $11h^{-1}$ Kg^{-1} (Hennessy et al., 1986).

Under conditions of chronic stress, feedback signals are weak and the system remains activated for longer periods, resulting in effects on body processes that can be long term and detrimental (Boonstra, 2004). These negative effects include alterations at

several physiological levels, from impaired growth and reproductive capacity to immune suppression (Tort, 2010). The effects of chronic stress on cortisol concentrations are reported by Becker et al. (1985) who found increased cortisol levels in pigs confined in a box for 1 h and electrically stimulated for 6 min., during 3 consecutive days of trial.

However, caution should be taken when interpreting the results due to several factors of variation that may influence cortisol concentrations. The cortisol secretion follows a circadian and ultradian rhythm, similar to that of other pituitary hormones with peak secretion occurring early in the morning (Irvine and Alexander, 1994). This statement have been confirmed by several studies which observed higher concentrations of cortisol in pig's blood in the morning compared to the afternoon and evening (Becker et al., 1985; Janssens et al., 1995; Ruis et al., 1997).

Furthermore, restraint and manual sampling of pigs can be extremely stressful and may affect cortisol levels (Marchant-Forde et al., 2012). Non-invasive sampling procedures, such as determination in the urine (Hay and Morméde, 1997), saliva (Cook et al., 1997) and faeces (Möstl and Palme, 2002) are then being used. However, cortisol levels in urine are dependent on urine volume and as the concentration is often expressed as units of cortisol over a defined period, the acute response of the HPA axis cannot be assessed.

The measurement of cortisol in blood also has some physiological limitations due to its biochemical state in blood. According to Cook et al. (1997), circulating cortisol in blood plasma is predominantly (90%) bound to cortisol-binding globulin (CBG) and albumin. The remaining 10% is in a "free" form, and it is this fraction that is available for uptake by target tissues. The measurement of "free" salivary cortisol appears more accurate than "total" plasma cortisol, even though the cortisol level in saliva is about 5 to 10% lower than in plasma and its response time is a few minutes slower due to conversion of cortisol to cortisone in the salivary gland by 11ß-deoxysteroid dehydrogenase type 2 (Broom 2000; Cook, 2012).

1.5.1.5. Enzyme and other metabolites

Creatine kinase and lactate dehydrogenase

Creatine kinase (CK) is found in the skeletal muscles of animals and is responsible for maintaining energy homeostasis at the sites of high ATP (Dieni and Storey, 2009). When the use of ATP is increased, the CK activity also increases in order to resynthesize ATP. In brief, creatine phosphate (CP) passes on a high energy phosphate group to adenosine diphosphate (ADP), forming ATP, in a reaction that is catalysed by the enzyme CK (Gregory, 1998).

The CK is measured in the blood serum or plasma as an indicator of muscle tissue damage and physical stress (fatigue), alone or in combination with other indicators (Payne and Payne, 1987; Gregory, 1998). The CK levels in blood increase in response of the demand of the mitochondrial phosphocreatine to resupply ATP for muscle activity (Broom and Johnson, 1993). However the disadvantage of measuring CK activity in serum is that serum is more prone to haemolysis than plasma, especially in stressed animals. When haemolysis occurs there is an artefactual slight increase in apparent CK activity (Gregory, 2016- Personal communication).

Lactate dehydrogenase (LDH) is found extensively in body tissues and its activity levels are exceptionally high in porcine muscles, having a low capillary and mitochondrial density (Pösö and Puolanne, 2005). Its release from sarcoplasm into the blood stream is triggered by vigorous physical exercise or muscle damage (Broom, 2000). The role of the LDH is to catalyze the anaerobic production of ATP through the reduction of pyruvate into lactate in the cell cytosol (Pösö and Puolanne, 2005).

Lactate

Lactate is either released into the blood flow in very disturbed or frightened animals or when there is some muscle damage (bruising) caused by vigorous physical exercise (Broom, 1995). It is the result of the glycogenolysis in the muscle which is initiated by epinephrine released from the adrenal medulla in response to intense physical exercise (Gregory, 1998). In this process, glucose is produced in the form of glucose-6-phosphate (G-6-P) through the breakdown of glycogen in the skeletal muscles and ATP is provided to the muscle cells as energy source (Nelson and Cox, 2008). The conversion of glucose into lactate occurs by an anaerobic process, during intense muscular activity where the oxygen supply is often insufficient and the muscles

become hypoxic (Gregory, 1998). Then, the anaerobic fermentation oxidizes the NADH (nicotinamide adenine dinucleotide + hydrogen) produced by glycolysis back to NAD^+ (Nicotinamide adenine dinucleotide), transferring two electrons from NADH to reduce pyruvate into lactate (Nelson and Cox, 2008). Lactate that is formed is either converted back into pyruvate to be used by an oxidative pathway via the tricarboxylate acid cycle or when there is lack of oxygen and/or mitochondria, transported out of the fibre (Pösö and Puolanne, 2005).

Blood lactate is considered as a very short-term physical stress indicator as it achieved the higher peak in 4 min and returns to basal levels in 2 h after physical exercise (Anderson, 2010).

Traditionally, lactate is measured in blood plasma and serum by enzymatic analytical procedures. However, these methods are time-consuming and only provide an assessment at the end of the slaughter process (exsanguination).

Based on the strong correlation (r = 0.97) between the blood lactate levels obtained with an enzymatic analytical method and the Lactate Scout Analyzer (Edwards et al., 2010a), which is an hand-held device allowing the real-time and rapid (15 sec) assessment of blood lactate, the latter is now used to monitor the quality of preslaughter handling for research and commercial purposes. For example, Edwards et al. (2010b) reported that specific handling behaviours of individual pigs within a *preslaughter* group were correlated with increases in blood lactate levels at exsanguination, such as jamming (r = 0.24), rearing (r = 0.27) and backing up (r = 0.25).

1.5.2. Long-term stress indicators

1.5.2.1. Corticosteroids

Adrenocorticotrophic hormone

The adrenocorticotrophic hormone (ACTH) is a polypeptide tropic hormone produced and secreted by the anterior pituitary gland, which in turn is regulated by the hypothalamic corticotrophin-releasing hormone (CRH). ACTH is synthesized by specialized cells of the anterior pituitary gland (corticotrophs) and its release is triggered by the coordinated action of two neuropeptides, the corticotropin-releasing hormone (CRH) and vasopressin (AVP), that are synthesized in specialized neurons

of the paraventricular nucleus of the hypothalamus (PVN) and released in the capillary bed of the median eminence from where they reach the pituitary directly via the hypothalamic–pituitary portal circulation (Manteuffel, 2002).

The ACTH is often released into the peripheral vasculature which allows it to travel from the cortex of the adrenal gland to bind the receptors, stimulating the production of glucocorticoids (Mills et al., 2010). The levels of ACTH is often released in response to biological stress as it increases the energy availability in the form of glucose (Mills et al., 2010), allowing animals to cope and respond to environmental challenges and stress, such as observed in pigs submitted to large changes in ambient temperature (above +40°C or below -5°C; Blatchford et al., 1978) and tight restrained (Janssens et al., 1995).

Blood levels of ACTH decrease in about 1 to 2 h, depending on the duration and intensity of the ACTH-releasing (Cook et al., 1997). Handling and restraint of the individual may be stressful for animals, *e.g.* eliciting secretion of ACTH and cortisol. Sampling procedures should, therefore, be considered carefully when sampling. To reduce this variation due to the stress induced by sampling, ACTH levels can be measured non-invasively in the urine (Hay and Morméde, 1997) or stress-free in the saliva (Meyerhoff et al., 1988).

1.5.2.2. Other hormones and neurotransmitters

Plasma lysine vasopressin

Lysin vasopressin (LVP) is a nine amino acid peptide hold by a disulfide bridge. However, its structure is species-specific. For example, in pigs lysine takes over arginine in the 8th position. This hormone is synthesized in the supra optic and paraventricular *nuclei* of the hypothalamus and is released from the posterior pituitary gland.

The main function of the LPV is to regulate the volume of water and the ionic composition of the body (Simon, 2008). For this reason, in humans and in pigs it has been used as an indicator of motion sickness and used to study the welfare condition during transportation (Rowe et al. 1987; Bradshaw et al., 1996a).

Prolactin

The reproductive hormone prolactin (PRL) is a peptide secreted from the anterior pituitary gland and from the placenta during gestation (Mills et al., 2010). The plasma PRL secretion may be influenced by behavioural factors in response to a physical or emotional stress, *i.e.* fasting (Tegelman et al., 1986; Matteri et al., 2000). Its response is more passive rather than active (Theorell, 1998). The half-life of PRL in blood varies from 20 to 60 min after stress exposition depending on the extent of the individual reactivity (Broom and Johnson, 2000).

Additionally, reports of increased PRL levels in response to acute stress of restraint and thermal stressors in castrated male pigs have been observed by Klemcke et al. (1987). Likewise acute psychological stress elevating PRL secretion was observed in a variety of species such as in rats (Neill, 1970) and sheep (Matthews and Parrott, 1994).

Beta-endorphin

Beta-endorphin (BE) is an endogenous opioid neurotransmitter with analgesic properties involved in the modulation of pain (Warriss, 2010). The synthesis of BE from the anterior pituitary gland is increased following painful stimuli (O'Benar et al., 1987; Shaw and Tume, 1992) or as sign of fear and excitement (Shaw and Tume, 1990). Bradshaw et al. (1996b) registered differences on the ratios of stress burden, especially the in the plasma concentration of beta-endorphin, of pigs submitted to differents ways of transport journey (rough and smooth).

1.5.3. Behavioural indicators of stress

Behaviour is a sensitive indicator of the physiological status of an animal and is the primary way for an animal to interact with its environment (Mench, 1998). The study of behaviour is not invasive (Mormède, 2008), is quick and appears technically easier to obtain compared with physiological measures (Rushen, 2000).

1.5.3.1. Agonistic behaviour

In group housed pigs, agonistic behaviour is defined as any behaviour associated with threat, attack or defence (Velarde and Geers, 2007). In general, aggressive behaviour is expressed when animal welfare is threatened (Haskell, 1996). Aggressive behaviour is often triggered by food competition (Baxter, 1989) or need to establish a new

hierarchy within the group, like in the case of pigs being mixed with unfamiliar conspecifics (Meese and Ewbank, 1973).

Although aggression is considered a normal pattern of pig's social behaviour, it may impair animal welfare (Mills et al., 2010) as it causes skin lacerations, wounds and damages on the animal body, particularly in the shoulder region (Warriss and Brown, 1985), possibly resulting in pain and, in extreme cases, death. Furthermore, aggressions may lead to physiological stress, as showed by the increased blood catecholamines, ACTH, cortisol and creatine kinase levels and reduction of feed intake (Fraser and Rushen, 1987; Arey and Edwards, 1998; Oliver et al., 1996).

1.5.3.2. Abnormal behaviours

Behaviour is defined as abnormal when it differs in pattern, frequency or context from those shown by most members of the species in normal conditions (Broom and Johnson, 2000). Examples of most common abnormal behaviours are: route-tracing, bar-biting, tongue-rolling (Fraser and Broom 1990), stereotypies (Mills et al., 2010) and tail biting (Beattie et al., 2005).

Stereotypies

The stereotypy is a repeated, relatively invariant sequence of movements with no obvious purpose (Broom, 1981; Mason, 1991; Broom and Johnson, 2000; Mills et al., 2010). Stereotypes are most often observed in confined animals being restricted in their ability to perform certain behavioural patterns (Mason, 1991). Many stereotypies of captive animals seem to stem from natural behaviour (Rushen et al., 1993). For example, stereotypies increase with increasing levels of food deprivation in pigs (Terlouw et al., 1991; Jensen et al., 1993) and decrease if substrates are provided to which naturalistic foraging behaviour can be directed (Spoolder et al., 1995). Furthermore, some generalisations have been made about timing, in which pigs are often typified as performing stereotypies before and after feeding (Terlouw et al., 1991). Terlouw et al. (1991) associated these stereotypies to the lack of space that may inhibit locomotor forms of pre-feeding stereotypies in pigs and to the high post-feeding appetitive behaviour of pigs that may be caused by lack of satiation following food.

According to Broom and Johnson (2000), the welfare status of an individual that shows stereotypes varies, depending on the amount of time dedicated to stereotypes,

reaching very poor status when it represents more than 40% of the activity time. In certain situations, stereotypies are reminiscent of earlier frustration, like mental scars, thus indicating brain dysfunction and chronic suffering or may even help the animals cope with adverse conditions (Fraser and Broom, 1990; Mills et al., 2010).

Tail biting

Tail biting is a major animal welfare and economic issue for the pork industry. Although the occurrence of tail-biting may indicate that some or all pigs within a pen are experiencing poor welfare (Widowski, 2002; Taylor et al., 2010), the major implications of tail biting are related to the welfare of bite receiving pigs, likely suffering from pain and discomfort. Additionally, tail biting has a negative economic impact on pork production economy, with losses due to reduced weight gain, increased on-farm veterinary treatment, culling and carcass condemnation due to physical damages caused to the tail and hindquarters of the bitten pigs (EFSA, 2007). These bruises may lead to death due to blood loss and trauma (Van Putten, 1969; Smulders et al., 2006) and to abscesses resulting from secondary infections (Pointon et al., 1999).

Tail biting is mainly observed in commercial indoor environments in unhealthy pigs (Geers et al., 1989), in pigs kept in barren environments, *i.e* higher stocking density or lack of objects/substrate to satisfactorily root, chew or manipulate (Hansen et al., 1982; Fraser et al., 1991; Beattie et al., 2000) resulting from increased attraction to blood (Edwards, 2006), or from poorly ventilated barns (Chambers et al., 1995).

1.5.3.3. Fear of humans

Humans interact with animals in many moments of life, especially under commercial conditions. In situations where these interactions are close and frequent, their quality may have a considerable impact both on the animal and the handler (Hemsworth and Barnett, 2000). Humans can evoke fear in animals by virtue of their relative size, and their propensity for quick or unpredictable movements (Rushen et al., 1999). Fear and anxiety are two emotional states, that are induced by the perception of a potential danger threatening the integrity of the animal (Boissy, 1995) and aimed to prepare the animal to cope with the danger through physiological and behavioural changes (Forkman et al., 2007). Sources of variation in the expression of fear and anxiety or depression are the genetic background and early life experience (Donald et al., 2011).

Based on the relationship with the stockperson skills, fear of humans is assessed at the farm and preslaughter (Dalmau et al., 2009; Grandin, 2010) using the human-animal relationship (HAR) test (Courboulay and Foubert, 2007) and the observation of fear behaviours, such as escape-avoidance (Hemsworth and Barnett, 2000), reluctance to move (Erhard et al., 1999) and turn-back (Dalmau et al., 2009), that are associated with lower easiness of handling and rough handling (Geverink et al., 1998a).

Practices, like walking in the finishing pens every day to get the pigs accustomed to handling or driving the pigs in the aisle at the farm proved to be efficient in reducing fear to humans and easing handling (Abbott et al. 1997; Grandin, 2000; Geverink et al., 1998a). However, they may also made pigs them harder to drive as they become too tame and not interested to the handler commands (Grandin, 1987).

1.6. Post-mortem animal welfare indicators

1.6.1. Bruises on the carcass

Bruises are subcutaneous lesions that may vary in number, distribution, seriousness, extent, colour, shape and grade (Strappini et al., 2012). The measurement of skin bruises is clearly relevant to welfare assessment and carcass quality, as they can reflect poor social and physical environments (Dalmau et al., 2009). Bruises on the carcass of pigs may be caused by several factors, such as handling on farm, transport condition, aggression, poor handling and poor design of facilities (Faucitano, 2001). A summary of the main causes of carcass bruises on pigs from farm to slaughter are shown on Table 1. The presence of bruises or lesions on the surface of the pork carcass also detracts from the appearance of the carcass leading to up to 6% carcass downgrading in case of severe bruises (MLC, 1985; Warriss, 2010). According to US pork industry estimates, bruises alone contribute to $0.08 value loss per carcass or more than $48 million in annual trim losses (Vansickle, 2002; Schultz-Kaster and Hill, 2006).

The degree of skin bruises on the carcass is usually assessed subjectively along the dressing line using different methods. The assessment can be conducted on the whole carcass or in specific carcass locations, such as head/shoulder, middle/loin and ham. For the whole assessment of carcass, the subjective photographic scales developped by the Meat and livestock commission (MLC, 1985; Fig. 3), based on 5 scores

Table 1. A summary of the main causes of carcass bruises on pigs from farm to slaughter

Preslaugther procedure	**Effects on carcass bruises**	**Reference**
On farm		
Mixing unfamiliar pigs	↑ Carcass bruises	Barton-Gade, 2008
Fasting (1h; 12h; 18h)	↑ Carcass bruises score	Brown et al. 1999
Loading		
Eletric prod use	↑ Carcass bruises	Geverink et al., 1996 Griot and Chevillon, 1997
Transport		
High stocking density (> 0.35 m^2/100 kg)	↑ Carcass bruises	Guise and Penny, 1989 Barton-Gade and Christensen, 1998 Gispert et al., 2000
Season (winter)	↑ Carcass bruises	Gosálvez et al., 2006 Dalla Costa et al., 2007
Truck Compartiment	↑ Carcass bruises	Barton-Gade et al., 1996
Lairage		
Lairage duration > 3h	↑ Carcass bruises	Geverink et al., 1996 Guàrdia et al., 2009
Stocking density	↑ Carcass bruises	Geverink et al., 1996
Stunning chute area		
Eletric prod use	↑ Carcass bruises	Rabaste et al., 2007

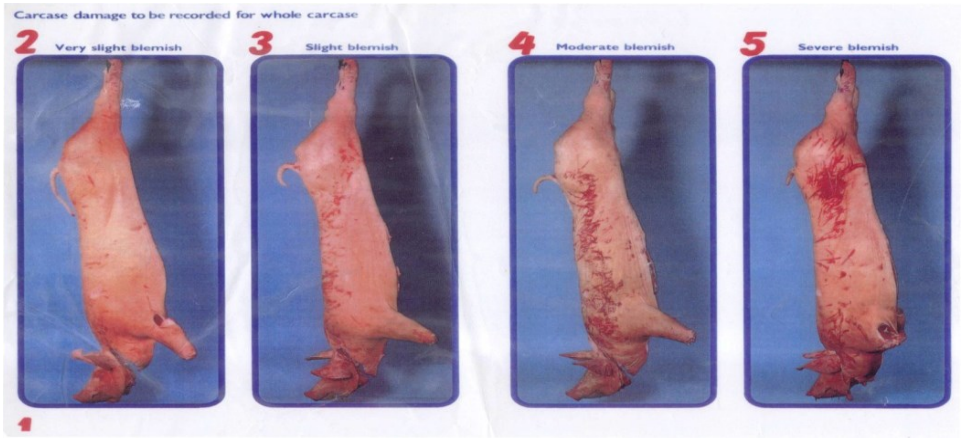

Figure 3. Chart for the assessment of carcass bruises on the whole carcass (from 1: none to 5: severe; MLC, 1985)

ranging from 1 (none) to 5 (severe bruises), can be used on the day of slaughter in the cooler. Furthermore, lesions can be scored by type (fighting-, density- and handling-type) and number according to the photographic standards of the Institute technique du porc (ITP, 1996) as follows: fighting-type bruises (score 1: < 10 bruises; score 2: ≥ 10 bruises), mounting-type bruises (score 1: < 5 bruises; score 2: ≥ 5 bruises) and handling-type bruises (score 1: 1 bruise; score 2: 2 bruises; score 3: 3 or more bruises). In order to distinguish between bruises inflicted at the farm or in the *ante-mortem* period, bruises may also be classified by colour change, indicative of the age of infliction, in terms of "red", corresponding to a fresh bruise (occurred within 10 h), and "dark" for an old bruise (more than 24 h older), as described by Gracey and Collins (1992) and Strappini et al. (2012).

Based on previous work of Barton-Gade et al. (1996), the WQ® (2009a) proposed an assessment by counting of the number of bruises in each of five different anatomical locations of a splitted carcass: 1) ears; 2) front (from the head to the back of the shoulder); 3) legs (from the accessory digit upwards); 4) middle (from the back of the shoulder to the hind-quarters) and 5) hindquarters (Fig. 4). Then, a bruises score is given according to the number of skin bruises at each locations as follows: level a, if all regions had up to 1 lesion; level b, when any location of the body showed from 2 up to 10 lesions; and level c, when at any location on the carcass it was observed more than 10 lesions or any wound which penetrates the muscle tissue. Hence, one of three possible final scores combining the five parts of the carcass is given: 0) all body parts

are considered as ‘a’; 1) when any body part is considered as ‘b’, or 2) when any body part is considered as ‘c’.

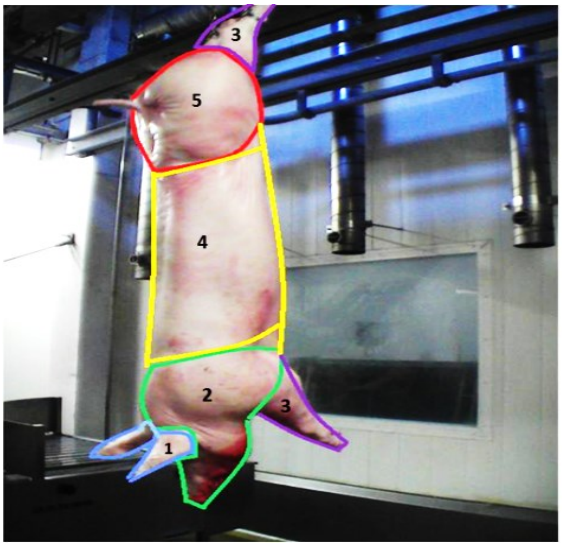

Figure 4. Bruises assessed at five different anatomical locations of the carcass: 1: ears; 2: front; 3: legs; 4: middle; and 5: hindquarters (WQ®, 2009a)

1.6.2. Post-mortem changes in the muscle during its conversion to meat

1.6.2.1. Muscle structure and organization

The skeletal muscle tissue is the most abundant type of muscle in a carcass. Contractile, structural, and regulatory proteins in this muscle type are highly organized into a distinct striated pattern (Weaver, 2012). A simplified scheme of the whole muscle structure is presented in Fig. 5. In summary, connective tissue sheaths are found at various structural levels of each muscle.

The entire muscle is surrounded by a connective tissue sheet, called epimysium, and the cell membrane, called sarcolemma, is surrounded by another connective tissue, called endomysium. The muscles fibres are collected into fibre bundles that are enveloped by other connective tissue, called perimysium. Within the fibre cell, the smallest contractile elements (sarcomere) are collected into a long thread, known as the myofibril (Tornberg, 1996).

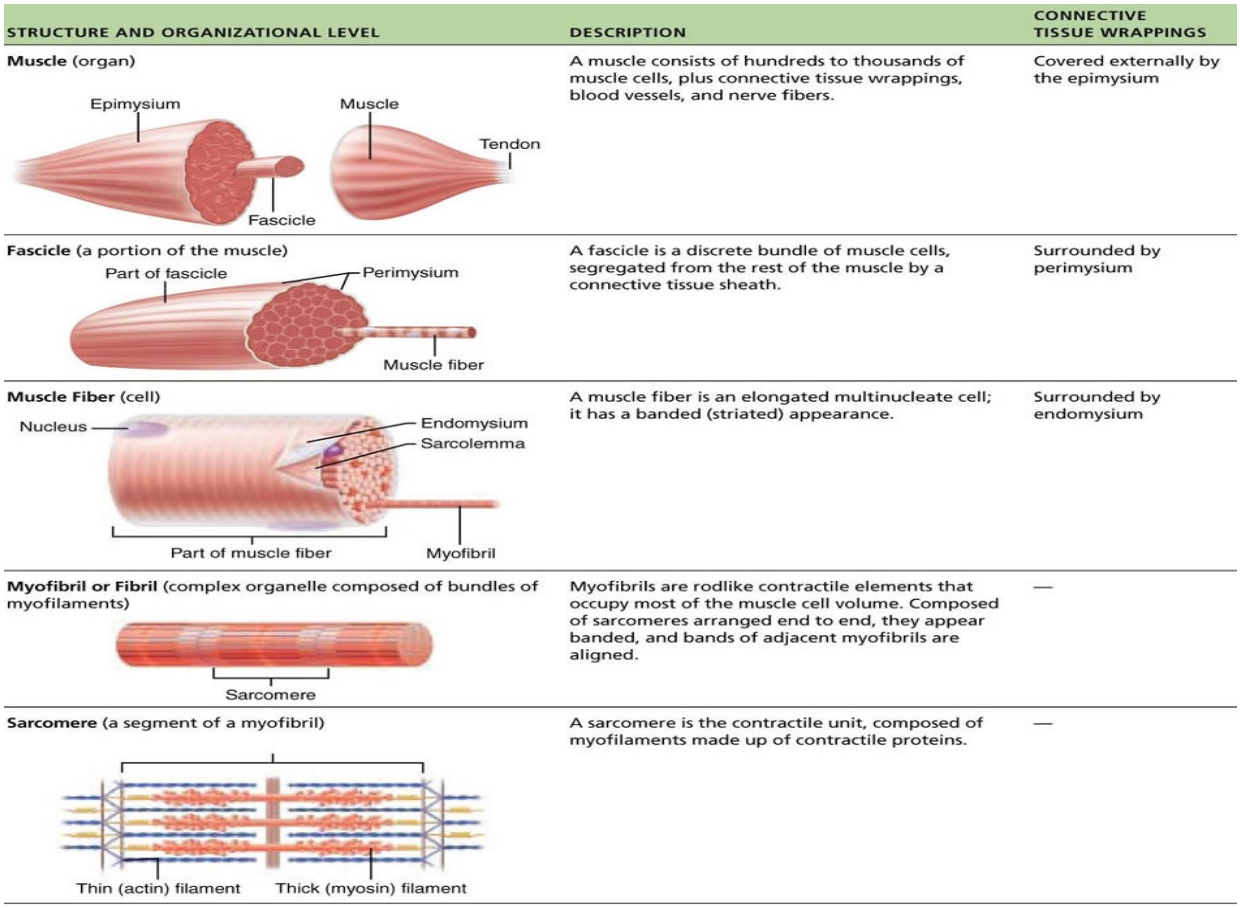

Figure 5. Structure and organization of the skeletal muscle (Marieb, 2006)

Within the myofibrils there are two types of protein myofilament: the thin -actin, and the thick- myosin filament. These are the basic structural components which perform muscle contraction. The myosin filaments have side branches which extend laterally to the actin filaments and they have an adenosine triphosphate (ATP) molecule in the terminal position (Fig. 6; Karlsson et al., 1999). The sliding of myofilaments relative to each other occurs in muscle from living animals with the contraction initiated by the nervous system, when a nerve impulse reaches the sarcolemma of the muscle (Gregory, 1998).

Muscle contraction is a rapid, energetically demanding process that requires the splitting of ATP in order to meet energy requirements for muscle contraction and relaxation, sequestration of calcium and maintenance of ion gradients (Scheffler and Gerrard, 2007). Briefely, the sarcoplasmic reticulum releases Ca^{2+} into the sarcoplasm, and from there it binds to troponin molecules in the actin myofilaments. The binding of Ca^{2+} causes a shape change in the troponin; this in turn causes tropomyosin to move deeper into the groove of the actin. During this binding process

a region of the myosin myofilament is exposed which bears an ATPase enzyme (Swatland, 1994). This enzyme causes the terminal ATP to be broken down to ADP + Pi, with the release of large amounts of energy. When the nerve impulses stop, Ca^{2+} is unbound from the troponin and passes back to the sarcoplasmic reticulum. Finally, when the ATPase in myosin is no longer active, no more ATP is broken down and the muscle relaxes (Gregory, 1998).

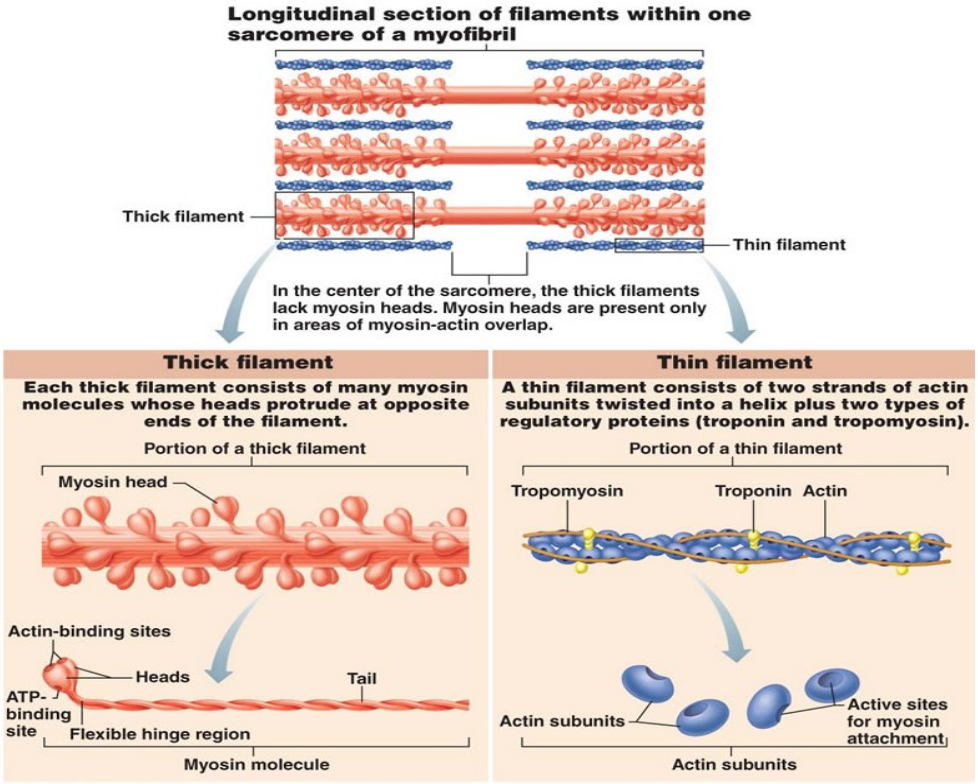

Figure 6. Structure and organization of the thick filament (Marieb, 2006)

1.6.2.1.1. Morphological and biochemical characteristics of muscle fiber

These morphological and biochemical characteristics of muscle fiber types together with glycogen reserves are major factors that influence the energy metabolism within the skeletal muscles of live animals, as well as during the *post-mortem* conversion of muscle to meat (Ryu and Kim, 2005, 2006).

One of the main factors determining muscle biochemical pathways is the fiber type composition (Chang et al., 2003). In principle, two major fiber types (I and II) of skeletal muscle can be divided in four types: I, IIa, IIx and IIb according to their myosin isoforms (Klont et al., 1998). Individual fibre types exhibit different

contractile, metabolic, physiological, chemical and morphological characteristics, as presented in Table 2. In principle, fibers type I low-twitch oxidative exhibit low myofibrillar and sarcoplasmic reticulum ATPase activities, and can sustain prolonged low power work in association with a well-developed oxidative metabolism, an efficient vascularization, and a small diameter. These fibres have a low excitation threshold (high Ca^{2+} sensitivity) and use a large amount of energy *in vivo,* since they are often recruited to tolerate low intensity contractions for basic movements. They are rich in myoglobine, poor in glycogen, and highly resistant to fatigue (Lefaucheur, 2010).

Table 2. Biological characteristics of individual fibre types (Adapted from Lefaucher et al., 2010)

	Fibre types			
Biological characteristics	**I**	**Ia**	**IIx**	**IIb**
Contraction speed	+	+++	++++	+++++
Myofibrillar ATPase	+	+++	++++	+++++
Oxidative metabolism	+++++	++++, +++++	+, ++	+
Glycolytic metabolism	+	++++	++++	+++++
Glycogen	+	+++++	++++	+++++
Vascularization	+++++	+++	+, ++	+
Myoglobine	+++++	++++	++	+
Buffering capacity	+	++++	+++++	+++++
Diameter	++	+, ++	++++	+++++
Fatigue resistance	+++++	++++	++	+

+, very low; ++, low; +++, medium; ++++, high; +++++, very high

On the opposite, glycolytic types IIb fibres exhibit high myofibrillar and sarcoplasmic reticulum ATPase activities, and can sustain brief and intense contractions fueled by immediate availability of phosphocreatine and degradation of local glycogen through the glycolytic pathway. These fibres exhibit a high excitation threshold and do not use a large amount of energy *in vivo* because they are only occasionally recruited to sustain violent movements of short duration, *e.g.* to escape immediate danger. In addition to the low vascularization and a large diameter, glycolytic types IIb fibres are rich in glycogen, highly fatigable and poor in myoglobine (Lefaucheur, 2010).

Finally, characteristics of type IIx fibres are close to those of IIb fibres, however they speed of contraction is lower and their oxidative metabolism slightly higher compared to IIb fibres whereas type IIa fibres exhibit intermediate contractile and metabolic properties between types I and IIx fibres (Lefaucheur, 2010).

1.6.2.1.2. Factors influencing muscle fibre number and size

It is suggested that animals with greater number of muscle fibres of moderate size produce a higher quantity and quality of meat (Rehfeldt et al., 2004). Whereas total muscle fibre number in mammals and birds is determined at birth and does not change further, postnatal growth and skeletal muscle mass is determined by the variation in muscle fibre size (hypertrophy; Rehfeldt et al., 2004).

Muscle fibre size is influenced by species-specific differences in muscle mass, primarily due to differences in the total number of muscle fibres (Rehfeldt et al., 2004). For example, studies of Vigneron et al. (1976), Fiedler (1983) and Wegner et al. (2000), comparing identical muscles between rabbit, pig and cattle, respectively, reported that the pig *longissimus* muscle contains 6 to 7 times as many fibres as rabbit *longissimus,* but fibre diameters only twice as large. Contrary, cattle exhibits 3 to 4 times as many fibre numbers as pigs, but same size, or even smaller muscle fibres. Possibly the evolutionary increase in muscle fibre size is limited by physiological reasons in that normal cell function is maintained until certain limits, which could limit cell size (Rehfeldt et al., 2004).

Moreover, differences in muscle fibre number may arise between males and females (Rehfeldt et al., 2004). Petersen et al. (1998) reported sex-related differences in the size of muscle fibres for pigs, in which partially larger fibres have been observed in females compared to entire males. This difference occurs because of hormonal action, especially androgen hormones, which are sufficiently high during the period of prenatal fibre formation (Rehfeldt et al., 2004).

Another important factor affecting muscle fibre number and size are the effects of maternal feeding, although, there are no consistent results of the effects of prenatal undernutrition caused by maternal constraints *in utero.* However, results of Rehfeldt and Kuhn (2006) suggested that transitional feed restriction has no permanent effect, whereas severely restricted feed intake throughout gestation shows long-term detrimental effects on postnatal (muscle) growth.

Physical activity, by housing system or activity-induced, is also an important factor that influences adaptations in muscle, causing hypertrophia. It is well know that different housing systems allow varied degrees of physical activity for farm animals (*e.g.* indoor *vs.* outdoor rearing systems; Petersen et al., 1998; Bee, 2002; Gentry et al., 2002). Activity-induced muscle growth has been reported to be accompanied by changes in muscle fibre size and number. For example, outdoor rearing system, which allows more physical activity, has been related to increases in the aerobic capacity of the muscle. In addition to this aerobic capacity, in some muscles, the physical activity leads to decreased muscle fibre diameters of type I and/or IIa (Bee, 2002; Gentry et al., 2002) or increased type I diameters (Petersen et al., 1998). Moreover, Petersen et al. (1998) also observed that 10 weeks of exercise training could increase fibre size in trained pigs.

Another factor that has been largely studied is the hypertrophy of muscle fibers caused by the use of β-agonist growth-promoting in pigs, which leads to a reduction of proteolytic activity and an increased protein synthesis in response to its administration (Adeola et al., 1992; Depreux et al., 2002; Rehfeldt et al., 2004; Rocha et al., 2013; Hui et al., 2015). Moreover, Depreux et al. (2002) also observed an increased abundance of type IIb myosin heavy chain and a decreased type IIx when pigs were fed a high dose (60 ppm) of ractopamine, whereas Hui et al. (2015) reported a larger type IIx fibers and lower glycolytic potential in ractopamine-fed pigs (20 ppm), compared with pigs not ractopamine-fed.

1.6.2.2. Muscle glycogen reserves

Glycogen is a polymer of glucose that is stored in muscle for ATP synthesis (Przybylski et al., 2006). Shortly after the commencement of the *post-mortem* phase anaerobic metabolism of glycogen into ATP takes over, and the waste product from this process is lactate. In brief, glycogen is broken down by glycogen phosphorylase and glycogen debranching enzyme. Glycogen phosphorylase cleaves the outer chains of glycogen, generating glucose 1-phosphate, which is isomerized to glucose 6-phosphate (G-6-P). Glycogen debranching enzyme breaks α-1,6 linkages, releasing free glucose. As a result, free glucose and G-6-P, as well as lactate, are the main metabolites from glycolysis that accumulate in the muscle (Scheffler et al., 2013).

The amount of glycogen in the muscle tissue depends on the animal species and type of muscle (Przybylski et al., 1994). The highest value was found on the *longissimus* muscle of pigs (154 ± 53 μmol/g fresh tissue), whereas the lowest value was found in the *semispinalis capitis* (54 ± 24 μmol/g fresh tissue), as describe on Table 3. Glycogen content can also be influenced by some genetic traits being greater in pigs carrying the dominant RN^- allele of the RN syndrome (Rendement Napole; Monin and Sellier, 1985) and the Hal gene (Przybylski et al., 2006).

Table 3. Glycolytic potential in different animal species and muscles (Przybylski et al., 1994)

Species	**Muscle**	**Glycolytic potential (μmol/g fresh tissue)**
Pigs	*Semimembranosus*	116 ± 39
	Semispinalis capitis	54 ± 24
	Longissimus	154 ± 53
Calves	*Psoas major*	133 ± 38
	Rectus abdominis	107 ± 55
Sheep	*Supraspinatus*	102 ± 37
	Pectoralis profundus	102 ± 30
	Semitendinosus	100 ± 33
	Rectus abdominis	70 ± 18

The glycogen level varies also among pig breeds. Modern pig breeds can be divided into three groups according to the level of glycogen and the meat quality (low, medium and high level). Breeds Large White (or Yorkshire), Piétrain and Duroc can be classified as having a low level of glycogen and good meat quality when they do not carry the Haln/RYR1T gene. Landrace pigs show medium levels with satisfatory pork quality when they are not carriers of that same gene, and Hampshire pigs exhibit frequently an abnormally high level of glycogen (about 70% more than Large White pigs in the white muscles) and may present the so-called “acid meat” (Monin and Sellier, 1985; Lundström et al., 1996; Przybylski et al., 1996).

Furthermore, muscle glycogen levels are also affected by other factors, such as fasting time and transport duration. Fasting-induced depletion of muscle glycolytic potential was reported in studies in which fasting times varying between 24 to 48 h (Warriss, 1982; Jones et al., 1985; Wittman et al., 1994; Partanen et al., 2007). Conversely, in other studies, muscle glycogen and glycolytic potential of pigs were not affected by up to 48 h (Warriss and Brown, 1983; Leheska et al., 2003) or 60 h (Bidner, 1999) of feed withdrawal.

Depletion of muscle glycolytic potential induced by transport time was reported in pigs submitted to 8 h transportation, compared to 0.5 h and 2.5 h (95.5 *vs.* 112.2 *vs.* 104.4 μmol lactate/g, respectively; Leheska et al., 2003). However, in other studies, muscle glycogen of pigs was not affected by up to 6 h transportation (Warriss et al., 1983).

Finally, another factor that affects muscle glycogen reserves is the rearing system and diet. Foury et al. (2005), for example, observed a greater residual glycogen in the muscle in pigs raised in enriched housing (*i.e.* straw-bedding) conditions and Rosenvold et al. (2001) reported that the use of high fibers content in diets of swine was related to a decreased glycogen level in the muscle at slaughter and to better meat quality

Muscle glycogen can be measured alone or within the glycolytic potential (GP), which is a measure of all compounds present in the muscle that can be converted into lactic acid. The GP is an indicator of the muscle's capacity for *post-mortem* glycolysis, and, therefore, of the potential extent of muscle pH decline after slaughter (Monin and Sellier, 1985).

1.6.2.4. Rigor mortis

The major physico-chemical change that occurs in the *post-mortem* muscle is the development of *rigor mortis*. The *rigor mortis* is the process resulting in the conversion, by biochemical reactions, of the muscle tissue from an extensible and metabolically active system into one that is inextensible and quiescent (Gueaser and Guo, 2012). In brief, after exanguination, the supply of oxygen, glucose and free fatty acids to the muscles ceases as the blood circulatory systems stops. In this condition, ATP can only be regenerated through the breakdown of glycogen by glycolysis

(anaerobic metabolism), generating and accumulating lactate in the muscle, which leads to a low intracellular pH (Warriss, 2010).

During *rigor mortis*, ATP decreases from approximately 6.0 to 0.7 μm/g and creatine phosphate from 6.3 to 0 μm/g at 4 h *post-mortem*. When the ATP levels drop at a very low level (5 mmol/kg $^{-1}$) and $Ca2^{+}$ levels raise due to disability of dying cells to exclude calcium (which is in higher concentration in the extracellular fluid), the calcium influx into muscle cells promotes formation of myosin cross-bridges (Maltin et al., 2003; Gueaser and Guo, 2012). However, as ATP levels are very low, myosin cross-bridges cannot be detached and actin and myosin become irreversibly cross-linked, producing the stiffness and the completion of *rigor mortis*, which gradually disappears as muscle proteins break down after death.

1.6.3. Meat quality

In general, meat is considered a highly unpredictable product, since its technological parameters are the result of muscle metabolism processes that occurs throughout the preslaughter procedure (Dingboom and Weijs, 2004; Ryu and Kim, 2005).

Meat quality is a generic term used to describe properties and perceptions of meat, such as combination of subjective and objective measurements of meat characteristics. These measures includes attributes such as carcass composition and conformation, the eating quality, health issues associated with meat production, animal welfare and environmental impact, among others (Maltin et al., 2003; Warriss, 2010). However, in this topic, the term "meat quality" does not refer only to quality properties of meat for human consumption, but will be also used as an indicator of the muscle physiology response to preslaughter stressors (Faucitano and Geverink, 2008).

1.6.3.1. Technological characteristics

1.6.3.1.1. pH

The pH rate is one of the most important *post-mortem* changes in muscles. The *post-mortem* pH of meat is determined by the amount of lactic acid, muscular physiologic condition at stunning and the capacity of the muscle to produce energy (Immonen et al., 2000; Honikel, 2004).

Muscle pH is generally measured within one hour after slaughter (pHi) and/or within 24 h (pH24 or ultimate pH, pHu) *post-mortem* (Barton-Gade et al., 1996). Overall, in

the pork loin muscle the desired pH values at 60 min *post-mortem* ranges from 6.3 to 6.7 (NPPC, 2001), whereas pHu is expected to range from 5.5 to 6.0 at around 24 h *post-mortem* (Warriss, 2010). A pH value of lower than 6.0 at 45 min *post-mortem* in the pork muscle is an indicator of abnormal muscle acidification due to ante-mortem acute stress. Whereas, a pHu value above 6.0 is an indicator of *ante-mortem* glycogen content exshaustion due to chronic stress.

Muscle pH is usually measured electrochemically in the muscles s*emimembranosus* or *longissimus dorsi or longissimus lumborum*, using either glass electrodes or solid-state chip electrodes (Andersen et al., 1999) in alternative to the obsolete and innacurate colouring methods (solution or paper strip pH indicators) with a range error of about ±0.2 pH units.

1.6.3.1.2. Colour

Colour and appearance of fresh meat are major factors in consumer purchase decisions because they are considered reliable indicators of meat freshness and quality (Brewer et al., 2002). Consumers associate bright red colour with freshness of raw meat, and grey or tan colour with cooked meat (Cornforth and Jayasingh, 2004).

The appearance, or colour impression, is determined by the scattering properties of the meat, which in turn are dependent on the amount of, and the chemical form of, myoglobin (Mb). Myoglobin is the primary meat pigment, but low levels of blood haemoglobin and other haem pigments may also be present (Cornforth and Jayasingh, 2004). Myoglobin is a single-chain globular protein, whose role is to store oxygen (O_2) in the muscle once it is released by hemoglobin in the capillaries and spreads inside the myocytes before entering the oxidative phosphorylation cycle to produce new energy (Castigliego et al., 2012).

The muscle myoglobin content varies by species, breed, sex, age, muscle fibers type and the biochemical state of the iron (Fe^{2+}ou Fe^{3+}; Ledward, 1992). According to Stewart (1965), myoglobin is present in the muscle under three main forms: deoxymyoglobin (Mb^{2+}), oxymyoglobin (OMb^{2+}) and metmyoglobin (MMb^{3+}), each one providing a different colour to the meat. The interconversion of the myoglobin form depends on pigment (myoglobin and hemoglobin) concentration, chemical states, association with the process of oxygenation and oxidation of myoglobin, and stress (Honikel, 1998; Klont et al., 1998; Lawrie, 2002).

Meat oxygenation or 'blooming' occurs within 10-30 min after the fresh cut is exposed to air allowing oxygen to bind to myoglobin (Cornforth and Jayasingh, 2004). Under very low O_2 tension, iron in the form of Fe^{2+} tends to interact ionically with H_2O when no other electron donors are present in the pocket of the protein (Mb^{2+}), providing a dark-red purple colour to the tissue. Under higher O_2 tension, the molecule tends to occupy the sixth coordination site and binds the iron, which maintains the lower oxidation state (Fe^{2+}):

$$Mb^{2+} + O_2 \Leftrightarrow Mb^{2+}O_2$$

This complex determines the bright red colour that is typical of fresh good quality meat. The two forms, Mb^{2+} and OMb^{2+}, are rapidly convertible, even if the reaction is influenced by the temperature, O_2 partial pressure, pH, and possible competitors binding the iron ions. The formation of MMb^{3+} occurs when iron interacts with a molecule of water, resulting in a covalent bound between Fe and a hydroxyl group. The formation of MMb^{3+} occurs rather slowly (over days) in the presence of air (20% oxygen; Cornforth and Jayasingh, 2004). This determines a shift in the meat colour towards brown and its extent is related to the proportion of MMb^{3+} and to the amount of the meat surface with a high percentage of MMb^{3+} (Castigliego et al., 2012).

Pork colour can be measured subjectively and instrumentally. The subjective evaluation is either carried out using the Japanese Pork Colour Standards (JCS), ranging from 1 (extremely light) to 6 (extremely dark; Nakai et al., 1975), or the National Pork Producers Council Pork Quality Standards (NPPC, 1999). The NPPC standard range from 1 (pale pinkish gray to white) to 6 (dark purplish red). The instrumental or objective colour assessment is based on the colourimetric scale CIE L^*, a^*, b^* (CIELAB, 1986). The L^* value refers to the lightness, where 0 is associated with black (complete absorption of light) and 100 with white (complete reflection). The a^* value indicates the level of redness or greenness, since the two colours are complementary, ranging from -60 (pure green) to +60 (pure red). Finally, the b^* value indicates yellowness (or blueness), also ranging from -60 (pure blue) to +60 (pure yellow).

1.6.3.1.3. Drip loss

Drip loss is an expression of the meat's lack of ability to hold on to the natural meat juices in the muscle and muscle fibers (Rasmussen and Andersson, 1996). A drip loss higher than 5% or lower than 2% are undesirable for further processing and fresh consumption resulting in economic losses, which in case of excessive exudation, are estimated at $5/carcass (Murray, 2001), and up to 40% unmarketable product (Grandin, 1993). The water-holding capacity (WHC) is one of the most important traits in pork quality and is a constant problem in the pork industry due to its financial implications.

Drip or purge is a red liquid with about 85% water containing an average of 10% of protein or 112 mg of protein per milliliter of fluid (Savage et al., 1990). Most proteins are water-soluble sarcoplasmic proteins, mainly myoglobin. Glycolytic enzymes, aminoacids and water-soluble vitamins are also present in the purge (Savage et al., 1990).

The most common methods used to measure the water-holding capacity of pork are the press method (Grau and Hamm, 1953), the filter paper or capillary method (Kauffman et al., 1986), the gravity method using a suspended plastic bag or the EZ-Drip Loss technique (Honikel, 1987; Rasmussen and Andersson, 1996; Correa et al., 2007).

Drip loss variation is influenced by intrinsic and extrinsic factors. Among the intrinsic factors, genotype, breed, sex and age, muscle type and location within muscle and *post-mortem* rate and extent of pH fall (Huff-Lonergan and Lonergan, 2005) are the most studied.

Muscle *post-mortem* acidification is directly related to main meat quality characteristics, especially meat color (Brewer et al., 2001) and WHC (Joo et al., 1999; Schäefer et al., 2002), due to its effects on protein structure and hydration properties. Decreases of pH towards values closer to the isoelectric point of most myofibrillar proteins (*i.e.* around 5) shrink myofibrils (Offer, 1991; Enfält et al., 1997), resulting in low pHu that will lead to meat proteins with reduced WHC and lighter colour (Hammelman et al., 2003). Fluids leakage from muscle cells and, consequently, drip loss is intensified by the shrinkage of myofibrils due to myosin denaturation,

enhancing meat lightscattering power and contributing to lightening of colour intensity (Monin, 2004). Conversely, a lower rate of pH decline results in high pHu which promotes a dark meat colour and increased WHC.

Besides the muscle pH fall rate and extent, other metabolic factors affecting the WHC of meat are the *post-mortem* muscle temperature, the degree of muscle shortening (Honikel et al., 1986) and the degradation of intermediate filaments and cytoskeletal proteins (Huff-Lonergan and Lonergan, 2005) during *rigor mortis* development. According to Offer and Knight (1988), severe denaturation affects the protein's ability to bind water and results in a poor WHC, even though only approximately 0.5 g of water *per* gram of protein is estimated to be tightly bound to proteins. Increased purge loss in meat have been also associated to the *post-mortem* degradation of proteins, such as talin and vinculin (Bee et al., 2004), and desmin (Davis et al., 2004; Huff-Lonergan and Lonergan, 2005). Additionally, the degradation of some membrane proteins, such as integrin, in the first hour *post-mortem*, has also been reported as contributing to the formation of drip channels and, thus, to the reduced ability of holding water from the muscle cell (Lawson et al., 2004). Finally, genetic effect is one of the most common intrinsic factors associated with excessive purge/drip loss in pork, such as mutations in the ryanodine receptor due to the halothane gene (Fujii et al., 1991) or in the porcine protein kinase adenosine monophosphate-activated c3-subunit gene, often referred as the Rendement Napole in Hampshire pigs (Monin and Sellier, 1985).

Moreover, based on the metabolic characteristics of fiber types, fast white fibers would be expected to be more actively recruited in the anaerobic *post-mortem* environment, resulting in higher extent of protein denaturation (lower protein solubility) with subsequent effects on meat color and drip loss. These effects have been demonstrated in practice in previous studies of Ryu and Kim (2005), Choi et al. (2006; 2007) and Choe et al. (2008). In contrast, muscle with a high proportion of slow red fibers and low proportions of fast white fibers have been shown to be associated with low levels of lactate accumulation at 45 min *post-mortem*, a higher protein solubility and corresponding darker muscle with lower drip losses (Choe et al., 2008).

Some extrinsic factors including nutrition, raising conditions, preslaughter handling and carcass handling (chilling, cutting, boning, slicling and retail packing) has also been related to increased drip loss in pig muscles (Huff-Lonergan and Lonergan, 2005). There is evidence that dietary modifications may offset the negative effects on pork quality, especially in the WHC. Feeding pigs low-starch/high-fat diets for 22–27 days before slaughter have effectively reduced muscle glycogen concentrations (Rosenvold et al., 2001), resulting in improvements in pork colour and WHC (Rosenvold et al., 2001; 2002). An energy restriction of 80% (Lee et al., 2002) did not alter pork *longissimus dorsi* drip loss percentages, but restricting feed intake reduced *longissimus* muscle drip loss (Wood et al., 1996). Additionally, drip loss percentage was reducted in *longissimus* muscle as the lysine content of the diet increased from 0.6% to 1.0% (Goodband et al., 1990). Conversely, drip loss percentage decreased in *semimembranosus* muscle as lysine content increased from 0.6% to 1.2% (Goodband et al., 1990). Moreover, previous studies reported that WHC of fresh pork does not appear to be affected by the cereal grain source (Lampe et al., 2004; Carr et al., 2005a) or protein source (Szabo´ et al., 2001; Apple et al., 2003) used to formulate swine finishing diets.

Regarding the raising systems, it has been reported that pigs raised under free-range conditions produce meat with less drip loss than pigs raised under intensively systems (Lambooij et al., 2004; Pugliese et al., 2005). Studies of Klont et al. (2001) also observed increased water-holding capacity in pork meat of animals housed in enriched environment, compared with that of animals kept in a conventional system.

Finally, animal handling before slaughter (Schäefer et al., 2002; Hambrecht et al., 2005) and *post mortem* carcass handling (Hambrecht et al., 2004) have also been investigated as means of alleviating stress-induced meat quality problems. According to Van der Wal et al. (1999) reduced water holding capacity 24 h *post mortem* was observed in pigs experiencing a short-term acute stress (1 min) immediately before stunning, which involved excitement or forcing pigs from the resting facility to the stunning pen.

Once animals are slaughtered, one of the first factors impacting upon pork quality is the carcass temperature. The carcass temperature decrease is vital to reduce or inhibit microbial growth. However, the rate and extent of carcass chilling has been shown to

have a negatively impact on fresh pork quality, by influencing sarcomere length and *post-mortem* proteolysis (Bendall, 1973; Shackelford et al., 2012). McFarlane and Unruh (1996) and Rybarczyk et al. (2015) observed that loins from blast chilled sides had reduced purge loss.

1.6.3.2. Meat quality classification

Traditionally, raw pork has been classified into three quality categories, namely reddish-pink, firm and non-exudative (RFN), pale, very soft and exudative (PSE) and dark, firm and dry (DFD) meat, according to three main technological parameters, namely ultimate pH (pHu), colour (L* value) and water-holding capacity (WHC) or drip loss. Over the past few years, for a more reliable quality assessment taking into account the variation in either colour or exudate, additional quality categories, such as reddish-pink, firm and exudative (RSE) and pale, firm and non-exudative (PFN) meat, have been described (Kauffman et al., 1992, 1993; Van Laack and Kauffman, 1999; Table 4).

Table 4. Pork quality classification according to pHu, drip loss (DL) and objective colour (L*) variation (modified from Correa et al., 2007)

Quality class	pHu	DL	L*
PSE	<5.5	>5%	>50
PFN	5.5 – 5.8	<5%	>50
RSE	5.6 – 5.8	>5%	42-50
RFN	5.6 – 5.8	<5%	42-50
DFD	> 6.1	<2%	≤42

PSE = pale, soft and exudative; PFN = Pale, firm, non-exudative; RSE = reddish-pink, firm, exudative; RFN = reddish-pink, firm, non-exudative and DFD = dark, firm, dry

Pork of the RFN quality class is described by a pHu value between 5.6 and 5.8, a desired colour (reddish-pink; Minolta L* value ranging from 42 to 50) and a drip loss percent between 2 and 5% (Kauffman et al., 1992; Warner et al., 1997; Joo et al., 1999; Correa et al., 2007). However, when the impact of preslaughter stressors on the *ante-mortem* metabolism of muscle cells is not controlled, RFN pork can turn into

PSE or DFD pork (Warriss, 2010), which are commonly considered as acute and chronic stress-related muscle disorders, respectively.

PSE quality class is described as having a pH value lower than 5.8 at 1 h *post-mortem* (pH1) and between 5.5 and 5.7 at 24 h *post-mortem* (pHu; Sellier and Monin, 1994), a drip loss higher than 5 % (Kauffman et al., 1992; Warner et al., 1997; Joo et al., 1999) and Minolta colour L* value higher than 50.

Pork of the DFD is defined by a high pHu (> 6.0), low drip loss (< 2%) and colour L* values lower than 42 (Warner, 1994; Correa et al., 2007). In addition to its unattractive appearance, DFD meat is susceptible to *post-mortem* microbial growth resulting in shorter shelf-life (Gill, 1976).

PFN pork has normal structure (firmness) as RFN pork, and pHu (5.5 – 5.8; Correa et al., 2007), but as pale as PSE (> 50; Kauffman et al., 1992; Correa et al., 2007). While RSE pork is characterised by a normal pHu value (between 5.6 and 5.8; Van Laack et al., 1994; Correa et al., 2007) and a reddish-pink colour (L* between 42 and 50; Warner et al., 1997; Correa et al., 2007), but presents a drip loss as high as PSE (>5 %; Kauffman et al., 1992; Warner et al., 1997; Joo et al., 1999).

The PFN and RSE meat quality classes have been recently reported as major quality defects in Canada, representing more from 13 to 47% of meat quality defects compared with PSE (13 to 21 %) and DFD pork (2 to 10%; Murray, 2001; Faucitano et al., 2010a). Moreover, Cheah et al., (1998) reported that the skeletal muscle test using biopsy *longissimus dorsis* muscle could be employed to reduce the incidence of RSE-meat, based on good correlations observed between biopsy fluid values, used as an indicator of water-holding capacity, and drip loss from *post-mortem longissimus dorsi* muscle, and between biopsy pH. However, due to the economic importance, the causes of the occurrence of RSE meat need to be deeply studied.

1.7. Assessing animal welfare

There is an increasing need for credible on-farm assessment systems to determine the welfare status of animals. These systems should provide a standard way of converting science-based welfare-related measures into information that is easily understood by the consumer, thereby addressing consumers' specific concerns and allowing the marketing of the product (Blokhuis et al., 2008).

1.7.1. Audit

Auditing is defined as an exercise aiming at validating the degree of correspondence between claims for economical actions and established assessment criteria for interested users (American Accounting Association, 1973). The audits are characterized by an emphasis on quantifiable and easily observable outcomes (FAWC, 2001).

The application of animal welfare auditing protocols allows the evaluation of handling and slaughter practices, resulting in a significant improvement in handling practices, facilities design and quality of work, besides increasing market opportunities due to the availability of certified products (Grandin, 2005, 2007; Ballantyne, 2006).

Because of these benefits, animal welfare auditing at slaughter plants has greatly increased since 1999 in the US, Australia, New Zealand and Europe (Grandin and Smith, 2004), and in Canada since 2001.

The audits within the meat industry started upon request from McDonald's Corporation and Wendy's International in response to demands from the People for the Ethical Treatment of Animals, which is an American animal rights organization (Grandin and Smith, 2004). This organization then, developed and implemented science-based animal care guidelines in response to consumer concerns that animals raised for food production must be treated humanely. However, since the documentation programs on animal well-being are relatively new, the welfare outcome of an animal welfare auditing is dependent on the level and specificity of the standards, rigour and consistency with which they are enforced. In fact, the transparency and the rigour with which an audit is conducted are central to the credibility of any audit protocol (FAWC, 2001).

1.7.1.1. Objectives of audit

Basically, on one side the audit is a verification tool for public inspectors as it helps to check the compliance to legislative requirements defined by societal demands (Edwards, 2008), but also helps to control whether the rules regulating farm animal production are actually enforced by the authorities (FAWC, 2001). For the production chain, it helps to monitor the conditions that may affect production efficiency and contributes to the marketing of differentiated products (Edwards, 2008).

1.7.1.2. Types of audits

There are three important types of audits, which can be performed by a first, second or third party. A first party or self-audit audit is usually performed by the company itself. Second party audit is external audits, which is performed by an organizational quality program and is usually performed by the customer upon its suppliers to ascertain whether or not the supplier can meet existing or proposed contractual requirements or specifications. Finally, a third party audit is an external audit as well. However, the assessment of a quality system is conducted by an independent, external auditor or team of auditors who, in case of successful audit, deliver a certificate of conformance to the company (Baysinger, 2000; Edwards, 2008).

1.7.1.3. Animal welfare audit protocols

Nowadays, the three main protocols used to assess animal welfare through the pork chain (from farm to slaughter) are the audit protocol developed by the American Meat Institute (AMI, 2012), the "Animal Care Assessment" developed by the Canadian Pork Council (CPC, 2011), and the Welfare Quality assessement developed by developed by researchers of the European project Welfare Quality® (WQ®, 2009a).

The AMI audit protocol (AMI, 2012) is based on the "Recommended animal handling guidelines and audit guide" and has the objective to evaluate animal welfare during transport and at the slaughter plant through measures based on the observation of animal behaviour and the assessment of facilities design. Its objective is to help improve the quality of transport and slaughter operations, while ensuring safety, efficiency and profitability for the industry.

The Animal Care Assessment (ACA) protocol (CPC, 2011), which is part of the Canadian Quality Assurance® (CQA), was developed for the evaluation of overall animal welfare at the farm level only. The ACA guidelines do not consider animal-based criteria, but only focus on the measurement of the resources supplied to the animal, *i.e.* resource and management-based measures, such as evaluation of staff training, quality of handling and facilities, comfort and nutrition of pigs at the farm based on the Canadian Codes of Practice (NFACC, 2014). Since 2012, self-auditing through this protocol has become mandatory for Canadian swine producers who wish to obtain the CQA certification.

The WQ® protocol (WQ®, 2009a) was developed to enable an overall assessment of animal welfare and standardised conversion of measures welfare into summary information, based on the following four animal welfare principles: good feeding, good housing, good health and appropriate behaviour, (Dalmau et al., 2009; Temple et al., 2011). This protocol provides a reliable on-farm welfare assessment system by integrating different measurements in an objective and scientific way. One of the innovations of the WQ® assessment system is its greater focus on animal-based measures (*e.g.* body condition, health, injuries, behaviour, etc.) rather than on resource and management-based measures (*e.g.* space allowance, number of feeders, etc.; Table 5).

1.8. Assessing animal welfare at the farm level

1.8.1. Good feeding

1.8.1.1. Feeding

Pigs must have access to balanced diets at each stage of growth (CPC, 2011). There are a variety of factors that influence feeding efficacy, such as feeding patterns, social facilitation, palatability, ambient temperature, digestive factors, hormonal effect and imbalance of dietary nutrients, among others (Houpt, 2010). The quality of the feed supplied can be evaluated through questionnaires or farm records, or by the conformity of diet specifications to nutritional standards. However, this evaluation does not ensure the correct implementation. For this reason, the evaluation should be made using parameters, such as the number of feeders and drinkers provided to each group of pigs and their state of cleanliness and proper functioning. Additionally, inappropriate nutrition can be easily detected by the observation of animal behaviour (Kyriazakis and Savory, 1997) and body conditions (Edwards, 2008). The body condition score is the result of the feeding strategy associated with well balanced diets and animal nutritional and fitness conditions (Courboulay, 2007).

1.8.2. Good housing

1.8.2.1. Housing

Many of the requirements for good physical and health conditions for pigs at the farm are closely associated with those for good biological function and good housing. Good housing does not only include the provision of an environment causing no

Table 5. Measures of the welfare assessment of growing pigs on farms using the Welfare Quality protocol (WQ®, 2009a)

Welfare criteria	Measures
Good feeding	
Absence of prolonged hunger	Body condition score
Absence of prolonged thirst	Water supply
Good housing	
Comfort around resting	Bursitis, absence of manure on the body
Thermal comfort	Shivering, panting, huddling
Ease of movement	Space allowance
Good health	
Absence of injuries	Lameness, wounds on body, tail biting
Absence of disease	Coughing, sneezing, pumping, twisted snouts, rectal prolapse, scouring, skin condition, ruptures, mortality and hernias
Absence of pain induced by management procedures	Castration, tail docking
Appropriate behaviour	
Expression of social behaviours	Social behaviour
Expression of other behaviours	Exploratory behaviour
Good human–animal relationship	Fear of human beings
Positive emotional state	Qualitative behaviour assessment

visible injuries, but also includes the provision of adequate physical comfort, in terms of thermal environmental, lighting, cleanliness, space allowance, flooring, air flow rate and speed, etc.

1.8.2.2. Thermal environment

The thermal environment in animal housing is important due to its direct effects on the animal metabolic rate and performance, and its indirect effects on the animal

health and comfort (Clark and McArthur, 1994). In this respect, the so-called "thermoneutral zone", representing the range of ambient temperatures within which the body temperature remains constant with minimal thermoregulation effort, has to be coinsidered. The thermoneutral zone for a growing-finishing pigs is between 18 and 21˚C, with temperatures above 27˚C and below 4˚C being critical (Leal and Nããs, 1992; Huynh et al., 2004a,b).

Compared with livestock species, pigs are more sensitive to high environmental temperatures, because they have a limited capacity to lose heat by water evaporation from the skin and do not pant effectively (Ingram, 1965). Therefore, in extreme situations of high temperature and humidity, when the heat loss is higher than the heat production, hyperthermia may occur, resulting in decreased feed intake, low performance and eventually death (Huynh et al., 2005). Conversely, in extreme situations of low temperatures, when the temperature loss becomes greater than the heat generation, pigs may suffer from hypothermia, resulting in pigs closer to each other to limit heat loss, increased food intake and may result in poor meat quality (Gosálvez et al., 2006; Houpt, 2010).

High relative humidity in itself had minor effects on physiological parameters and on swine performance (Huynh et al., 2005). However, high humidities combined with high temperatures can enhance the negative effects of the high temperatures and be detrimental for pig performance (Huynh et al., 2005). The higher the humidity level in the air, the less effective is the process of evaporative cooling (less moisture can evaporate into humid air than dry air). Thus, when relative humidity is 50% or higher, the pig will feel the effects of heat stress at a lower temperature than when the air is drier. Huynh et al. (2005) observed that at high relative humidity, the inflection point temperature for incresead respiration rate was lower than it was at lower relative humidity (21.3˚C at 80% relative humidity, 22.6˚C at 65% relative humidity and 23.4˚C at 65% relative humidity). The daily gain per pig was also affected, being lower at high relative humidity, compared to low relative humidity. Therefore, in pig production a relative humidity level between 50 and 70 % is desirable under most practical circumstances (Bottcher et al., 2001), whereas relative humidity values of more than 80% and less than 40% should be avoided.

1.8.2.3. Lighting

Light is essential source for life and as such can have a profound effect on animal behaviour and welfare (Mills et al., 2010). The intensity of lights at the farm should be kept at 50 lux at least (Taylor et al., 2006; CPC, 2014).

According to the Danish Agriculture and Food Council (2013) and the CPC (2014), a minimum period of 8 h per day under natural or artificial lighting and at least 6 consecutive hours per day in the dark (*i.e.* ~5 lux or less) are beneficial for pigs' performance and well-being (Taylor et al., 2006).

Baldwin and Start (1985) showed that pigs prefer light rather than darkness, and dimmed rather than bright light. Additionally, when awake, pigs prefer a lit environment, but prefer to sleep in the dark (Taylor, 2010). However, continuous and particularly very bright lighting, and continuous dark conditions have negative effects on pig welfare (Taylor, 2010). Previous investigations demonstrated that a moderate increase in light intensity and/or light duration can positively affect pig welfare and/or growth parameters of finishing pigs (Martelli et al., 2010) without affecting meat quality (Sardi et al., 2012). Additionaly, recent results of Martelli et al. (2015) reported that a further increase in the duration of the photoperiod (up to 16 h of light per day) can, even at the minimum recommended light intensity (40 lux) and given an adequate dark period for rest, improve pigs' growth parameters and technological meat quality, without affecting animal behaviour.

1.8.2.4. Cleanliness

For hygienic reasons, *i.e.* control of *Salmonella*, finishing units should be cleaned and disinfected regularly (Danish Agriculture and Food Council, 2013). However, pigs soiled by faeces and/or urine can be observed inside the pen. Animal cleanliness is considered as an animal welfare indicator (WQ®, 2009a; Dalmau et al., 2009; Temple et al., 2011) as the presence of pigs dirty with feaces may indicate poor housing conditions, such as low space allowance or high temperature, which force them to rest in the dirty area of the pen (Courboulay, 2007).

1.8.2.5. Space allowance

In commercial housing systems, space is a resource which is limited in the interests of efficiency of building utilisation. The space allowance could be defined as the available space for the animal to live in (Turner et al., 2000). Reduced space

allowance has been related to stress, mediated through the endocrine, autonomic nervous and immune systems (Black et al., 2001) and to negative behaviours, such as agonistic interaction (Mattiello et al., 2003), tail biting (Moinard et al., 2003) and skin bruises (Turner et al., 2000). Likewise, it may altere physiological parameters, reducing health, performance (Hörning, 2007) and meat quality (Maw et al., 2001).

1.8.2.6. Flooring

The type of flooring used in housing production affects the welfare of animals in a number of ways (Mills et al., 2010). Floors that have abrasive, slippery, wet, dirty, or uneven surfaces, protruding sharp edges, exposed aggregate, and uneven or inappropriately sized slats (for the size of the pigs) predispose animals to claw lesion and lameness (Straw et al., 2004). Furthermore, pigs that are raised on partially or fully slatted floor are more likely to have bursitis than pigs raised on other floor types, such as solid concrete with or without bedding (Smith, 1993; Lyons et al., 1995; Mouttotou et al., 1999; Guy et al., 2002). This effect has been associated to the pig's effort to support its weight with its legs on the slat, which is a smaller area compared with a full solid floor (Mouttotou et al., 1997). This physical effort increases the risk of trauma of the superficial lymphatic vessels and capillaries which eventually results in bursae development (Mouttotou et al., 1997). Furthermore, according to Mouttotou et al. (1999) the importance of bursitis is more determined by the floor quality, than by the floor type.

When exposed to heat stress, animals increase the rate of heat loss to maintain core temperature (Nichols et al., 1982). In this situation, a strategy applied by pigs is to increase their contact with surfaces having a lower temperature, such as the floor. Under heat stress, the flooring type is very important, since a slatted floor is a cooler for pigs to lie on than an insulated solid floor. Huynh et al. (2004a) and Huynh et al. (2004b), comparing lying behaviour in pigs housed indoors on partially slatted flooring, observed that as temperature increases (>18.8 °C) pigs will shift their lying behaviour towards the slatted floor which is about 3.6 °C cooler than solid floors. Another important factor is the presence of straw, which despite many positive effects on the welfare of pigs, Fraser (1985) and Tuyttens (2005) reported that under high ambient temperatures pigs prefer to lie in bare floors, indicating that under heat stress situation, straw may lead to animal welfare problems if pigs have no provisions to cool off in a bare floor (Tuyttens, 2005).

1.8.2.7. Ammonia

Similar to other intensive farm operations, pig production generates substantial quantities of manure (faeces and urine) and mortalities, which lead to a production of mixture of vapours, gases and dust combination, in which ammonia emissions are of particular environmental concern. The most important factors influencing ammonia emissions in pig production, approximately 50% (van der Peet-Schwering et al., 1999), are the concentration of nitrogen excreted via urine (50% of nitrogen ingested) and in the slurry (20% of nitrogen ingested; Jongbloed and Lenis, 1992). The nitrogen observed in the urine is mainly in the form of urea, which is easily converted into ammonia and carbon dioxide by the enzyme urease present in faeces (Canh et al., 1998).

The pattern of airflow through the building is also considered a very important factor for the pigs' welfare (Smith and Crabtree, 2005), since an approriate ventilation ensures minimal gaseous concentration and maximal cooling rate. According to Robertson (1998) the poor ventilation at the farm, associated with presence of wet bedding, may lead to increases in ammonia levels.

A lower ammonia concentration in pig houses are desired due to its benefits to the health of pigs, resulting in associated improvements in pig performance and safety for stockmen. Studies of Robertson (1998), Straw et al. (2004) and Smith and Crabtree (2005) related respiratory diseases to high ammonia levels and poor ventilation at the farm. The exposition of pigs and humans to high concentrations of ammonia can impair respiratory system. According to Straw et al. (2004), concentrations of ammonia greater than 25 ppm may irritate the respiratory mucosa and causes excessive lacrimation, serous nasal discharge, and shallow respiration.

Some feeding practice alternatives it is possible to reduce the urea concentration in the urine and the pH of the slurry, such as feeding low protein diets and including fibrous feedstuffs in the diet. Sutton et al. (1996) proposed that manipulating the diet of finishing pigs by reducing the crude protein may reduce the total nitrogen excretion, reducing the ammonia emissions and altering the components of volatile fatty acids and other odorous compounds, while not influencing the animal growth. Hayes et al. (2004) reported reduction of 62.4% of ammonia emission per animal when dietary crude protein was decreased from 220 to 130 g kg^{-1} in finishing pigs.

Canh et al. (1997) reported decreased ammonia emissions when nonstarch polysaccharides levels were increased in the diet. In this last study, pigs fed diets with the lowest nonstarch polysaccharides content excreted more nitrogen in the urine compared to pigs fed with the highest nonstarch polysaccharides content.

Some other studies have proposed measures as including acidifying salts to the pigs diet to reduce ammonia levels at barn, by reducing the slurry pH (acidification; Colina et al., 2000). Canh et al. (1996) observed a significantly lower pH in urine and slurry, as well as a lower ammonia emission when CaCO3 in the diet was replaced by CaSO4, CaCl2 or Ca-benzoate, under laboratory conditions.

In Denmark, emissions of ammonia from solid manure heaps can be reduced by covering the heaps with either air-permeable or airtight material (Sommer, 2001; Chadwick, 2005). Other alternatives used include the use of extra straw to reduce ammonia emission by reducing airflow across surfaces soiled by urine and by bacteria using a high carbon: nitrogen material as substrate (Dewes, 1996).

1.8.3. Good health

Health refers to the state of the body and brain in relation to the effects of pathogens, parasites, tissue damage or physiological disorder (Broom, 2006). Since all of these effects involve pathology, the health of an animal is its state as regards its attempts to cope with pathology (Broom, 2000). If the welfare of an individual is its state as regards its attempts to cope with its environment (Broom, 1986) and pathology is one of the effects of environment, then it is clear that health is a part of welfare.

The relationship between health and animal welfare requires inferences about subjective feelings, such as pain, discomfort and distress (Cockram and Huges, 2011). Indicators of the health condition of pigs on farm include body conformation, postures, nutritional status, respiration rate, and body functions, such as urination, defecation, sneezing and coughing (Straw et al., 2004).

1.8.3.1. Bursitis

Bursa is defined by Adams (Adams, 1974, cited in Mouttotou et al., 1999) as an acquired fluid-filled sac that develops in the subcutaneous connective tissue and that becomes solid after the formation of granulation tissue. Bursitis is usually observed

on the front and hind legs, on the latero-plantar or medial aspect of the hock and on the edge of the hock (Jorgensen, 2000), but it does not appear to produce pain.

Bursitis does not result from an infection (Smith and Smith, 1990), bone disorders or infectious arthritis (Mouttotou et al., 1999), but appears to be caused by poor housing conditions, *i.e.* lower space allowance and slatted floor (Smith, 1993; Lyons et al., 1995; Mouttotou et al., 1999; Guy et al., 2002; Courboulay, 2007). Raising pigs on straw has been shown to reduce the incidence of bursitis (Mouttotou et al., 1997). Thus, bursitis is an indicator of floor comfort and comfort around resting area (Smith, 1993; Lyons et al., 1995; Mouttotou et al., 1999; Guy et al., 2002).

The prevalence and severity of bursitis can be estimated by a physical examination using a scoring system. There are two approaches used, the first proposed by Lyons et al. (1995) where the adventitious bursitis can be scored from 0 to 5 by observing and feeling the limb and the second, proposed by Smith and Smith (1990), in which the severity of swelling is observed and scored in: 0: no bursitis; 1: small raised swelling; 2: moderate swelling; 3: fairly extensive swelling; 4: very severe swelling and 5: eroded bursa with infection. Recentely, the WQ® protocol, based on the assessment methods proposed by Smith and Smith (1990) and Lyons et al. (1995), developed a visual assessement from a maximum distance of 1 m from the animal (WQ®, 2009a; Temple et al., 2011).

1.8.3.2. Lameness

Lameness refers to abnormal gait caused by painful lesions of the limbs or back or to mechanical defects of the limb, indicating a painful state and intense discomfort (Fraser and Broom, 1990). The occurrence of lameness causes the inability to use one or more limbs in a normal fashion, while generally displaying a normal degree of alertness and coordination in the other unaffected limbs. Moreover, animals may present reduced ability or inability to bear weight, alteration or shortening of stride, or recumbency (Straw et al., 2004). Frequently, lame pigs are reluctant to rise or are seen leaning against the pen, they spend more time lying and less time on activities such as standing, walking, playing or fighting (Mouttotou et al., 1999).

The causes of lameness can be divided in four main factors: housing, hygiene, genetic selection and nutrition (Mills et al., 2010). Furthermore, in finishing/growing pigs, the causes for lameness fall within the classes of infectious (Mycoplasma hyosynoviae;

Nielsen et al., 2001), non-infectious (physical injuries; Mouttotou et al., 1997) and genetic (osteochondrosis; Grøndalen, 1974).

The risk of lameness increases on rough flooring or poorly maintained slats and in case of inherent leg weakness or infection, such as infectious arthritis, resulting in swollen joints and abscesses (Velarde and Geers, 2007). Additionally, Munsterhjelm et al. (2015) observed that feed intake starts decreasing, about 20 days before diagnosis in pigs becoming lame, indicating a more profound decrease in animal welfare.

1.8.3.3. Coughing and sneezing

In pigs, coughing can be an indicator of respiratory problems, due to parasitic, bacterial, or viral invasion of the lungs, whereas sneezing can be related to atrophic rhinitis, respiratory infection, or environmental contaminants, such as dust, ammonia, or other noxious gases (Straw et al., 2004; Smulders et al., 2006). The assessment consists in recording the number of animals sneezing and coughing during a 5 min observation (Ekkel et al., 1995; WQ®, 2009a). Although the causes of coughing and sneezing are considered multifactorial, their presence is a useful indicator of welfare on-farm, since their presence has been linked to poor environment control (WQ®, 2009a; Temple et al., 2011).

1.8.3.4. Twisted snouts

Twisted snouts are characterized by stunted development or total disappearance of the nasal turbinates mostly caused by infectious atrophic rhinitis (Jong, 2004). Besides infectious ethiology which is the prevailing cause, extrinsic factors, such as dietary imbalance of calcium and phosphorus, nutritional deficiency, high density and poor ventilation can also contribute to the clinical expression of the atrophic rhinitis (Brown et al., 1966; Penny, 1977; Jong, 2004). Its expression can be also favoured by the genetic predisposition (Jong, 2004).

1.8.3.5. Rectal prolapse

Rectal prolapse is a disorder characterised by the protrusion of one or more layers of rectum through the anus. It may be either partial or complete depending on the structures involved. Only the rectal mucosa protrudes in partial prolapse, whereas all the layers of the rectum are involved in the complete prolapse. Rectal prolapse in pigs occurs when support and fixation mechanisms (fascia, muscles, ligaments) are

overcome by pressure caused by constipation, diarrhoea or coughing, or the support tissues are weakened due to fat or tumour infiltration, genetic predisposition, certain drugs (antibiotics; *e.g*. lincomycin and tylosin) or oedema due to mycotoxins infection, particularly zearalenone (Smith and Straw, 2006; Thomson and Friendship, 2012).

Sudden changes in the diet (*e.g.* from corn meal to wheat) may lead to occasional cases of rectal prolapses. Low fiber diet, may cause constipation and may result in staining and rectal prolapse (Thomson and Friendship, 2012). Other nutrition-related assossiation have been reports by Amass et al. (1995) including diet containing excess of lysine (20% more than required).

Rectal prolapse can occur during transport when intra-abdominal pressure is too high and as a result of a vigorous exercise (climbing ramps) or are squeezed in a high density situation (Becker and Van der Leek, 1988; Gregory, 1998). Other factors influencing the occurrence of rectal prolapses are the ambient conditions, gender and birth weight, with greater incidence in winter (Gardner et al*.,* 1988), in males than females and in lighter piglets (less than 1000 g) at birth.

The length of the docked tail may otherwise affect the physiology of the pig. Studies in lambs showed that when tails were docked as close to the body as possible, rectal prolapses were significantly more likely to occur (Thomas et al., 2003). Likely, due to compromised innervation of the anal sphincter and perianal muscles (Anderson and Miesner, 2008). However, results observed by Bovey et al. (2012) reported that that pigs with tails docked to 1.2 or 4.5 cm are equally likely to be affected by prolapse of the rectal mucosa and then, further investigation are required.

1.8.3.6. Enteric disorders

The normal colour of pig faeces ranges from brownish yellow or brownish green to dark brown. Mechanisms of enteric disorders include hypersecretion, malabsorption, inflammation, and incresead intestinal permeability (Thomson and Friendship, 2012). A change in its consistency (watery or dry hard) and colour (reddish, black, or yellow feces associated with blood or mucus) may depend on the feed ingested, but also to enteric disorders caused by nutritional factors or infection of large or small intestine (Straw et al., 2004). The main causes of of diarrhea in growing-finishing pigs are summarized in the Table 6.

Table 6. Certain common causes of diarrhea in Growing-Finishing pigs (adapted from Ramirez, 2012)

Causes	Nutritional	Viral	Bacterial	Others
	Iron toxicity	Porcine epidemic diarrhea virus	*Campylobacter* spp.	Antibiotic-induced colits
	Biotin deficiency	Porcine circovirus type 2	*Salmonella* spp.	Water quality
	Selenium deficiency	Classical swine fever virus	*Lawsonia intracellularis*	Gastric ulcer
	Vitamin D toxicosis		*Brachyspira spp*	Ascaris suum
				Toxoplasma gondii

Alvarez et al. (2015) reported a poorer performance of growing pigs weaned after a porcine epidemic diarrhea virus outbreak compared with those weaned within the previous 14-120 days, suggesting that in addition to the mortality induced by the diarrhea in suckling pigs, the disease also impairs the performance of surviving pig. Additionally, an increase of 11% in the mortality rate and 0.5 % in the feed conversion ratio were observed in infected-pigs compared with control batches, likewise a decrease of average daily gain of 0.16 lb/day.

Cleanliness and animal welfare of the pigs may also be impaired when pigs present enteric illness, since diarrhoeic pigs are more likely to become soiled. When pigs are dirty with feaces it shows an inadequate environment and a poor hygiene, *i.e.* risk for disease (Courboulay, 2007).

1.8.3.7. Hernias

Hernia is one of the most common congenital and developmental undesirable defects in pigs and has been related to poor environmental conditions and genetic variability. Hernias are classified as indirect or direct, depending on whether intestinal loops outside the abdomen are covered by peritoneum or vaginal tunic or intestines are directly in contact with the skin, respectively (Grindflek et al., 2006).

The presence of hernias is often related to welfare problems, such as intense discomfort and pain, as well as to economic losses due to mortality or carcass

condemnation, due to the ruptured of the intestines during the slaughter process and the carcass contamination (Searcy-Bernal et al., 1994; Keenliside, 2006; Zimmerman et al., 2012). Umbilical hernias are related to reduce performance in pigs, due to adhesions that can interfere with normal digestion (Bates and Straw, 2008; Straw et al., 2009). The presence of hernia in pigs, especially during the finishing phase indicates a poor welfare, since hernias can cause suffering, intense discomfort and pain, especially, if the intestine becomes completely obstructed or if the hernial sac is injured or abscessed (Keenliside, 2006; WQ®, 2009a), and eventually death (Searcy-Bernal et al., 1994).

Umbilical hernias and scrotal hernias are the two most common hernias in pigs, with frequencies ranging from 1.7 to 6.7% (Thaller et al., 1996). The umbilical hernias are generally associated with weakened supportive muscles around the umbilical stump or navel area of the pig (Bates and Straw, 2008). Umbilical infections from the early postnatal period may also contribute to failure of the umbilical cord opening to close and could be exacerbated by traum exerted by piglets to reach the udder of sows (Done et al., 2012).

In the pig breeding industry, infrequent incidence of inguinal and scrotal hernias can happen for certain pig breeds and lines, and genetic factors are believed to drive the hernia development. Scrotal hernias are caused by failed obliteration of the process vaginalis after descent of the testis (Clarnette et al., 1998), or from failed involution at the internal inguinal ring that does not close off properly after the testes descend into the scrotum (Clarnette and Hudson, 1997). Inguinal hernia is most frequently due to hereditary predisposition and can be observed in both genders, although in females is rare and usually associated with intersexuality (Tiranti et al., 2002; Done et al., 2012).

1.9. Critical points within preslaughter period

During the last 24 h prior to slaughter pigs may experience stress from a variety of handling practices, such as feed withdrawal, loading and transport, mixing, human interventions and slaughter.

1.9.1. Good feeding at farm level

1.9.1.1. Feed withdrawal

Feed withdrawal is the first practice for on-farm preparation of pigs before harvest, which is regulated by codes of practice in Canada (AAFC, 1993). Although, divergence exists between recommendation regarding the most efficient duration of fasting, ranging from 5 h in Canada (AAFC, 1993) to 48 h in the USA (Miller et al., 1997), Faucitano et al. (2010b) suggest a period of 24 h between the last meal and slaughter as an acceptable compromise to obtain optimal carcass yield and pork quality, including food safety.

The main advantages of feed withdrawal include higher well-being of animals during transport, notably by reducing motion sickness (Bradshaw et al., 1996a; Guàrdia et al., 1996; Stewart et al., 2008) and animal losses during transport (Chevillon, 2001; Stewart et al., 2008). Under practical conditions, Correa (2011) reported that the application of the appropriate fasting interval at the farm reduced by half the proportion of animal losses during transport. Similar results were found by Stewart et al. (2008), in which fasting pigs by 16 h resulted in 0% of total transport losses compared to 0.39% of total losses in pigs with full stomach. Studies of Bradshaw et al. (1996a) also showed that handle pigs with full stomach (shorter than 8 h fasting) resulted in motion sickness or even death during transport. Ritter (2007) observed that applying a correct fasting interval at the farm resulted in a reduction of 50 % of downers on arrival at the pant, evidencing its importance.

Moreover, appropriate fasting are also related to reduced carcass contamination due to lower risk of gut contents spillage during carcass evisceration (Eikelenboom et al., 1991; Lambooij, 2000; Saucier et al., 2007), to greater easiness of handling pigs (Eikelenboom et al., 1991) and to improved pork quality (Guàrdia et al., 2004, 2005).

However, despite these potential advantages, feed withdrawal is sometimes not used or misapplied by producers, because of concerns related to carcass weight losses resulting in reduced revenue from the carcass sale (Faucitano et al., 2010b). A report from the late 90ies, for example, shows that in Quebec only 15% of pigs were withdrawn of feed before slaughter and for a maximum interval of 12 h (Viau and Champagne, 1998). Faucitano et al. (2010b) has reported that the application of 20-24

h feed withdrawal time allows producer to save 2 kg of feed per pig at no detriment of carcass yield and enables pork processors to get a two-fold lower stomach weight at slaughter, reduced waste to be disposed of at the plant and better meat quality.

The effect of prolonged periods of feed withdrawal on carcass weight loss is still a subject of debate. It has been reported that between 18 and 48 h of feed withdrawal a weight loss rate of 0.11% per hour is expected (Warriss and Brown, 1983). However, Beattie et al. (2002) and Kephart and Mills (2005) reported only 1 kg lower carcass weight in pigs fasted for 20 and 24 h, respectively. Furthermore, according to Chevillon et al. (2006) this weight reduction only became significant after 24 h of fasting (360 g/pig), in which this loss resulted in an equivalent to 30 g/h of cold carcass weight loss for a pig weighing 110 kg.

There is also evidence that long fasting preslaughter is related to poor animal welfare. This evidence was observed by an increased aggressiveness resulting in greater risk of skin damage in fasted pigs kept in mixed groups in lairage for 15 h compared to 3 h (18 *vs*. 10 %; Guàrdia et al., 2009). Similar results were found by Dalla Costa et al. (2015) observing pigs submitted to 22 h fasting at slaughter plant, those pigs spent more time fighting than pigs submitted to 3 h fasting at plant. Moreover, feed restriction prior to slaughter was related to increase drinking rate during lairage time, suggesting that pigs which arrive at slaughter with empty stomachs feel hungry (Brown et al., 1999; Saucier et al., 2007).

According to Guàrdia et al. (2005) when fasting time is longer than 22 h the risk of DFD pork may increases. The prevalence of DFD pork meat in this case can be explained due to the depletion of muscle and liver glycogen levels (Eikelenboom et al., 1991, Gispert et al., 2000; Leheska et al., 2003). However, Warriss (1989) observed that only 20% of muscle glycogen was lost over 24 h of fasting, which may explain the confliting results of a number of other studies which reported no or very little impact of feed withdrawal on meat quality (Murray et al., 2001; Beattie et al., 2002).

1.9. 2. Good housing

1.9.2.1. Loading

Loading pigs onto the truck is considered the most critical stage during the transport period as showed by the increased heart rate and higher levels of blood stress indicators (*e.g.* cortisol and lactate), with these effects lasting until slaughter (Bradshaw et al., 1996b; Chevillon, 2001; Correa et al., 2010; Edwards et al., 2010b). The stress associated with loading procedures results from the combination of different factors, such as group splitting, distance from the pen to the loading deck (Ritter et al., 2008), group size (Lachance et al., 2005; Lewis and McGlone, 2007; Berry et al., 2009), handling system (Correa et al., 2010), design of facilities for loading (Grandin, 1990; Johnson et al., 2010) and strong human-animal interaction combined with the change of environment (Chevillon, 2001).

1.9.2.1. 1. Facilities

The main problems experienced by pigs during loading procedures involve, besides the human interaction, the inadequacy of facilities. Difficulties are often experienced in ascending and descending ramps outside and inside the trucks, because of their steepness (Gregory, 1998). Lambooij (2000) reported that pigs urged to climb slopes above 20° slow down and show increased heart rate due to strong physical efforts.

Another problem often experienced is the hardness to handle pigs onto the truck due to lack of adequate illumination. Pigs do not move from a well-illuminated loading deck into a dark vehicle, since changes in light level lead to frighting and reluctance to move (Broom, 2000). Ellis and Ritter (2006) also observed that long walking distances from home-pen to loading and the loading ramp (> 60m) are related to increases in the proportion of non-ambulatory and non-injured pigs at the loading point. Finally, aisles too narrow or having some sharps edges or protrusions also have been related to handling difficulties, due to the fact that pigs are hesitating to move along in those conditions (Broom, 2000). Therefore, good gates, corridors, raceways, lighting and ramps design and slope are essential for calm and successful loading procedures (Gregory, 1998).

1.9.2.1.2. Handling practices

Among the stressors associated with the loading procedure, the quality of the handling system has a major impact on pork quality (Faucitano, 1998). Poor handling

throughout the preslaughter period is both an animal welfare and a meat quality issue. Many handling problems are caused by pigs that are hard to handle, and can lead to alterations on the physiological state of pigs at slaughter. Hambrecht et al. (2004), for example, reported increased plasma lactate concentrations, in pigs subjected to the high preslaughter stressor level compared to the minimal stressed group (15.6 mM vs. 27.7 mM).

Factors of variation in the easiness of handling are pig genetics background, on farm handling conditions and preparation of pigs at the farm before loading (Grandin, 1993). Genetic background has been associated with increased responsiveness in pigs. Certain lines of lean pigs, which contain the stress gene, tend to be more nervous and excitable than lean lines without the stress gene. Grandin (2002b) observed at large packing that lines containing Duroc genetics tend to be calmer and lines with Hampshire or Piétrain genetics tend to be more nervous and hard to handle. Moreover, Terlouw et al. (1997) observed that Large White pigs react more actively to the approach of humans, than Duroc pigs. Similar results were observed by Weschenfelder et al. (2012) and Rocha et al. (2013) under commercial conditions, in which they observed that as the proportion of Piétrain genetics (25–50%) increased, it resulted in higher responsiveness to handling, regardless of the presence of the Hal gene.

There is ample evidence that reactivity to humans is influenced by prior experience (Hemsworth and Barnett, 1992). Repeated positive handling increases pigs' willingness to be approached by a human (Tanida et al., 1994), or their motivation to approach or physically contact a human (Hemsworth and Barnett, 1992; Hemsworth et al., 1994, 1996). On the other hand, very negative behaviour by the human towards the pig, as the use of an electrical prods, was related to reduce this motivation to approach humans (Hemsworth et al., 1986). Therefore, practices, like walking in the finishing pens every day to get the pigs accustomed to handling can lead pigs ease to handle (Abbott et al., 1997; Geverink et al., 1998a; Grandin, 2000). Another example, under commercial conditions is applying a correct fasting interval at the farm, which has been related to an increased ease of driving pigs to slaughter (Eikelenboom et al., 1991).

Poor handling practices and/or poor facility design (see *1.10.2.1. 1. Facilities* section) during preslaugther period may result in loss of profits due to reduction in carcass value related to 1) occurrence of the "downer" (non-ambulatory) syndrome, resulting in carcass condemnation due to bruises (Ritter et al., 2009), or residual blood in the carcass (up to 30 % price depreciation; Faucitano and Geverink, 2008); 2) weight losses due to the misapplied of the feed withdrawal prior to slaughter (Chevillon et al., 2006); 3) skin bruises ($0.44 value loss per carcass; Riendeau et al., 2010) and 4) meat quality defects, such as PSE and DFD pork. Therefore, providing a quiet and calm handling before slaughter may result in 10 to 12% reduction of the incidence of PSE carcasses (Warriss, 1998).

1.9.2.1.3. Moving devices

Poor farm facilities combined to the presence of large groups of pigs and lack of personnel training may cause handling problems at loading and lead to undiscriminated use of electrical prods (Gregory, 1998). It has been consistenly reported that handling pigs using electric prods reduces the easiness of handling (Rabaste et al., 2007), increases body temperature (Brundige et al., 1998), heart rate, blood lactate and salivary cortisol levels (Hemsworth et al., 2002; Küchenmeister et al., 2005; Correa et al., 2010). Eventually, their use may results in animal losses (*i.e.* fatigued pigs; Benjamin et al., 2001; Correa et al., 2010), carcass bruises and poor meat quality (Rabaste et al., 2007; Correa et al., 2010).

Pigs should be encouraged to move forward by pushing the group from behind with boards, paddle and/or flag, whereas the use of electric prods have to be a last resort to move pigs (Faucitano and Geverink, 2008). Correa et al. (2010) observed that, compared to the paddle, the electric prod helped move pigs out of the finishing pen quickly, but pigs slipped, fell and overlapped more, which may result in body injuries and poor meat quality. Additionally, McGlone et al. (2004) reported that the electric prod and paddle were equally effective for pig handling, but less effective than the board.

Correa (2011) reported that the implementation of an animal welfare program, including training of personnel, removal of electric prods and economic incentives, within the pork chain was effective to reduce the proportion of fatigued animals at

arrival from 0.23 to 0.11%, and the dead on arrival pigs decreased from 0.11 to 0.04%, as well as the proportion of condemned carcasses by 10%.

1.9.3. Transport conditions

Transportation is a novel situation for pigs and inherently stressful for them and recognized worldwide to influence behaviour and welfare of the animals (Kim et al., 2004) as well as economic losses related to mortality rate (Rademacher and Davies, 2005), carcass damage (Dalla Costa, 2006) and poor pork meat quality (Warriss, 2003; Schwartzkopf-Genswein et al., 2012; Correa et al., 2013). Transport negative effects are generally aggravated by unavoidable circumstances such as loading and unloading, vibration, coping with a new environment, restricted space, mixing groups, lack of ventilation, and deprivation of food and water (Stephens et al., 1985; Piñeiro et al., 2007; Bench et al., 2008).

1.9.3.1. Good housing

1.9.3.1.1. Temperature and ventilation inside the truck

Pigs are are more vulnerable to the ambient temperature rise than to changes in humidity and, since pigs cannot sweat, they must rely on other means of thermoregulation, such as moving away from the heat source, changing posture and/or wallowing (Knowles and Warriss, 2000).

The combination of high temperatures and high humidity during transportation can result in severe heat stress for pigs, which according to Schrama et al. (1996) is more important than the cold stress, in terms of transport losses. Variation in temperature and humidity within the vehicle, particularly during the hot season (30°C), have been reported as resulting in greater mortality (Brown et al., 2011). This greater mortality under higher temperatures may be explained by the poor air flow (ventilation) inside the truck, especially when the vehicle is stationary. Sällvik et al. (2004) reported that the temperature inside a standing vehicle with natural ventilation increase by 0.15 °C/min.

Furthermore, it has been evidenced that the animal location (deck and/ or compartment position in the truck) during transportation has an impact on its welfare and meat quality. According to Weschenfelder et al. (2012), the compartments in the middle and bottom front of a stationary pot-belly trailer, were up to 6 °C warmer than

the external ambient temperature in Canadian commercial transports. Brown et al. (2011) reported 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 can be explained by reduced ventilation and poor insulation (increased thermal radiation), respectively. Therefore, these finds may contributed to explain the higher incidence of poor quality pork from pigs located in these mentioned compartments, as reported in previous transport studies of Correa et al. (2013).

Thus, to prevent poor welfare, fluctuations of temperature and relative humidity, the temperature and humidity balance inside the compartments of the truck should be keept. The installation of forced ventilation systems rate using fans in combination or not with water sprinkling may improve animal welfare in terms of reduced risk of heat stress during the hot seasons (Barton Gade et al., 2007). A recent study of Fox et al. (2014) reported that the application of water sprinkling in a stationary truck (after loading and before unloading) at an ambient temperature below 23°C in a pot-belly trailer resulted in an imporved animal welfare at slaughter plant. Moreover, in this study sprinkled pigs spent more time standing during transport and lying down during lairage, compared to non-sprinkled pigs, whereas the non-sprinkled pigs spent more time lying down during transport and more time drinking during lairage. Additionally, the application of water sprinkling in a stationary truck also resulted in lower blood lactate levels at exsanguination and in improved meat quality parameters, especially in pigs transported in some of the critical compartiments (Nannoni et al., 2014).

1.9.3.1.2. Trailer design

Truck designs can vary widely, from small single-deck trucks to large 3-deck punch-hole trailers (often referred to as "pot-belly" trailers; Torrey et al., 2013). Pot-belly trailers are quite common in North America, and consists of 2 straight decks and a smaller deck (the 'belly') between the front and rear tires. Pot-belly trailers are often dual purpose (transporting either pigs or cattle) and can hold a large number of animals, up to 230 pigs on three decks (10-13 compartments), in a single journey. However, these vehicles incorporate multiple (up to five) and steep (up to 40° slope) internal ramps and 180° turns, which result in a lower easiness of handling during loading and unloading, increasing the use of electric prods and extending the load and unload time (Torrey et al., 2008).

It has been evidenced that the vehicle type can be detrimental for animal welfare and meat quality, as observed in a previous Canadian long-distance transport trials (8 h trip in winter and summer), which showed that the use of the pot-belly trailers may result in increased heart rate and blood lactate concentration at exsanguination (Correa et al., 2013; Goumon et al., 2013). Moreover, pigs also displayed more open-mouth breathing when transported in pot-belly trailers rather than flat deck trailers, presumably due to the exercise required to navigate the extra ramps (Kephart et al., 2010; Correa et al., 2013). There are few results on the effects of vehicle design on pork quality, wich are inconclusive. Weschenfelder et al. (2012) and Correa et al. (2008), for example, found no effect on animal welfare or pork quality when pot-belly trailers were used for long distance transportation under controlled conditions and when comparing a pot-belly trailer with a compact truck, respectively.

1.9.3.1.3. Stocking density

Stocking density is one of most easily manipulated and regulated variables in the transport of pig, affecting the carcass quality (Barton-Gade and Christensen, 1998) and animal welfare (Warris, 1998). Basically, the criteria for acceptable loading densities are based on the provision of adequate ventilation and minimum space required by pigs to lie down (Bench et al., 2008).

Due to differences in codes of practices, legislation and scientific evidence from different reports, the range of densities for pig transportation varies extremely around the world. According to Collins (1993), the ideal density is one which allowed all pigs to lie down all together. However, recommended loading densities for pigs during transport are often a compromise between economic pressure to increase loading density in order to maximise profit from a single journey (Bench et al., 2008) and animal welfare requirements. In Canada, the recommended density ranges from 0.36 m^2 to 0.45 m^2/100 kg, according to the environmental temperature (CARC, 2001). As the temperature is lower than >16°C space allowance should be decreased by 10% (AAFC, 1993), while when it is very hot (>24°C) and humid it should be decreased by 25%, in order to proveide more ventilation during transport to prevent dangerous levels of heat buildup (AAFC, 1993; CARC, 2001).

Kim et al. (2004) reported that stocking density are related to physiological changes in pigs during transport, in which they observed lower plasma glucose, CK and LDH

concentration in the low density group compared to the medium and high- density group (0.39 m^2 *vs.* 0.35 m^2 *vs.* 0.31 m^2/100 kg BW).

Moreover, some recommendations for stocking density levels have been proposed by Riches et al. (1997) and Ritter et al. (2006). When densities are > 2.5 pigs/m^2 (285 kg/m^2 at an average shipping weight of 114 kg, or 0.4 m^2/pig or 0.4 m^2/100 kg), there is an increase in average in-transit loss from 0.04% to 0.77% (Warris, 1998). On other hand, a very low stocking density is not recommended during transportation, as pigs might be thrown around during motion (Penny and Guise, 2000). Ritter et al. (2006) also observed that losses (DOA and non-ambulatory pigs) during transport were more than two times greater at low space allowances (0.40 m^2/pig) compared with high (0.50 m^2/ pig) accounting for 0.88 *vs*. 0.36% of total losses.

1.9.3.1.4. Transport duration

Transport duration are associated with poor animal welfare (Bench et al., 2008), poor meat quality (Mota-Rojas et al., 2006), increased carcass bruising and rectal temperature (Mota-Rojas et al., 2006) and increased death losses (Werner et al. 2007; Averós et al., 2008; Haley et al., 2008). Some authors agree that shorter transit time may have some effects on meat quality by depletion on the muscle glycogen stores. It has been reported that pigs transported short distances (< 30 min) may produce more PSE pork than pigs transported for longer distances (3 h; Grandin, 1994; Pérez et al. 2002). Conversely, in pigs hauled for long distances (> 6 h) appears that transport, especially during winter, may result in muscle energy depletion with pigs exhibiting dehydration (Averós et al., 2007) and an increased incidence of meat quality defects related to the production of dark pork (Gispert et al., 2000; Mota-Rojas et al., 2006). However, depletion of muscle glycolytic potential induced by transport time was only reported in pigs submitted to 8 h transportation, compared to 0.5 h and 2.5 h (95.5 *vs.* 112.2 *vs.* 104.4 μmol lactate/g, respectively; Leheska et al., 2003). Agreeing with other studies in which muscle glycogen of pigs was not affected by up to 6 h transportation (Warriss et al., 1983).

1.9.3.2. Good health

1.9.3.2.1. Animal losses

The term transport loss refers to pigs that die or become non-ambulatory at any stage of the marketing process. Transport losses include DOA, pigs that are injured (NAI),

and pigs which are not injured but unwilling or unable to walk (NANI; Sutherland et al., 2008). Both NAI and NANI are defined by the general term "downer". Transport losses are not only a welfare issue, but also an economic concern for producers, with an annual cost estimated in up to $100 million for the US pork industry (Ellis et al., 2003). Across the USA, approximately 0.5-1.0 % of pigs are characterized as NAI or NANI on arrival at the plant (Carr et al., 2005b), whereas within North America, 1/1,000 shipped pigs to dies in transit and 1/2,000 dies in the rest pen (Benjamin, 2005). A recent review of field trials conducted in the United States and Canada between 2000 and 2007 reported that of all pigs marketed, between 0.27 and 0.44 % were non-ambulatory/non-injured (NANI) or fatigued at arrival at the plant (Ritter et al., 2009). A pig death during transit is a major economic loss for producers and transporters. With approximately 109 million pigs being slaughtered annually in the United States, the proportion of DOA (0.17%) cost pork producers more than $3 million in 2010.

An US epidemiological study identified the major source of DOA and non-ambulatory variation (0.17 and 0.25%, respectively) as being the farm (25%) followed by transporter (19%) and the packer (16%; Sunstrum et al., 2006; Haley et al*.,* 2008).

Factors affecting the percentage of DOA and downers on arrival at the plant are a multi-factorial problem that can be influenced genetics (Ellis et al., 2003; Ritter et al., 2008). The contribution of genetics on the occurrence of the NANI syndrome has only been assessed in one study so far (Ritter et al., 2008). Ritter et al. (2008) tested 2,109 pigs at US packing plants to determine the impact of the HAL-1843 mutation (causal mutation of PSS) on the incidence of NANI pigs. They demonstrated that 98% of NANI pigs were negative for the HAL-1843 mutation, suggesting that the HAL-1843 mutation has only minor effects on the overall incidence of NANI pigs at the packing plant. Owing to the characteristic symptoms exhibited by NANI pigs, it is plausible that other genes (mutations) and biological pathways may be involved.

Many other factors, including transport conditions have also been associated with the percentages of dead and NANI pigs at plant, such as vehicle design, trailer micro-environment and season, space allowance, transport duration. Correa et al. (2013) observed a numerical increase in death losses and NANI pigs when pot-belly trailer

was used compared to straight trailers. However, within the pot-belly trailer, no effect of the deck on transport losses has been reported (Ritter et al., 2006).

Transportation deaths were reported to increase at temperatures above 20°C (Sutherland et al., 2009; Haley et al. 2010, Conte et al., 2015) and decreased as the temperature increased above 0°C (Rademacher and Davies 2005). Furthermore, it can double from 0.15 to 0.30% when the outside temperature was >35 °C (Grandin, 1994). Barton-Gade et al. (2007) reported that 42% of the mortality in Danish pigs transported to slaughter may be explained by the temperature effects. Ellis and Ritter (2006) found that the rate of NANI pigs was affected by the season, increasing in the Midwest during late fall and early winter. Whereas, in Canada the higher losses during transport were reported during the month of August (0.40%), when the maximum ambient temperature recorded was 33.6 °C (Haley et al., 2008). Studies of Haley et al. (2010) observed that the losses during transport more than doubled when pigs were transported at temperatures above 21°C, and at increased stocking density (from 0.52 to 0.44 m^2/pig).

A recent study examinating of 302 trailers transporting 48,143 pigs in order to determine the amount of ventilation, or varied side-wall boarding, required to keep pigs within their thermal comfort zone, found that pig losses were highest when low boarding levels (open sides) were used in cold air temperatures (<5 °C). However, in mild air temperatures (5 to 26 °C), boarding levels had little impact on pig losses (McGlone et al., 2014).

The conditions during the journey are also a major factor, for example, Pilcher et al. (2011) found that pigs transported for short journey times (≤40 min) at reduced floor spaces (0.415 and 0.437 m^2/pig) in the trailer are more prone to develop the NANI condition than those transported for a long journeys time (3h), suggesting that pigs that died on shorter journeys were unable to recover from loading stress.

An increased stocking density was also related to an increase in the death losses and rates of NANI pigs at slaughter (Ritter et al., 2006). Some other important factor that affect pigs' welfare and contribute for increase the numbers of DOA and NANI pigs are the handling skill, which was confirmed by Benjamin et al. (2001) and Gonyou (2004), who observed a greater percentage (20.4 % and 34%, respectively) of fatigued

pigs when pigs were aggressively handled compared to gentle handling (0.0% and 2%).

Finally, the muscle acute acidosis in response to physical stress (Hambrecht et al., 2002), which results in NANI or fatigued pig, can directly affect meat quality, and lead to dark meat, high ultimate pH and low drip loss (Carr et al., 2005b) or higher incidence of pale and exudative pork depending on when (long- or short-term stress) the pig becomes fatigued during the marketing process.

1.9.4. Good housing at the slaughter plant

1.9.4.1. Unloading

According to Marchant-Forde and Marchant-Forde (2009), one of the main factors impairing animal welfare at slaughter plant is the waiting time between arrival and unloading. It is usually recommended to unload pigs as soon as possible after arrival at the plant. However, under commercial conditions waiting time to unload after arrival at the abattoir is variable. In Canada, some loads waited for up to 4 h before unloading (Aalhus et al., 1992). Driessen and Geers (2001) and Ritter et al. (2006) showed a higher muscle acidification in pigs unloaded after 30 min of wait at high ambient temperatures (> 20°C). This occurs because when the vehicle stops moving, the temperature and humidity inside the truck quickly begins to rise (Ellis et al., 2010; Lewis et al., 2010). Fox et al. (2014) and Nannoni et al. (2014) reported that sprinkling pigs during the last 5 min prior to unloading at the slaughter plant was effective in reducing heat stress response and fatigue, and in improving pork quality. However, in order to limit the effects of waiting time, Faucitano and Geverink (2008) suggest that a better arrival schedule should be applied or that the number of unloading quays should be equal to the number of row of lairage pens so that more than one vehicle can unload at the same time.

At unloading, special attention should be paid to ramp slopes (<15-20°), lighting (Grandin, 1996), noises (Grandin, 2002a) and floor gaps due to height difference (>15 cm step) between the truck deck and the unloading ramp (SCAHAW, 2002). The lack of adequate environment can induce physiological and behavioural responses. For example, novel noises between 80 and 90 dB caused increased heart rate in pigs (Spensley et al., 1995). Lippmann et al. (1999) observed that when the sound pressure

was reduced by 50 % pigs were less aroused and moved more easily without the use of driving aids. Recently, van de Perre et al. (2010a) reported a clear relationship between the high noise level produced during unloading and in lairage and the reduction of pH in the loin muscle as a result of fear response. Moreover, Vermeulen et al. (2015c) found that a cut-off value of 85 dB during the preslaughtering phase is not only a threshold to evaluate animal welfare but it is also associated with a slower drop of pH, when sound levels are <85 dB.

1.9.4.2. Lairage

Given its importance for the pork chain economy precautions must be taken in lairage to ensure adequate handling and environmental control to take advantage of its function as a resting area, enabling pigs to recover from the stress of transport and be fit at the time of slaughter (Faucitano, 2010; Warriss, 2010).

However, the response of pigs to lairage may be influenced by several factors, such as the concurrent fasting, the previous preslaughter treatments (transport, unloading, mixing) and the genetics (De Smet et al., 1995; Nanni Costa et al., 2002). Hence, to avoid economic losses due to death, skin damage and poor meat quality in lairage, factors such as lairage time, environment, handling and slaughter system must be controlled (Faucitano, 2010).

1.9.4.2.1. Lairage time

Keeping pigs in lairage for a period of time is necessary to optimise animal welfare and pork quality (AMI, 2012). However, the optimal lairage duration is not easy to quantify because the interaction with conditions during transportation. While some authors conclude that there is no need of lairage when low stress preslaughter handling is used (Aaslying and Barton-Gade, 2001), others recommend a lairage duration ranging from 1 to 3 h to allow pigs to recover from previous stress prior to arrival at the abattoir. Overall, these lairage durations are considered ideal to get optimal meat quality (Warriss, 2003; Young et al., 2009).

The application of no or very short lairage (< 60 min) is not recommended as it results in more fatigued and excitable pigs (based on higher blood cortisol levels at slaughter and aggressiveness), increased skin damage due to fighting, higher (+ 1°C) muscle temperature immediately before slaughter and higher level of lactic acid in the muscle resulting in increased incidence of PSE pork (Fraqueza et al., 1998; Owen et al., 2000;

Pèrez et al., 2002; Hambrecht et al., 2005; Shen et al., 2006; Faucitano, 2010). Whereas, although longer lairage (< 22 h) effectively reduces PSE meat (Nanni Costa et al., 2002), it may compromise the welfare of pigs, as showed by the increase of blood indicators, such as acute phase proteins levels, cortisol, lactate and CK (Nanni Costa et al., 2002; Saco et al., 2003). During lairage, high CK were associated with long lairage duration (2 *vs.* 24h; Salajpal et al. 2005) and poor lairage conditions (Warriss et al., 1994). Warriss (1995) also reported elevated circulating concentrations of CK in pigs after stressful experience (fights during lairage) at slaughter plant. Its levels in blood increase in response of the demand of the mitochondrial phosphocreatine to resupply ATP for muscle activity (Broom and Johnson, 1993).

Moreover, longer time resting has been associated with increased aggressiveness and fighting rate in mixed groups of pigs after more than 9 h of lairage (Nanni Costa et al., 2002), greater risk of DFD pork (Warriss et al., 1998; Guàrdia et al., 2005), losses in carcass weight and yield (Gispert et al., 2000; Pèrez et al., 2002; Warriss, 2003) and skin blemishes due to fighting (Nanni Costa et al., 2002; Guàrdia et al., 2009).

1.9.4.2.2. Lairage density

Space allowance has been reported to have a greater impact on social behaviour of pigs in the lairage pen than group size (Faucitano, 2010). The stocking density recommended in Canada ranges from 0.34 to 0.41 m^2 /100 kg liveweight. In commercial conditions, this recommendation is hardly respected, and space allowance can vary from 0.3 to 2.7 m^2/pig (Gispert et al., 2000; Weeks, 2008). However, in order to limit fighting in lairage, recent studies have shown that the space allocations should vary depending on animal size and holding time. As an example, a stocking density of 0.42 m^2/pig is recommended when short lairage is applied (≤ 3h) and 0.66 m^2/pig for long lairage (≥ 3h; Weeks, 2008) based on the observation that at lower stocking density, fighting may increase due to the increased opportunities for pigs to interact with more individuals. However, in large space allowance conditions, subordinate pigs also have more opportunities to escape from the dominant ones (Weeks, 2008). At low space allowance, fighting is limited because pigs can only interact with the neighbour pigs rather than moving around the pen (Weeks, 2008).

1.9.4.2.3. Environment

The ideal lairage temperatures and humidity for finishing pigs should range from 15 to 18°C and 59 to 65%, respectively (Honkavaara, 1989), considering the results of studies of Leal and Nããs (1992) and Huynh et al. (2004a,b). According to AMI (2012), death losses in lairage, so called "dead-in-pen", almost doubled when temperatures above 32°C were recorded and compared with 16 °C.

Fraqueza et al. (1998) reported that when pigs are held at 35°C for 3 h in lairage, there is a greater risk for PSE pork production, which is reduced when they are kept for longer time (3h *vs*. 0.5 h) at 20°C. Therefore, when temperatures exceed 21°C, pigs should be cooled off by water-sprinkled/misting to avoid animal losses and meat quality defects. According to Huynh et al. (2006), sprinkling pigs using cold water (approx. 10˚C) reduces heat-related respiration rate and leads to a 2°C drop in muscle mass temperature at 40 min *post-mortem*, reducing the incidence of PSE pork (Long and Tarrant, 1990). Furthermore, water sprinkling increases the electrical stunning efficiency as wetting the skin lowers its impedance, resulting in an easy and rapid unconsciousness prior to slaughter (Wotton, 1996).

Furtehrmore, the respect of ventilation (135 m^2 h^{-1}; Brent, 1986) criteria is not only necessary to remove excessive heat and humidity, but also to keep low the ammonia levels and other noxious gases (Weeks, 2008). Wathes et al. (2002) reported that ammonia levels higher than 10 ppm are aversive to pig. However, it is acceptable on animal welfare science the exposure to moderate levels of ammonia (10 ppm) in lairage for a short period (1 h; Weeks, 2008).

1.9.5. Good health

1.9.5.1. Mixing pigs

Even though mixing unfamiliar animals is recommended to be avoided, it is a common practice in lairage pens either because of the lack of adequate holding facilities (adjustable pen size) or because changes in this practice are not perceived by the abattoir managers as economically important (Faucitano, 2010).

Mixing unfamiliar pigs during lairage will result in poor welfare and carcass and meat quality. A poor animal welfare is suggested by the greater cortisol levels in blood and

saliva that have been reported in response to the preslaughter mixing practice (Geverink et al., 1998b; De Jong et al., 2000).

Moreover, mixing pigs may lead to social stress within the group and is usually followed by fighting (mainly during the initial hours) to create a new hierarchy (Fàbrega et al., 2013). The elicited aggression (Turner et al., 2006; Barton-Gade, 2008), not only results in physical injuries, such as skin damage in the shoulder area and in the whole carcass (Barton-Gade, 2008), but also results in poor pork quality (Faucitano, 2010; Stukenborg et al., 2011). These effects on pork quality can be explained by the glycogen exhaustion caused by strenuous physical activity and the significant rise in body temperature during the *peri-mortem* phase (Jones et al., 1994; De Jong et al., 1999).

One of the proposed strategies used during lairage to control the fighting rate in mixed groups of pigs is the group size (Faucitano, 2010). However, the evidence about the ideal group size in the lairage pen is contradictory. Rabaste et al. (2007) observed that groups of 30 pigs spent more time standing and fighting in the lairage pen than groups of 10 pigs, at the same stocking density (0.59 m^2/pig). Whereas, Grandin (1990) observed that mixing very large groups (up to 200 pigs) in the pen also reduced fighting, because the use of larger group allows that an attacked pig has the opportunity to escape.

1.9.5.2. Moving pigs to restrainer

Prestunning handling facilities are of primary importance, given the need to handle pigs faster, so as to follow the speed of the slaughter-line. The combination of higher speeds, poorly designed handling systems and large groups is detrimental to animal welfare and pork quality (Faucitano, 2010).

The progressive passage from a free-moving group situation to a single line of aligned and restrained individuals in the *peri-mortem* period have been shown to be very stressful to pigs (Griot et al., 2000). Critical factors at this point are the entrance into the race and the ‘‘stop-start’’ forward motion of pigs towards the stunner (Faucitano, 2010). At this point, handlers use, often indiscriminately, electric prods device to speed up the procedure and feeding the restrainer. However, it is well know that the use of electric prods is very aversive, as showed by the significantly increase in blood

cortisol and lactate exsanguination concentrations of pigs submitted to harsh handling using electric prods (Hambrecht et al., 2004).

Results of Rabaste et al. (2007) revealed that the use of the electric prod reduce the ease of handling increasing escape attempts without necessarily speeding up the flow of pigs to the stunner. Moreover, when the electric prod is used in a decreased space, such as the entrance of restrainer, turning around behaviour is limited and pigs try to escape from the stressor (*e.g.* the electrical shock and the handler) by climbing over the backs of other group mates in search of protection within the group (Guise and Penny 1989; Lambooij and Engel 1991). This increase in mounting behaviour may result in more fatigued pigs (Benjamin et al., 2001; Rabaste et al., 2007) and in a greater number of bruised carcasses and poor pork quality (Rabaste et al., 2007). In order to limit these negative effects of electric prod use by handlers, alternative handling devices to the electric prod, such as board, paddle, and flag, to move pigs along the plant alleys shoul be adopted. These devices are known by induce fewer behavioural problems, compared to electrical prods and by reduce the time required to move pigs (McGlone et al., 2004; Correa et al., 2010).

Another strategy for minimize the stressfulness of the experience and minimize the time required for driving pigs forward, it is moving 5 or 6 pigs per group (Lewis and McGlone, 2007). Lewis and McGlone (2007) demonstrates that as group size increases (> 6 pigs per group), the heart rate of pigs being moved also increase. Moreover, the authors reported that there is no time savings advantage to moving more than 5 pigs per group, and moving of pigs in larger groups may also cause skin damage caused by pig movement (in large groups) and fighting (due to pigs turning).

1.9.5.3. Restraint systems

According to EC Directive (1993), animal restraint prior to slaughter is a mandatory requirement in order to achieve maximum efficiency during electrical stunning. Animal restraint allows adequate positioning when the electrodes are applied.

For practical and economical reasons the stun-pen holding 3 to 8 pigs is used for "on-floor" manual electric stunning at small plants (Faucitano, 2010). Disadvantages of this system are the risk of bone fractures (up to 40%), return to consciousness *post-stunning* and delayed exsanguination due to inaccurate positioning of the electrodes and delayed exsanguination (Anil et al., 1997; Griot, 1998).

At plants with high slaughter volume and speed, the restrainer systems used for electrical stunning are the V-type restrainer (specific to head-only stunning) and the monorail conveyor or band restrainer (specific to head-to-heart/chest stunning). In the V-type restrainer pigs are lifted up and carried by the flancs to the stunner. In the monorail restrainer pigs walk onto a belt, which they straddle, and are then conveyed off the ground (Faucitano, 2010). It has been observed that pigs in the monorail system feel more comfortable as indicated by the lower heart rate (180 heart beats min^{-1}) compared with those conveyed with the V-type restrainer (220 heart beats min^{-1}; Lambooij et al., 1992; Griot et al., 2000). The effect of the V-type restrainer may be explained by the physical reaction induced on the pigs by the lateral compression on its body (OIE, 2014). The increased muscle activity, finally, results in blood-splashed and PSE pork (Griot et al., 2000).

1.9.5.4. Stunning

Stunning before slaughter is a legal requirement according to the European legislation (EU Council Directive 93/119/EC, 1993) and the Humane Slaughter Act (1978). In farm animals, stunning is applied in order to establish an unconscious state (Gregory et al., 2009) so as to ensure that animals do not suffer unnecessarily and are insensible to the slaughter procedure (Raj, 2008).

Nowadays, the two methods most used for stunning pigs at commercial slaughter plants are the electrical and carbon dioxide (CO_2) stunning.

1.9.5.4.1. Eletrical stunning system

Electrical stun can be carried out by applying an electric current across either the head only (between the base of the ear and the corner of the eye; head-only stunning) or across the head followed by a second current applied to the chest, behind the position of the heart (head-to-chest/brisket stunning) or on the back (head-to-back stunning; Faucitano, 2010). Differently from the head-only electrical stunning, which is a reversible procedure that is optimally effective for only few seconds (15-20 s) after stun completion, the electrical stun method head-to-heart (or head-to-chest) consists in applying an electric current (one current cycle) that is immediately followed by a second current cycle applied across the chest to induce cardiac ventricular fibrillation, and, consequently, the death of the animal (two current cycles; Raj, 2008; Faucitano, 2010). At the application of an electrical current of sufficient magnitude to the brain

generalised epilepsy is induced (Raj, 2010), causing the inhibition of impulses from both the reticular activating and the somatosensory systems (Heath et al., 1994; Raj et al., 1997). The state of unconsciousness induced by electricity is caused by a massive neurotransmitters (glutamate and aspartate) discharged to the sensory cortex of the brain (Cook et al., 1995).

Insensibility is induced by passing the electric current (expressed as amperage) through the brain, whereas the voltage (V) and current flowing from the electrodes into the brain determines the depth and onset of unconsciousness (Warriss, 2010). For both head-only or head-to-chest stunning the amperage that is required to induce epilepsy in market pigs is the minimum of 1.25 amps (Hoenderken, 1983; Gregory, 1988). However, the original studies were performed on small (100 kg) pigs and the larger pigs that are being processed today will often require a greater amperage setting. Insufficient amperage can lead the animal to be paralyzed, but not unconscious (Grandin, 1999). A sufficient voltage is necessary to gain the resistance and allow a smooth flow of current into the brain. The recommended minimum voltage for pigs is 250 V (Troeger and Woltensdorf, 1989), whereas the frequency of the electric current should range from 50 to 60 Hz (Gregory, 1998).

When the electrical stunner is efficiently applied, pigs go through three post-stun phases: 1) tonic: the animal becomes rigid and rhythmic breathing ceases, the head is raised, the forelegs are extended and hind legs are flexed towards the body and eyeballs are fixed. This phase lasts 15-20 s; 2) clonic: involuntary kicking or paddling movements, gradual relaxation of muscles, downward movement of eyeballs, urination and/or defecation. This phase lasts between 15-45 s; and 3) recovery when head-only stunning method is used: if the animal is not killed by exsanguination, slowly the animal resume normal rhythmic breathing, in response to painful stimuli it becomes visually aware and attempts to stand. This phase starts about 30 to 60 s after stunning (McKinstry and Anil, 2004; Warriss, 2010).

1.9.5.4.2. Carbon dioxide stunning system

Carbon dioxide is a gas that has been used for stunning pigs, salmon and, to a limited extent, chickens (Gregory, 1998). When CO_2 is inhaled at high concentrations, it changes blood chemistry, depressing brain activity and inducing loss of consciousness (Raj, 1999). The loss of consciousness is induced by hypercapnic hypoxia and leads

to changes in blood parameters, such as pH, carbon dioxide partial pressure (pCO_2), oxygen partial pressure (pO^2), oxygen saturation ($SatO^2$) and bicarbonate concentration (HCO^{-3}; Martoft et al., 2001). Although studies reported that the sticking time for pigs exiting from a CO_2 system depends on the time of exposure to the gas and its concentration, the maximum sticking time should not be longer than 30 s following stunning and the gas concentration should never fall below 70% to avoid recovery of consciousness (Velarde et al., 2000).

The use of CO_2 for stunning pigs is approved in many countries. However, there is no consensus about its acceptability from an welfare point of view. According to Raj and Gregory (1995, 1996) and Becerril-Herrera et al. (2009), the CO_2 stunning has some animal welfare disadvantages as the gas inhalation is aversive and induces a severe respiratory distress in animals. Moreover, Llonch et al. (2012) reported that CO_2 induction of unconsciousness is not immediate taking up to 45 s. During this interval, animals typically exhibit agitation with forced breathing, stretch the head upwards and vocalize loudly triggering catecholamine release, increasing cardiac rate, oxygen consumption and body temperature and diminishing pH, and causes accumulation of lactic acid in muscle (Hambrecht et al., 2004).

For a more humane stunning, the use of different gases proportion mixtures, *e.g*. 30% carbon dioxide and 60% argon, have been proposed based on the low respiratory distress, lack of aversiveness and higher gas effectiveness in rapidly abolishing brain responsiveness (Raj et al., 1997). However, Llonch et al. (2012) reported inconclusive results with pigs exhibiting fewer signs of aversion when sttuned using 70% nitrogen and 30% CO_2 stunning than 90% CO_2, but longer time to reach unconsciousness, which may negatively affected animal welfare, meat and carcass quality.

However, if on one side the animal welfare aspect of CO_2 stunning is debated, on the other side the positive effects of CO_2 stunning on pork meat quality when compared with electrical stunning are clear. Research has showed that CO_2 stunning reduces the incidence of PSE meat and the occurrence of blood splashes in loins and hams (Velarde et al., 2001). Furthermore, Facco Silveira et al. (1998) observed higher muscle pH and water-holding capacity was when pigs were CO_2 stunned, compared to when pigs were electrically stunned. However, Henckel (1998) comparing electrical stunning and CO_2 stunning of market hogs found that for pigs with similar genetic

background (free from the Hal gene) and with the same environmental exposure prior to stunning, electrical stunning resulted in twice the drip loss from the *longissimus* muscle with the same muscle pH and meat color.

1.9.5.5. Bleeding procedure

In order to avoid recovery of consciousness after head-only electrical stunning pigs should be bled promptly (within 15-20 s; Wotton and Gregory, 1986; Gregory, 1998; Pérez-Chabela and Legarreta, 2001). Differently from head-only stunning, when head-to-chest/back stunning is used, it induces cardiac arrest and, consequently, the death of the animal (Faucitano, 2010). Thus, the exsanguination procedure has to be performed within a maximum of 3 min in order to obtain a satisfactory bleeding and to prevent blood clots in the large vessels (Gregory, 1998). In this case, however, the exsanguination procedure is only performed to drain the carcass of its blood.

Post-stun convulsions must be controlled because they not only delay the bleeding process and the quality of bleeding (*i.e.* shoulder sticking), but also increase risks of muscle acidification and undesired blood splashes (Gregory, 1998). Therefore, according to recommendations of Grandin (1994), in order to limit post-stun convulsions the animal should be bled promptly whilst still in the tonic phase after stun and in the lying position.

Vertical bleeding is usually observed when CO_2 stunning is as there are no convulsions and the extremities of pigs are relaxed at the exit of the system (Faucitano, 2010). Woltersdorf and Troeger (1987) observed improved pork quality traits when animals were bled out in a lying position, indicating that bleeding position (vertical *vs.* prone position) may have an important effect on quality of bleeding. Interestingly, a significant increase in the incidence of PSE pork was observed after delayed exsanguination (>15 s) in the vertical position compared with a short stun-to-stick interval (<15 s) combined with exsanguination in the prone position (Faucitano et al., 1998). The skills of the operator also play an important role in the efficiency of the bleeding procedure. Troeger and Meiler (2007) reported improved bleed-out efficiency when the bleeding procedure was performed by a skilled operator compared with an unskilled one (4.3 *vs.* 4.1% carcass weight loss).

1.10. Relationship between stress indicators and pork meat quality

Animal welfare can be assessed by using a large number of stress indicators (Broom and Johnson, 1993), such as behavioural (WQ®,2009a) and physiological (Hambrecht et al., 2004; Becerril-Herrera et al., 2010; Edwards et al., 2010a,b; Choe and Kim, 2014; Vermeulen et al., 2015b) responses. These responses are not only indicators of animal welfare, but may also have effects on muscle metabolism and, thereby, on meat quality (Mormède, 2008; Faucitano and Geverink, 2008).

1.10.1. Heart rate

In most farm animal studies, tachycardia has been found to be associated with disturbing situations, *e.g,* as a result of electrical prods use to drive pigs forward during loading (Griot and Chevillon, 1997; Correa et al., 2010) and related with increases in hormones releasing and negative effects on ultimate potential of hydrogen (pHu) in pork meat quality (Correa et al., 2010). Detrimental effects of increased HR on meat quality is supported by a significant, positive correlation between pHu and HR ($r = 0.25$; $P < 0.005$) observed in veal calves during unloading after being transported between 30 and 140 min (Lensink et al., 2001). This effect is due to the releasing of adrenaline by the sympathetic nervous system, which increases the HR and stimulates the breakdown of skeletal muscle glycogen, leading to reduced pHu (Voisinet et al., 1997; Lensink et al., 2001).

1.10.2. Body temperature

It is well known that under stress situation, muscle glycogenolysis is increased by adrenergic mechanisms resulting in accelerated glycolytic rate *post-mortem* and muscle and body temperature increase. These effects lead to poor meat quality due to a severe denaturation of muscle protein causing a rapid decline in muscle pH while the muscle temperature is still high. The increased pH decline due to the production of lactic acid can also originate from the ATP hydrolysis, which produces free protons and heat (Scheffler and Gerrard, 2007; Scheffler et al., 2011) and this might result in poor meat quality (Van der Wal et al., 1999). Therefore, non-invasive stress indicators, such as meat temperature or body temperature, have also been widely studied and, according to Guàrdia et al. (2009, 2012) and Vermeulen et al. (2015b), could be appropriate to indicate possible PSE meat.

Recent results of Weschenfelder et al. (2013), measuring the surface temperature of the ocular region with an infrared devide just before slaughter, were weakly correlated with with pH taken 1 hour *postmortem* ($r = -0.18$; $P = 0.03$) and drip loss ($r = 0.20$; $P = 0.02$) in the LD muscle, and with pHi in the SM muscle ($r = -0.20$; $P = 0.02$). On the other hand, Vermeulen et al. (2015b) had indicated that measuring rectal temperature of pigs just before slaughter allows the identification of pork with PSE traits. This conclusion was based on a linear correlation between rectal temperature and ham and LT muscles temperatures at 30 minutes *post-mortem*.

1.10.3. Catecholamines and corticosteroids

The stress response has the potential to activate the hypothalamic-pituitary adrenocortical axis, secreting ACTH hormone, which regulates cortisol synthesis and secretion (Warriss, 2010). Although some experiments studying the relationship between cortisol levels and meat quality found no or low correlations, Shaw et al. (1995) reported that greater plasma and muscle cortisol levels were positively correlated with pHu ($r = 0.25$; $P < 0.01$; and $r = 0.51$; $P < 0.001$, respectively) and negatively related to drip loss ($r = -0.20$; $P < 0.05$; and $r = -0.35$; $P < 0.001$, respectively). Furthermore, Choe et al. (2015) recently reported that pigs showing high levels of serum cortisol exhibited low pHu ($r = -0.22$; $P < 0.05$) and increased drip loss ($r = 0.37$; $P < 0.001$).

In addition to the effects on cortisol, stress prior to slaughter can also activate the sympatho-adrenal medullary axis releasing catecholamine, among them noradrenaline and adrenaline (Warriss, 2010; Terlouw, 2005). Catecholamines by turn, increase heart rate, blood glucose and lactate level through the rapid breakdown of glycogen in the liver, which can also have some effects on meat quality (Warriss, 2010; Mota-Rojas et al., 2012). For example, Foury et al. (2005) observed that high levels of noradrenaline and adrenaline in urine were, rather low, positively correlated ($r = 0.16$ and $r = 0.17$, respectively; $P < 0.01$) with pHu measured in the *longissimus lumborum* muscle of pigs.

1.10.4. Enzyme and other metabolites

Several studies have reported that, partly due to the activation of the sympatho-adrenal system (Warriss, 2010), changes in blood glucose levels were associated with stress (Mota-Rojas et al., 2012) and poor pork quality (Choe et al., 2009; Choe et al., 2015). In Choe et al. (2009) for example, greater blood glucose levels at

exsanguination resulted in lower pork muscle pH45 min (r^2 = 0.32, P < 0.001) and greater drip loss (r^2 = 0.38; P < 0.001) *post-mortem*. However, Choe et al. (2015), using a hand-held device, recently reported that greater blood glucose levels at exsanguination were related to low pork muscle pHu (r = - 0.51, P < 0.001) and greater drip loss (r = 0.32, P < 0.001).

In contrast to the metabolic response to acute stress, exposing animals to long periods of stress may cause a restricted formation of muscle lactate *post mortem*, due to the depletion of muscle glycogen reserves, increasing the incidence of DFD pork (Van de Perre et al., 2010b; Warriss, 2010). Indeed, lower glycogen levels in muscle, measured by the glycolytic potential, have been related to greater pHu (r = 0.60; P < 0.01) at 24 h *post mortem* (Wittman et al., 1994), whereas higher contents of muscle glycogen and blood lactate at early *post-mortem* were related to paler colour and higher drip loss of pork (Ryu et al., 2005; Choe et al., 2008).

As already commented, factors prior to slaughter such as fights while in lairage pens, have also been related to poor meat quality. The unusual physical activity during fighting leads to increased blood levels of cortisol and CPK (Oliver et al., 1996) as well as higher muscle glycogen depletion and, consequently, a progressive higher ultimate pH and DFD meat (Faucitano et al., 1998; Gispert et al., 2000). The CPK level will influence metabolic rates during the conversion from muscle to meat (Pösö and Puolanne, 2005). Indeed, CPK delays meat acidification by the retardation of lactate production and by buffering H^+ (Pösö and Puolanne, 2005).

Studies have shown that a plasma lactate levels (related to glucose metabolism) at exsanguination were associated with poor pork quality by a strong positive correlation with muscle pH at 30 min *post mortem* (r = 0.60; Hambrecht et al., 2005). However, Edwards et al. (2010a) fail in finding strong correlations between blood lactate levels at exsanguination, measured using the hand-held lactate analyser, and meat quality traits pHi (r = - 0.32; P < 0.001) and drip loss (r = 0.22; P = 0.02).

Chapter 2: Issue, hypothesis and objectives

2.1. Issue

Nowadays, poor handling throughout the preslaughter period is both an animal welfare and a meat quality issue. The combined impact of several stressors before slaughter can affect the metabolism of muscle cells and lead to carcass downgrading and meat quality defects (pale, soft and exudative meat or PSE and dark, firm and dry or DFD pork). This negative impact on meat quality is of paramount importance as it is associated to the economic losses resulting from the downgrading or even rejection of poor quality products within the fresh meat trade. Additionally, the importance of animal welfare in livestock production has increased in recent decades and it has become a key criterion for consumers and the pork industry. Despite the progress on the subject, further research is necessary to better understand the mechanisms of the animal stress response and its relationship with pork quality variation through reliable assessment techniques. The stress condition in animals can be monitored by recording physiological changes, such as blood pressure and levels of metabolites (hormones and enzymes), heart rate and body temperature, and behavioural changes (as described in the literature review of this thesis). However, these techniques, besides being time-consuming and needing trained manpower for their execution, are invasive and as such may impose some additional stress on the animal biasing the results. Therefore, considering the conditions in commercial settings, there is a need for the development of non-invasive and easy-to-perform procedures and tools that can be used as stress and physiological indicators at the farm and the slaughter plant.

2.2. Hypothesis

Non-invasive techniques can be accurately used to monitor the well-being of animals through the assessment of the physiological status of pigs in response to stress preslaughter and finally predict meat quality variation. These techniques may be useful to study the impact of on-farm raising conditions on the behavioural response to preslaughter handling at the slaughter plant and identify its contribution to pork quality variation.

2.3. Objectives

2.3.1. General objective

To provide the pork industry with tools that could be used under commercial conditions to monitor animal welfare preslaughter and to predict pork quality variations.

2.3.2. Specific objectives

In order to validate the hypothesis stated above, the work plan presented in this thesis unfolded through different approaches targeting the validation of three techniques for animal welfare monitoring preslaughter:

- ✓ Lactate Scout Analyser

  - To establish the relationship between lactate levels in blood, collected at different sampling points at the slaughter plant (from arrival to slaughter), and pork quality variation (in loins and hams) in a large sample size and under commercial preslaughter handling conditions.
  - To evaluate the reliability of the Lactate Scout Analyser as a tool to help explain the variation in pork quality.

- ✓ Animal Welfare Audits protocol

  - To determine the relationship between the animal welfare conditions on farm and preslaughter behaviour, as assessed by audit protocols, and pork quality variation.
  - To evaluate the relationship between audits scores on farm and at the slaughter plant, and between the audits scores and pork quality variation.

- ✓ Infrared Thermography

  - To identify the best anatomical location for infrared thermography body temperature image captured that could be used to explain the variation in the physiological status of pigs subjected to stressful conditions.

Chapter 3: Hand-held lactate analyzer as a tool for the real-time measurement of physical fatigue before slaughter and pork quality prediction

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Although the effects of stress in animals can be monitored by physiological and behavioural changes, a major issue in terms of animal welfare research is that most assessment techniques imply invasive procedures that may be stressful for the animal and may bias the results. Furthermore, they are laborious and time-consuming, and require trained personnel for their execution. For these reasons and based on the relationship between exsanguination blood lactate and pork quality, the use of the hand-held Lactate Scout Analyzer (LSA) was evaluated to monitor the impact of preslaughter handling on pork quality variation. Thus, this chapter presents the results of a study aimed at investigating the use of the hand-held lactate analyzer as a tool for the real-time measurement of lactate, indicator of fatigue, before and at slaughter and prediction of pork quality variation.

A short paper including these results was published in the proceedings of the 58th International Congress of Meat Science and Technology (ICOMST), 13-17 August, 2012, Montreal, Canada. The communication was in the poster format and was also selected for the oral student competition.

Contributing authors:

Luiene Moura Rocha (Ph.D. candidate): experiment planning, data collection, data analysis and manuscript preparation.

Dr. Linda Saucier and Dr. Luigi Faucitano (Research director and co-director, and corresponding author, respectively): student supervision, revision and correction of the manuscript.

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Sophie Horth (Research assistant): supervision of data and sample collection at the plant and supervision of laboratory work.

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Hand-held lactate analyzer as a tool for the real-time measurement of physical fatigue before slaughter and pork quality prediction

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Short title: Blood lactate and pork quality

Abstract

The objectives of this study were to assess the relationship between blood lactate variation measured at the plant, and pork quality variation on a large sample size and under commercial preslaughter handling conditions. A total of 600 pigs were randomly chosen on arrival at a commercial slaughter plant and blood samples taken from the ear vein at unloading (UN), after lairage (LA), in the restrainer (RE; before stunning), and at exsanguination (EX) were analysed for lactate content using a Lactate Scout Analyzer (LSA). In order to have a large range of measures, pigs were distributed into two groups; one kept in lairage overnight (G1) and the other for 2-3 h (G2) before slaughter. Meat quality was assessed in the *Longissimus thoracis* (LT), S*emimembranosus* (SM) and *Adductor* (AD) muscles by measuring the pH 30 min *post-mortem* (pH1) and at 24 h *post-mortem* (pHu), the colour and the drip loss. Blood lactate levels did not differ between G1 and G2 ($P > 0.05$). A reduced muscle lactate

and glucose contents ($P = 0.02$ and $P = 0.004$, respectively) resulting in a lower ($P < 0.001$) glycolytic potential (GP) was observed in the LT muscle of G1 pigs when compared to G2 loins. In the LT muscle of G1 pigs, the lower GP resulted in an increased pHu ($r = - 0.67$; $P < 0.001$), decreased drip loss ($r = 0.57$; $P < 0.001$) and darker colour ($r = 0.50$; $P < 0.001$) compared to G2. In both G1 and G2 pigs, the lower GP was correlated to higher pHu value in the SM and AD muscles ($r = - 0.73$; $P < 0.001$). The greatest correlation was observed in G2 between blood lactate levels at LA and pHu value of the SM and AD muscles ($r = 0.46$ and $r = 0.44$, respectively; $P < 0.001$ for both muscles). The second greatest correlation was found between blood lactate levels at EX and pH1 value in the SM muscle in both groups ($r = - 0.37$ and $r = - 0.41$, respectively; $P < 0.001$ for both groups). Based on the results of this study, it appears that blood lactate levels, as measured by the LSA, reliably reflect the physiological response of pigs to *peri-mortem* stress and may help explain the variation in pork quality.

Keywords: stress, lactate, blood, meat quality, pigs

Implications

The majority of meat quality defects are directly related to preslaughter procedures, which are known to influence the physiological state of pigs before and at slaughter. Hence, the Lactate Scout Analyzer used in this study may be an accurate tool to assess the physiological condition of pigs under commercial conditions, and to predict the variation of meat quality traits. Furthermore, it may allow plant managers to identify critical points to be controlled in the preslaughter procedures in order to improve animal handling, facilities design, etc., and ultimately to limit meat quality losses.

Introduction

Muscular activity requires energy, which is provided by the breakdown of glycogen in the skeletal muscles. During intense muscular activity, the oxygen supply is often insufficient, so the energy is released through an anaerobic process which converts pyruvate to lactate (Nelson and Cox, 2008). Therefore, lactate is either released into the blood flow in very disturbed or frightened animals or when there is some muscle damage (bruising), caused by vigorous physical exercise (Broom, 1995), and indicates an acidosis status of the pig as showed by the low blood pH (Ritter et al., 2009).

Earlier studies have associated greater values of exsanguination blood lactate to poor pork quality (Correa et al., 2010; Edwards et al., 2010a). As blood lactate is not influenced by post-stunning handling (Aalhus et al., 1991) and is a very short-term stress indicator (higher peak in 4 min and return to basal levels in 2 h after physical exercise; Anderson, 2010), the greater lactate level in blood at slaughter definitely mirrors the physiological state of pigs prior to slaughter. For practical reasons, there is a need to develop a blood lactate measurement in the bleeding rail at the slaughter plant alternative to the traditional time-consuming enzymatic analytical procedure. The hand-held Lactate Scout Analyzer (LSA; EKF Diagnostic GmbH, Magdeburg, Germany) is being increasingly used for the measurement of blood lactate at swine slaughter plants, based on its strong correlation (r = 0.97; Edwards et al., 2010a) with the enzymatic procedure. This device would allow the monitoring of lactate variation in commercial conditions and assist in the development of improved animal handling methods before stunning. LSA blood lactate levels proved to be significantly correlated, although weakly, with a few pork quality traits, such as pH value 1 h *post-mortem* and drip loss in the loin muscle (Edwards et al., 2010a). The low correlations reported in this study may be explained by the small sample size (n = 128 pigs), the low-stress handling conditions and by the fact that only the loin muscle was used for meat quality evaluation. There is evidence that the *longissimus* muscle may not be the most suitable muscle to study meat quality variation in relation to physical stress (Correa et al., 2010).

Therefore, the objectives of this study were two-fold: 1) to assess the relationship between lactate levels in blood collected at different points on the slaughter line and pork quality variation (in loins and hams) on a large sample size and under commercial preslaughter handling conditions and 2) to evaluate LSA's reliability as tool to prevent meat quality losses.

Materials and methods

Animal ethics

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 (2009).

Animals and treatments

In a 6 week trial, a total of 600 market weight pigs (crossbreed F1 Yorkshire female × Landrace sired with Duroc boar) were randomly chosen on arrival at a commercial slaughter plant (slaughter speed of 500 pigs per hour; 7,000 pigs/day) located in Eastern Canada over 6 slaughter days (1 day/week and 100 pigs/day). On each slaughter day, multiple trucks were randomly sampled to get 100 pigs (10 - 15% of total load/truck). Random selection of pigs was chosen in order to ensure the largest variation in pigs' physical conditions on arrival at the plant, with pre-transport fasting interval ranging from 6 to 12 h and transport time ranging from 0.5 to 5 h. Animals were identified by a numbered plastic ear tag to facilitate their identification at each sampling point and to track the carcasses for the meat quality assessment after slaughter. Pigs were distributed into 2 main groups of 50 pigs each. The first group of 50 pigs was kept in one pen in lairage overnight (G1; n = 300), whereas the second group was kept in two pens, with 25 pigs each, and kept in lairage between 2 and 3 h before slaughter (G2; n = 300). In lairage, stocking density in the pen was 0.58 m^2/ pig for both groups. The stocking density in the lairage pen was controlled in this study as it may interfere, more than group size, on the effects of lairage time on pigs' resting behaviour (Moss, 1978). During lairage water was available through nipple type drinkers. Both lairage groups were sprinkled in the rest pen during the 30 – 45 min of end of lairage. Pigs were electrically stunned (head-to-chest electrical stunning) prior to exsanguination in the prone position.

Blood lactate analysis

Blood samples were collected from each pig by pricking 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 four different sampling points: at unloading (UN; n = 600), after lairage at the exit of the resting pen (LA; n = 600) and in the restrainer before stunning (RE ; n = 600). 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; n = 600) 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. The bleeding wound was preferred for blood sampling at exsanguination instead of the ear based on the positive correlations between lactate content in the ear venous blood and that in the jugular venous and arterial blood (r = 0.80 and r = 0.74, respectively; $P < 0.001$ for both sampling locations) obtained in a preliminary study (unpublished results).

Meat quality measurements

Each slaughter week, twenty-five (25) carcasses were selected from each lairage groups (50 carcasses/slaughter day; total of 300 carcasses) according to the blood lactate level at exsanguination with the objective to ensure a large range of blood lactate levels and meat quality traits. About 35 min after slaughter, carcasses were blast chilled (-20˚C) for 90 min and then transferred to standard chilling rooms (3˚C) where they were kept until the next day.

Meat quality was assessed in the *Longissimus thoracis* (LT; at the 3rd/4th last rib), *Semimenbranosus* (SM; in the middle region) and *Adductor* (AD) muscles. Muscle pH was measured at 30 min *post-mortem* (pH1) in the LT and in the SM muscles by means of a portable pHmeter (Oakton Instruments Model pH 100 Series, Vernon Hills, IL) fitted with a Cole Parmer spear tip electrode (Cole Palmer Instrument Company, Vernon Hills, IL) and an automatic temperature compensation (ATC) probe. This measurement was repeated at 24 h *post-mortem* (pHu) in the same muscles and in the AD muscle. At 24 h *post-mortem*, colour data were collected on the LT and SM muscles at the afore-mentioned anatomical locations after 30 min blooming time. Visual colour was evaluated using the Japanese colour standards (Nakai *et al.,* 1975) in the LT muscle only, whereas instrumental colour (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 in the LT and SM muscles. Drip loss was measured in a LT muscle chop removed at the 3rd/4th last rib level and in the middle region of the SM muscle by a modified EZ-Driploss procedure (Correa *et al.*, 2007). Briefly, three 25 mm diameter cores were removed from the center of a 2.5 cm thick LT and SM muscle cross-section, 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 re-weighed, and drip loss percentage was calculated by dividing the difference between initial and final core weights by the initial core weight.

The floppiness score of the LT muscle was assessed by finger testing before dissection by a trained evaluator using a subjective scale ranging from 1 to 3 (1 = very soft and watery to 3 = very firm and dry; NPB, 2000).

A sample of the LT muscle was also harvested in the region of the $3^{rd}/4^{th}$ last rib and immediately frozen in liquid nitrogen at 24 h *post-mortem* for the analysis of the glycolytic potential (GP). The analysis was performed according to the method described by Monin and Sellier (1985) with some modifications and following the extraction protocol described by Bergmeyer (1974). Briefly, 1 *g* of the LT muscle was homogenized in a Polytron device (System Polytron® PT 3100, Kinematica AG, Luzern, Switzerland) and then the samples were centrifuged at 2,000 x g for 20 min at 4°C. For the enzymatic determination of glycogen, glucose and glucose-6-P, 500 µl were transferred to glass tubes and the rest of homogenate was filtered with a filter paper (Whatman # 4; Buckinghamshire, UK) and the homogenate was kept at 4°C for the enzymatic determination of lactate. The samples were homogenized in buffer containing Rhizopus amyloglucosidase to decompose glycogen to glucose and glucose 6 phosphate. Lactate concentration in the homogenized samples was determined using nicotinamide adenine dinucleotide (NAD) and lactate dehydrogenase. Glucose concentration was determined using a NAD, glucose-6-phosphate, adenosine triphosphate (ATP) and enzymatic solution of hexokinase. The GP was quoted in terms of potential lactate formation according to the following formula proposed by Monin and Sellier (1985): 2 ([glycogen] + [glucose] + [glucose 6 phosphate]) + [lactate]. GP is expressed as µmole glucose equivalent /g of fresh muscle.

Statistical analyses

All statistical procedures performed in the current study were carried out using the Statistical Analysis Software (SAS Institute Inc., Cary, NC, 2002). Blood lactate values were log-transformed (log10) for data normalization before analysis. Log values were analyzed for each sampling point with the MIXED procedure of SAS using sampling points as repeated measures in a one-way analysis of variance for the

group effect with the animal as the experimental unit and the week as random effect. Resulting adjusted means and confidence limits were back-transformed to the original scale and used to build up Figure 7. Multiple comparisons between sampling points were adjusted with a Tukey-Kramer correction.

Analysis of variance for quality traits, potential glycolytic, muscle lactate and muscle glucose were carried out using the MIXED procedure of SAS. The model included the group as a fixed effect, the animal as the experimental unit and the week as a random effect. For variables showing a non-normal distribution of residuals, the analysis was performed with the non-parametric Wilcoxon Mann-Whitney test, using the NPAR1WAY procedure with the WILCOXON option. Spearman correlations were performed between blood lactate concentration at different sampling points and meat quality. Floppiness scores were analyzed by the FREQ procedure of SAS using the Cochran-Mantel-Haenszel statistic to determine the effect of group on the mean score.

Results and discussion

Blood lactate variation

The physical activity associated with handling and fighting in lairage may cause physiological changes in pigs during the preslaughter period. As showed in Fig. 1, in this study average lactate levels were of 3.66 mM (range: 3.50 to 3.83 mM) at unloading, dropped to 2.88 mM (range: 2.77 to 3.00 mM; $P < 0.001$) after resting in the lairage pen, regardless of the resting time, and increased to 5.00 mM (range: 4.81 to 5.19 mM; $P < 0.001$) prior to stunning and to 8.71 mM (range: 8.37 to 9.08 mM) at exsanguination. The increase in blood lactate concentration between LA and RE reflects the progressively higher level of muscle activity and stress as the animals are handled and pass from a free-moving group situation to a single line of aligned and restrained individuals. Other studies also reported increased blood concentration of lactate at exsanguination (Hunter et al*.,* 1994; Edwards et al*.*, 2011), and body temperature (Stewart et al*.*, 2005) in pigs being moved forward in a single line to the stunning point.

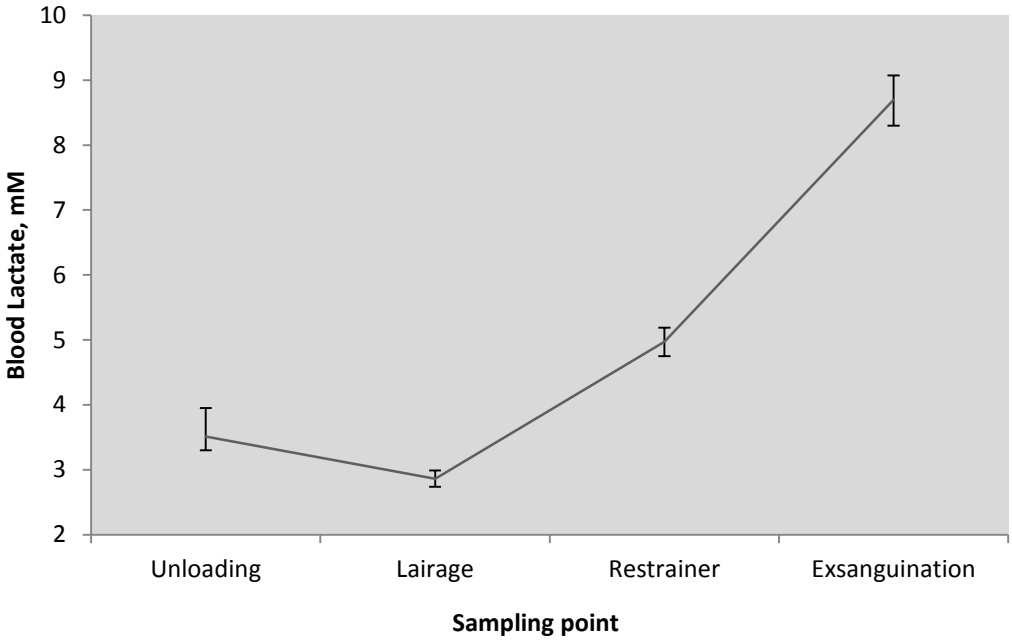

Figure 1. Preslaughter variation of lactate levels (mM; ± Confidence limits) in blood collected at different sampling points on the dressing line*.

*Between sampling points, means with a different letter differ significantly ($P < 0.001$).

Based on the highest correlation between RE and EX blood lactate levels ($r = 0.60$; $P < 0.001$; Table 1), the measurement of blood lactate level using the LSA at the entrance into the restrainer appears to be the best indicator of physical fatigue of pigs at slaughter. However, our results also showed an increase in the blood lactate level between RE and EX ($P < 0.001$; Fig.1), meaning that electrical stunning may have an impact on the rate of lactate release into the blood flow at slaughter in this study.

Greater blood lactate levels have been also reported in electrically *vs.* gas stunned pigs by Bertoloni et al. (2006). This difference can be explained by the greater muscle contraction (tonic phase) in response to electrical current application.

Similarly to Edwards et al. (2011), EX blood lactate levels as measured by the LSA in this study were lower than those reported by Hambrecht et al. (2004, 2005) which reported lactate values ranging from 12 to 31 mM in exsanguination blood analyzed with the traditional enzymatic procedure.

Table 1. Spearman correlations between lactate concentrations collected in pigs at four different sampling points[1] at the slaughter plant

Sampling point *R*	**UN**	**LA**	**RE**	**EX**
UN	1.00	0.20***	0.17***	0.22***
LA		1.00	0.45***	0.23***
RE			1.00	0.60***
EX				1.00

[1]UN = Unloading (n = 600); LA = End of lairage (n = 583); RE = Restrainer (n = 581); EX: Exsanguination (n = 583).

*** $P < 0.001$.

Similarly to Edwards et al. (2011), EX blood lactate levels as measured by the LSA in this study were lower than those reported by Hambrecht et al. (2004, 2005) which reported lactate values ranging from 12 to 31 mM in exsanguination blood analyzed with the traditional enzymatic procedure. The explanation for these differences between studies may be two-fold: 1) the different distribution of lactate between whole blood (*i.e.* blood from which no constituent, such as red blood cells, white blood cells, plasma, or platelets, has been removed according to the American Heritage® Science Dictionary, 2005) and plasma resulting in the underestimation of blood lactate concentrations when whole blood instead of plasma alone is use for analysis and 2) the difference in stress level (high vs. minimal) experienced by pigs prior to slaughter in the two studies. Indeed, results obtained in a preliminary study showed that LSA is an efficient tool to detect pig fatigue after physical exercise based on the significant ($P > 0.001$) increase in blood lactate levels from rest to post-handling stress, *i.e.* pigs were imposed to walk at a fast pace for 250 m (2.41 ± 0.84 mM *vs.* 7.63 ± 3.98 mM, unpublished results).

According to Pösö and Puolanne (2005), blood lactate concentration may vary between 5 and 25 mM in meat animals. Furthermore, the distribution of lactate in blood does not appear to be homogenous (Harris and Dudley, 1989). For example, it was reported that whole blood lactate is approximately 40 % lower than plasma

lactate concentration, although they are strongly correlated (r = 0.993; Foxdal et al., 1990). The greater concentration of lactate in plasma compared to whole blood may explain the difference in lactate values reported by Hambrecht et al. (2004, 2005) in blood plasma and those found in our study and in Edwards et al. (2010a, 2011) where lactate content was analyzed by the LSA in the whole blood. The underestimation of the blood lactate content as measured with the LSA may be also explained by the significant delay of the transfer of lactate from plasma into red cells in the whole blood after it is generated in the muscle tissue until a balance is reached (Forrest et al., 1990).

Considering the speed rate of lactate to reach the maximum concentration after stress in blood (4 min; Anderson, 2010), the stress level applied in the *peri-mortem* phase may be another possible explanation for the difference in blood lactate contents between this study and those reported in the literature. Greater lactate concentrations in exsanguination blood have been reported in pigs aggressively moved (use of electric prods and yells) to the stunner (Hambrecht et al., 2004). Whereas, similarly to Edwards et al. (2010a), in this study where the *peri-mortem* handling conditions were controlled (*i.e.* driving small groups without electric prods), the stress level applied on pigs prior to stunning does not appear to have been sufficient to produce an elevation of lactate levels in blood at exsanguination. Benjamin et al. (2001) also reported no variation in blood lactate concentration analyzed by the LSA in pigs that were pushed to walk a long distance (300 m), but were handled gently (natural pace without electric prods).

Effect of lairage time on blood lactate concentration

Differently from Warriss *et al.* (1998) and Edwards et al. (2010a) who reported greater exsanguination blood lactate levels in pigs after long lairage (overnight *vs.* 4 h), blood lactate levels did not differ between lairage groups in this study, meaning that lairage time did not influence blood lactate concentration at slaughter (Table 2). Pèrez et al. (2002) and Hambrecht et al. (2005) did not find significant effect on blood lactate concentration at exsanguination between long (up to 9 h) and short (< 45 min) lairage groups either.

It is worth mentioning that blood lactate levels recorded at LA in this study were lower than 4 mM, which is the resting level of blood lactate reported for market-

weight pigs in previous studies (Edwards et al., 2011). Based on the speed of blood lactate level to return to rest level (120 min; Anderson, 2010), the low blood lactate levels after lairage recorded in this study would indicate that pigs had the adequate lairage conditions to recover from the stress of transport and unloading, regardless of the lairage time.

Table 2. Descriptive statistics of blood lactate levels (mM) per lairage group[1] of pigs at four sampling points[2]

	G1				G2			
Sampling point	**n**	**Mean**	**Lower**	**Upper**	**n**	**Mean**	**Lower**	**Upper**
UN	300	3.65	3.44	3.87	300	3.64	3.43	3.86
LA	299	2.98	2.82	3.17	284	2.74	2.58	2.90
RE	297	4.98	4.69	5.28	285	4.96	4.67	5.27
EX	299	8.76	8.26	9.29	285	8.59	8.09	9.13

[1]G1= Group kept in lairage overnight; G2 = Group kept in lairage between 2 and 3 h

[2]UN= Unloading; LA= End of lairage; RE = Restrainer; EX: Exsanguination

Meat quality

Effect of lairage time on meat quality traits

The purpose of lairage is to allow an opportunity for stressed and (or) fatigued animals to recover from loading and transport and to improve pork quality (Warriss, 2003). No difference was observed in pH1 between lairage groups. As expected, compared to 2-3 h lairage (G2), overnight lairage (G1) resulted in a greater pHu in the LT, SM and AD muscles ($P = 0.03$, $P = 0.005$ and $P = 0.005$, respectively) and lower L* and drip loss values in the LT and SM muscles ($P = 0.002$ for both muscles and $P = 0.001$ and $P < 0.001$, respectively; Table 3). Moreover, a greater ($P = 0.02$) proportion of firm and dry (score 3) loins was found in G1 loins compared with G2 (37.0 *vs*. 25.5 %; Fig. 2). Increased incidence of greater pHu and darker and firmer pork after long lairage has been extensively reported in the literature (Warriss, 2003) and is explained by muscle glycogen depletion caused by extended feed restriction and muscle fatigue (Fernández and Tornberg, 1991; Hambrecht et al., 2004).

Table 3. Variation of meat quality characteristics in the *longissimus thoracis* (LT), *semimembranosus* (SM) and *adductor* (AD) muscles of pigs according to the lairage group[1]

Variable	G1			G2			
	n	Mean	SD	n	Mean	SD	*P value*[2]
LT muscle							
pH1	133	6.64	0.22	156	6.63	0.20	NS
pHu	135	5.74	0.14	157	5.70	0.14	0.03
L*	134	51.26	3.54	157	52.39	3.26	0.002
Drip loss, %	134	2.69	2.12	157	3.25	1.88	0.001
SM muscle							
pH1	132	6.81	0.18	156	6.79	0.21	NS
pHu	135	5.92	0.18	156	5.86	0.17	0.005
L*	135	49.52	2.94	156	50.35	2.82	0.002
Drip loss, %	135	1.91	1.19	156	2.41	1.41	0.0009
AD muscle							
pHu	135	6.13	0.27	156	6.06	0.25	0.005

[1]G1= Group kept in lairage overnight; G2 = Group kept in lairage between 2 and 3 h.
[2]Z -Wilcoxon test.

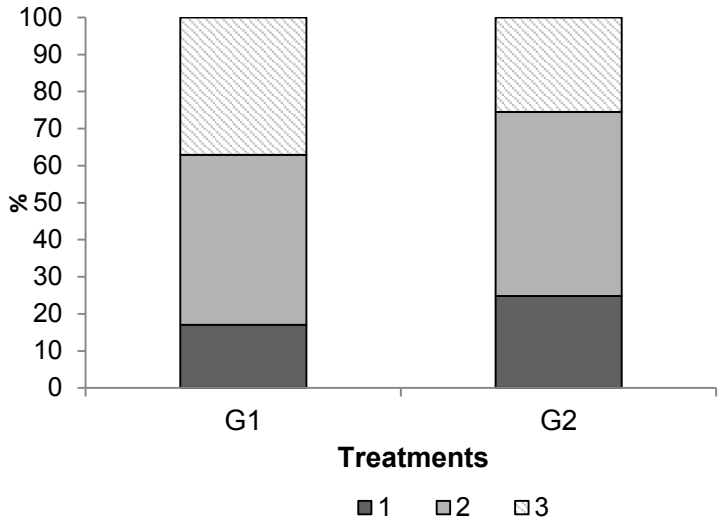

Figure 2. Comparison of scores* frequency for floppiness in the LT muscle between lairage groups**.

* Floppiness scores: 1 = very soft and watery; 2 = normal and 3 = very firm and dry.

**G1= Group kept in lairage overnight; G2 = Group kept in lairage between 2 and 3 h.

Overall, the GP values obtained in this study (Table 4) are within the range reported for the LT muscle of pigs in the literature (128-154 μmol/g fresh tissue; Przybylski et al., 1994; Hambrecht *et al.*, 2004). However, similarly to meat quality traits, lairage time had an effect on the GP of the LT muscle, with muscle lactate and glucose contents and GP values being lower ($P = 0.02$, $P = 0.004$ and $P < 0.001$, respectively) in the LT muscle of pigs kept in lairage overnight (Table 4). Zhen et al. (2013) also reported decreased lactate and glucose concentrations and GP value in the LT muscle of pigs as lairage time increased. The GP variation reflects the greater ante-mortem muscle energy exhaustion in the loin muscle of G1 pigs and contributes to explain the variation in pHu in the LT, SM and AD muscles ($r = -0.67$ and $r = -0.73$ for both SM and AD muscles, respectively; $P < 0.001$ for all muscles), in drip loss ($r = 0.57$; $P < 0.001$) and L* value ($r = 0.50$; $P < 0.001$) in the LT muscle compared to G2 (Table 5).

The correlation between GP of the LT muscle and pHu in the SM muscle is not surprising as these muscles have comparable metabolic characteristics (Laborde et al., 1985; Monin et al., 1987). Indeed, similarly to the LT muscle, in the SM muscle pHu variation follows a curvilinear regression when GP increases ($r = -0.80$; $P < 0.001$; Przybylski et al., 1994).

Table 4. Variation of lactate content, glucose content and glycolytic potential measured in the *longissimus thoracis* (LT) muscle of pigs from two lairage groups.

	G1	G2	SEM	*P-value*
N	125	150		
Lactate, µmol/g[1]	90.90	95.01	5.16	0.02
Glucose, µmol/g	5.42	6.48	0.30	0.004
GP[2], µmol/g	124.32	134.60	5.96	<0.001

[1]All results are presented by µmol/g of meat from the LT muscle at 24h *post-mortem*.
[2]GP = Glycolytic potential.

Table 5. Spearman correlations between glycolytic potential and meat quality characteristics as assessed in the *longissimus thoracis* (LT), *semimembranosus* (SM) and *adductor* (AD) muscles by lairage group[1,2]

	G1			G2		
Parameters *R*	GP[3]	Lactate	Glucose	GP[2]	Lactate	Glucose
LT muscle						
pH1	-0.18*	-0.39***	0.10	-0.31***	-0.30***	-0.16
pHu	-0.67***	-0.48***	-0.53***	-0.45***	-0.20*	-0.56***
L*	0.50***	0.37***	0.35***	0.32***	0.01	0.43***
Drip loss	0.57***	0.38***	0.47***	0.15	0.13	0.01
SM muscle						
pH1	-0.21*	-0.44***	0.11	-0.20*	-0.23***	-0.12
pHu	-0.73***	-0.47***	-0.68***	-0.56***	-0.30***	-0.62***
L*	0.24***	0.18*	0.19*	0.27***	0.03	0.38***
Drip loss	0.46***	0.11	0.61***	0.39***	0.10	0.51***
AD muscle						
pHu	-0.73***	-0.46***	-0.68***	-0.35***	-0.13	-0.47***

[1]G1= Group kept in lairage overnight; G2 = Group kept in lairage between 2 and 3 h.
[2] LT muscle (n = 124 for G1 and n = 148 for G2); SM muscle (n = 123 for G1 and n = 148 for G2) AD muscle (n = 125 for G1 and n = 148 for G2).
[3] GP = Glycolytic potential.
* $P < 0.05$; *** $P < 0.001$.

Correlations between blood lactate levels and meat quality

Spearman correlations between blood lactate concentration at different sampling points and meat quality traits by lairage event are showed in Table 6. Similarly to Edwards et al. (2010a), in this study the correlations between blood lactate levels and meat quality traits in the LT muscle were generally low for both lairage groups. The greatest correlation was found between blood lactate level recorded at the end of the resting period when exiting the lairage pen (LA) and the pHu value in the SM and AD muscles ($r = 0.46$; $P < 0.001$ and $r = 0.44$; $P < 0.001$, respectively) in the G2 group.

The second greatest correlation was found between blood lactate levels at EX and pH taken at 1h post-slaughter in the SM muscle in both G1 and G2 groups ($r = -0.37$ and $r = -0.41$, respectively; $P < 0.001$ for both lairage groups), suggesting a decreased pH1 as blood lactate levels increase at exsanguination. The contribution of exsanguination blood lactate levels to early *post-mortem* acidification rate found in this study confirms what was already reported in previous studies (Hambrecht et al., 2005; Edwards et al., 2010a). However, the correlations obtained in this study are greater than those reported by Edwards et al. (2010a) using the LSA and the LT muscle as meat quality indicator ($r = -0.32$).

Overall, the greater correlations between blood lactate levels and meat quality traits in the ham muscles are not surprising as they are locomotors muscles and thus more prone to rapid glycogen exhaustion after physical exercise rather than postural muscles, such as the LT muscle. These results, similar to others from previous studies (Hambrecht et al., 2005; Correa et al., 2010), show that the effects of a specific stress on meat quality, either physical or psychological, are muscle-dependent.

Conclusions

Overall, our results suggest that the hand-held scout analyzer is capable of measuring blood lactate levels variation associated with the physiological condition of pigs in the *peri-mortem* phase. However, although significant, the magnitude of the correlations between blood lactate and meat quality traits found in this study is rather low, meaning a poor reliability in predicting pork quality variation. Possible reasons for these low correlations can be either the small range of variation in the preslaughter stress levels applied in this study or the use of whole blood for lactate analysis

Table 6. Spearman correlations between blood lactate level at different sampling points[1] on the dressing line and meat quality characteristics as assessed in the *longissimus thoracis* (LT), *semimembranosus* (SM) and *adductor* (AD) muscles by lairage group[2,3]

	G1				G2			
Parameters *r*	**UN**	**LA**	**RE**	**EX**	**UN**	**LA**	**RE**	**EX**
LT Muscle								
pH1	-0.06	0.01	-0.04	-0.23*	0.00	0.16	-0.01	-0.20*
pHu	0.04	0.24*	0.22*	0.19*	0.18*	0.29**	0.07	-0.02
L*	0.02	-0.11	-0.09	0.14	-0.14	-0.18*	0.03	0.14
Drip loss	-0.14	-0.16	-0.04	0.03	-0.17*	-0.09	0.11	0.18*
GP, µmol/g [4]	-0.14	-0.33***	-0.26***	-0.16	-0.19*	-0.19*	0.05	0.00
Lactate, µmol/g	0.03	-0.13	-0.16	-0.02	0.07	-0.11	0.06	-0.01
Glucose, µmol/g	-0.26***	-0.30***	-0.27***	-0.29***	-0.41***	-0.19*	-0.04	-0.05
SM Muscle								
pH1	-0.18*	0.06	0.06	-0.37***	-0.09	0.13	-0.08	- 0.41***
pHu	0.23*	0.28**	0.26**	0.29**	0.33***	0.46***	0.07	-0.07
L*	-0.18*	-0.02	-0.08	0.10	-0.22*	-0.26**	-0.04	0.17*
Drip loss	-0.23*	-0.21*	-0.19*	-0.09	-0.24**	-0.27**	0.01	0.22*
AD Muscle								
pHu	0.25**	0.29**	0.32**	0.28**	0.30**	0.44***	0.28**	0.13

[1]UN = Unloading; LA= End of lairage; RE = Restrainer; EX: Exsanguination.
[2]G1= Group kept in lairage overnight; G2 = Group kept in lairage between 2 and 3 h.
[3] LT muscle (n = 134 for G1 and n = 156 for G2); SM muscle (n = 133 for G1 and n = 155 for G2) AD muscle (n = 135 for G1 and n = 156 for G2).
[4]GP = Glycolytic potential (n = 124 for G1 and n = 148 for G2).
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

resulting in an underestimation of lactate concentrations in blood. Thus, for a more reliable validation of the LSA technique for the monitoring of the preslaughter conditions and control of pork quality variation, further studies in which the LSA is used as stand-alone measurement or in combination with other non-invasive tools (*e.g.* Infrared thermography) under more variable preslaughter conditions are needed.

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Chapter 4: Can the monitoring of animal welfare parameters through the supply chain (from farm to slaughter) predict pork meat quality variation?

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Consumers are expressing an increased concern about animal welfare issues in livestock production. The shift of consumer confidence towards food safety and animal welfare from government control to trusted brand products pushed the pork chain stakeholders to adopted auditing systems, which validate standards throughout the pork supply chain. However, it is unknown whether or not the the implementation of animal welfare audit criteria set by audit protocols is contributing to high and uniform quality pork. Therefore, this chapter investigated the animal welfare conditions by audit protocols through the supply pork chain and their relationship with preslaughter behaviour at the plant and pork meat quality variation.

Upon invitation from the Canadian Meat Science Association (CMSA), the preliminary results of this study were presented orally at the 2014 Canadian Society of Animal Science (CSAS)-CMSA joint meeting in Banff (AB). Two oral communications were also given at the 2014 Midwest Meeting of the American Society of Animal Science (ASAS) in Des Moines (IA). Rocha, L.M., Velarde, A., Dalmau, A., Saucier, L., and Faucitano, L. (2014). Can animal welfare assessment at the farm be a good tool to control pork quality variation? , Journal of Animal Science, 92(Suppl.1), p. 24. (Abstr.) and Rocha, L.M., Velarde, A., Dalmau, A., Saucier, L., and Faucitano, L. (2014). Effect of the farm system on the behavioural response preslaughter and on meat quality variation in pigs, Journal of Animal Science, 92(Suppl.1), p. 5. (Abstr.).

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Can the monitoring of animal welfare parameters predict pork meat quality variation through the supply chain (from farm to slaughter)?

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ABSTRACT: The objective of this study was to assess the relationship between the animal welfare conditions evaluated through the supply chain and pork quality variation. A total of 4,680 pigs from 12 farms, 5 animal welfare-improved (AWIRS) and 7 conventional (CON) farms, were assessed from farm to slaughter through a comprehensive audit protocol merging the European Welfare Quality®, the Canadian Animal Care Assessment and American Meat Institute Audit Guide criteria. At the abattoir, a sub-sample of 1,440 pigs (120 pigs/farm) was randomly chosen out of 24 loads (2/farms per wk) transported by two drivers (driver A and driver B) for the assessment of stunning effectiveness, carcass bruises, blood lactate levels and meat quality traits. Meat quality was assessed in the Longissimus lumborum (LL) muscle 24 h post-mortem by measuring ultimate pH (pHu), colour (L*, a*, b*) and drip loss. Data were analysed by MIXED, GLIMMIX and NAPAR1WAY procedures of SAS. Spearman correlations were calculated to determine the relationship between audit scores and meat quality traits. Better animal welfare conditions, as showed by greater final scores for good housing (GHO; $P = 0.001$) and good health ($P = 0.006$) principles, were recorded at AWIRS farms. Pigs from AWIRS farms handled by driver B displayed a greater percentage of turning-back ($P = 0.01$) and slips ($P < 0.001$) during unloading and greater ($P = 0.02$) frequency of falls in the stunning chute. A greater ($P = 0.02$) reluctance to move at loading was found in CON pigs loaded by driver A compared to driver B, whereas a greater ($P < 0.001$) reluctance to move was found in these pigs at unloading when they were unloaded by driver B. Drip loss was higher (P = 0.003) and PSE pork percentage was greater ($P < 0.001$) in the LL muscle of the heavier AWIRS pigs. The GHO principle was best correlated with pHu ($r = - 0.75$; $P = 0.01$) and Minolta L* value ($r = 0.87$; $P < 0.001$) of the LL muscle. Overall, drip loss variation in the LL muscle was correlated with the frequency of slips at unloading ($r = 0.63$; $P = 0.001$) and in the restrainer area ($r = 0.74$; $P < 0.001$). The results of this study showed that the quality of the raising system and truck driver skills as assessed by animal welfare audit protocols are important sources of variation in the behavioural response of pigs to preslaughter handling and may affect pork quality variation. However, the different liveweight between CON and AWIRS pigs may have biased the meat quality results in this study.

Keywords: animal welfare, audit, behaviour, meat quality, pigs

INTRODUCTION

The shift of consumer confidence in food safety and animal welfare from government control to trusted branded products pushed the pork chain stakeholders to adopt auditing systems, which check standards through the pork supply chain. In North America, the Animal Care Assessment (ACA) protocol developed by the Canadian Pork Council (CPC, 2011) and the Animal Handling Audit Guide of the American Meat Institute (AMI, 2012) are used by the pork industry to monitor animal welfare at the farm and at slaughter, respectively. In Europe, the Welfare Quality protocol (WQ®, 2009a) has been developed for the animal welfare assessment at the farm and at the slaughter plant.

According to Grandin (1993), to have quiet handling, it is essential to bring easy to handle pigs to the plant. Raising conditions at the farm are considered a major source of variation in the easiness of handling pigs commonly observed between batches of pigs at the slaughter plant (Grandin, 1993; Grandin and Vogel, 2011). On-farm animal welfare audit protocols, such as those developed by the WQ® and the CPC, may be valid tools to assess the quality of the raising conditions at the farm of origin, and may allow the consistent delivery of easy to handle pigs at the slaughter plant. However, on-farm audit scores were never matched with handling audit scores at the slaughter plant to assess the efficiency of these protocols as tools to predict preslaughter animal behaviour. Furthermore, overall it is not known whether the respect of the animal welfare audit criteria set by these audit protocols are conducive to high and uniform quality pork either.

The overall objective of this study was to assess the relationship between the animal welfare conditions on farm and preslaughter behaviour as assessed by audit protocols and pork quality variation, and more specifically to evaluate the relationship between audit scores on farm and at the slaughter plant, and their impact on pork quality variation.

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 (2009).

Farms, Animals and General Preslaughter Procedures

A total of 4,680 crossbred pigs of mixed genders (barrows and gilts) were audited in this study. On both farm types animals were of the same genetics and were fed the same diet. Pigs were raised in 12 growing/finishing farms belonging to a swine integrated company located in Eastern Canada. Of the 12 selected farms, 5 farms (A, B, C, D and E) were classified as animal welfare improved raising system (AWIRS; *n* = 1,943 pigs) and 7 (F, G, H, I, J, K and L) were classified as conventional raising system (CON; *n* = 2,737 pigs) according to the swine company internal audit protocol. Farms were classified as AWIRS based on the company audit protocol using the following animal welfare standards: antibiotics and growth promoters-free feeding, minimum space allowance of 0.85 m^2/pig, mandatory presence of bedding, use of trained handlers and frequent management operations (*i.e.* periodical change of bedding). Furthermore, according to these internal standards, pigs must be handled quietly (no loud sounds) and firmly using paddles and sorting boards in order to reduce fear and improve animal welfare. The use of electric prods in this housing system is prohibited, except when animal or human safety is in jeopardy. Whereas, conventional farms strictly met the animal welfare legislative requirements, such as more confined housing on partially slatted floors (ranging from 33 to 100% of slatted floor) and lower floor space allowance (around 0.74 m^2/pig). Furthermore, AWIRS farms were of wean-to-finish type, while CON farms were of grow-to-finish type. A description of housing facilities and pigs' performance at each commercial farm within farm type and between farm types is provided in Tables 1 and 2, respectively.

Pigs were withdrawn of feed for 16.3 h ± 2.6 at AWIRS farms and for 14.3 h ± 2.1 at CON farms before loading. At loading, pigs were sorted out from the home pen in cohorts of 5-6 pigs and driven through the alley up to the loading ramp (walking distance: 39.8 m ± 11.5 at AWIRS farms and 45.7 m ± 7.7 at CON farms). A total of 24 loads, in terms of 2 loads/farm per wk, were transported using two similar pot-belly trailers, but driven by two different drivers (driver A and driver B). To avoid the confounding effects of handling and driving skills on the audit results driver A and driver B were rotated between farms and between loads within the farm each wk. Transport distance between AWIRS and CON farms and the slaughter plant was of 322 km ± 157 and 284 km ± 117, respectively.

Table 1. Description of farm management and facilities, and pigs' performance by farm type and farm unit within farm type[1]

	AWIRS					CON					
Farm	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Management system												
Pigs/farm, n	816	809	770	620	634	1134	1400	624	819	760	504	1110
Pigs/pen, n	18	16	175	13	15	17	26	25	126	15	19	17
Grow-to-Finish time, d	153	153	147	156	157	107	106	103	102	93	101	106
Farm Facilities												
Pen dimension, m^2	15.8	16.1	371.5	15.6	16.4	12.7	18.1	18.5	254.2	12.1	12.3	15.6
Full floor:slatted floor	1/3	1/3	1/3	1/3	1/3	1/1	1/3	4/5	2/3	1/2	1/3	2/3
Space allowance, m^2/100 kg	0.65	0.73	1.67	0.86	0.79	0.58	0.57	0.59	1.69	0.68	0.54	0.73
Performance												
Start weight, kg	7.76	6.90	7.88	7.88	8.48	30.39	32.12	30.85	26.90	36.31	32.51	27.82
Final weight, kg	135.3	138.62	126.93	139.7	138.48	128.9	122.02	125.94	119.34	118.21	120.53	125.43
ADG[2], kg	0.852	0.875	0.792	0.854	0.866	0.943	0.862	0.910	0.925	0.956	0.891	0.940
Feed conversion	2.6	2.5	2.5	2.5	2.5	2.4	2.7	2.6	2.3	2.5	2.8	2.5

[1]AWIRS = Animal welfare improved raising system; CON = Conventional raising system

[2] ADG = Average daily gain

Table 2. Descriptive statistics of farm management and facilities, and pigs' performance by farm type

System[1]	AWIRS				CON			
	Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Management system								
Pigs/farm, n	729.8	95.59	620	816	907.29	318.23	504	1400
Pigs/pen, n	47	71.3	13	175	35	40.34	15	126
Grow-to-Finish time, d	153.03	3.91	146.9	156.63	102.61	4.87	92.27	106.92
Farm Facilities								
Pen dimension, m^2	87.08	159.02	15.58	371.55	49.10	90.49	12.07	254.23
Space allowance, m^2/ 100 kg	0.94	0.42	0.65	1.67	0.77	0.42	0.54	1.73
Full floor:slatted floor	1/3	0	1/3	1/3	3/5	1/4	1/3	1/1
Performance								
Start weight, kg	7.78	0.57	6.90	8.48	30.99	3.14	26.90	36.31
Final weight, kg	135.81	4.93	126.93	139.70	122.91	3.78	118.21	128.9
ADG[2], kg	0.850	0.630	0.790	0.880	0.920	0.030	0.860	0.960
Feed conversion	2.54	0.03	2.51	2.59	2.55	0.16	2.34	2.81

[1]AWIRS = Animal welfare improved raising system; CON = Conventional raising system

[2] ADG = Average daily gain

After arrival at the commercial slaughter plant (slaughter speed of 400 pigs/h) pigs were kept in the lairage pen for 82 min on average (ranging from 75 to 90 min) at an average density of 0.76 m^2/pig (ranging from 0.65 to 0.88 m^2/pig). During lairage, water was available at all time through nipple drinkers. Pigs were water sprinkled for 10-15 min before the end of lairage and were driven in groups to the electrical stunner (head-to-chest electrical stunning). After stunning, pigs were exsanguinated in the prone position.

Audit Protocols at the Farm

Over the period from October 2012 to January 2013 the 12 selected farms were audited once, one wk before slaughter, by two trained assessors using the Welfare Quality® (WQ®) and the Animal Care Assessment (ACA) audit protocols (WQ®, 2009a; CPC, 2011). At each farm 10 pens holding a maximum of 15 pigs (total of 150 pigs per farm) were assessed using the WQ® and ACA protocols. These pens were chosen in order to have the best representation of the farm. The number of sampled pigs was a proportion of the total number of pigs per room (up to 75 pigs/room). Hospital pens were not included in the sampling plan.

Welfare Quality Audit Protocol

The WQ® protocol was developed to enable an overall assessment of animal welfare and standardised conversion of welfare measures based on four animal welfare principles, such as good feeding (GF), good housing (GHO), good health (GHE) and appropriate behaviour (AB), using animal-based measures (Table 3). Criteria and individual measures for GHO and GHE conditions and AB are described in Table 3. In order to have information about the thermal comfort of pigs in the pen, behaviours, such as shivering, panting and huddling, were observed before the assessor entered the pen since these behaviours are more reliably assessed in resting animals.

Coughs and sneezes, measures of GHE, were counted during 5 min and the procedure was repeated at six different sampling points chosen randomly in the farm. Each sampling point corresponded to 20-40 pigs. The scouring presence was assessed inside the pen and at group level by walking inside the pen and looking for areas presenting diarrhea and fresh feces. Data on mortality rate (excluding euthanized pigs) were

obtained from on-farm records of the last 12 months as per WQ® guidelines (WQ®, 2009a).

Table 3. Animal welfare measures assessed on farms using the Welfare Quality and the Canadian Care Assessment audit protocols (WQ®, 2009a; CPC, 2011)

Welfare criteria	Measures
Welfare Quality	
Good feeding	
Absence of prolonged hunger	Body condition score
Absence of prolonged thirst	Water supply
Good housing	
Comfort around resting	Bursitis, absence of manure on the body
Thermal comfort	Shivering, panting, huddling
Ease of movement	Space allowance
Good health	
Absence of injuries	Lameness, wounds on body, tail biting
Absence of disease	Coughing, sneezing, pumping, twisted snouts, rectal prolapse, scouring, skin condition, ruptures, mortality and hernias
Absence of pain induced by management procedures	Castration, tail docking
Appropriate behaviour	
Expression of social behaviours	Social behaviour
Expression of other behaviours	Exploratory behaviour
Good human–animal relationship	Fear of human beings
Positive emotional state	Qualitative behaviour assessment
Care Assessment Program	
Environmental control	
Ammonia Level	Mg/Kg
Lighting provided	Lux
Pig Comfort	
Space allowance	$m^{2/}$ 100 Kg

Appropriate Behaviour. Behavioural measures were assessed by averaging the results of social and exploratory behaviour observations, of the human-animal relationship (HAR) test and of the qualitative behaviour assessment (QBA) from two trained auditors. Pigs were initially scored as either active or inactive. The following behaviours were recorded from active pigs: positive and negative social behaviour (PSB and NSB), exploratory behaviour (EB) and others (*e.g.* eating, drinking, etc.). The measure of EB was divided into investigation of the pen and investigation of enrichment material. Social and exploratory behaviours were assessed by means of scan samplings at three different observation points (40-60 pigs/observation point) of the farm so as to provide a reliable overall representation of the farm. Each point was observed five times consecutively with an interval of 2.5 min between scans (Courboulay and Foubert, 2007).

The HAR evaluation was evaluated according to the Fear of Human test (Courboulay and Foubert, 2007) that allows the assessment of pigs' panic response to human presence by doing two laps inside the pen. In this study, this assessment was done in 10 selected pens (each pen evaluated as a whole) at each farm using a two point score: 0 or no panic response in the presence of humans and 2 or > 60% of the animals in the pen showing panic response in the presence of humans, *e.g.* when pigs faced away from the observer or huddled in the corner of the pen.

The QBA observations were carried out at six observation points per farm for a total of 20 min as described by Wemelsfelder (2007). A rating scale was used to score pigs at group level on the basis of the following 20 different terms: 1: active, 2: relaxed, 3: fearful, 4: agitated, 5: calm, 6: content, 7: tense, 8: enjoying, 9: frustrated, 10: sociable, 11: bored, 12: playful,13: positively occupied, 14: listless, 15: lively, 16: indifferent, 17: irritable, 18: aimless, 19: happy and 20: distressed. Scores were obtained using a 125 mm long scale. A value on the left side (or minimum) of the scale indicated that the expressive quality of the term was entirely absent in any of the pigs observed, whereas a value on the right side (maximum) of the scale indicated that the given descriptor was dominant across all pigs.

Scores Systems. On farm the GF, GHO and GHE principles of the WQ protocol were assessed in the pen or at the individual level using a 3-point scale ranging from 0 to 2, where the score of 0 was awarded if the animal welfare was good, a score of 1 if animal welfare was compromised and score 2 when the animal welfare was poor or unacceptable. In some cases, where a condition was either present or absent, a binary scale (0: absent or 2: present) was used. The above-mentioned score for each criterion was then used to obtain an overall final score using an algorithm of the WQ scoring system (WQ, 2009b). Briefly, once all the measures have been performed on an animal unit, a bottom-up approach was followed to produce an overall score of animal welfare on that particular unit. The approach was the following: first, the data collected *(i.e.* values obtained for the different measures on the animal unit using the 0 to 2 scale) were combined to calculate criterion scores; then, criterion scores were combined to calculate principle scores (expressed by a 0 or worst to 100 or best scale). Farms units were then assigned one of four possible animal welfare category ("Not Classified", "Acceptable", "Enhanced" or "Excellent"), based on reference profiles for the principle-scores it obtained.

A list of definitions and descriptions of all assessed WQ parameters and their related scores are shown in Tables 4 and 5, respectively. The AB principle was assessed by counting the number of pigs showing social and exploratory behaviours, and scores of QBA and HAR test.

Animal Care Assessment Protocol

The ACA which is part of the food safety Canadian Quality Assurance® has been developed by the Canadian Pork Council (CPC) for the evaluation of staff training, general handling, raising and loading facilities, health, comfort and nutrition quality of pigs at the farm level. Since 2012, self-auditing through this protocol has become mandatory for Canadian swine producers who want to obtain the accreditation of the Quality Assurance Program of the CPC. The CPC audit protocol criteria and their measures are shown in Table 3.

Table 4. Definition of the measures for the welfare assessment of pigs on farms using the Welfare Quality audit protocol (WQ®, 2009a)

Criteria	Definition
Body condition	Evaluation where the pigs are scored considering level of visible bones
Bursitis	Bursa is a fluid filled sac that develops as an inflammatory result of a pressure injury on the weight-bearing points of the legs
Pig dirtiness	Characterized by the presence of manure/faeces on the body of the pig
Shivering	Slow and irregular vibration of any body part, or of the body as a whole
Panting	Short gaps carried out with the mouth when the pig is breathing rapidly
Huddling	When a pig is laying with more than half of its body in contact with another pig
Lameness	Inability to use one or more limbs in a normal manner
Skin damage	Evaluation of skin condition, regarding to scratches and round lesions
Tail biting	Parameter related to damages to the tail, ranging from superficial bites along the length of the tail to absence of the tail
Pumping	When the pigs' breathing is heavy and labored, and it is easy to see the chest rising and falling with each breath
Twisted snouts	Characteristics of a atrophic rhinitis, and can vary in severity from a slight deformity of the snout to severe nasal distortion
Rectal prolapse	When internal tissues extrude from the rectum
Scouring	When the faeces become more fluid in consistency than normal
Hernias	Occurs when there is protrusion of a bodily structure or organ through the wall that normality contains it, resulting in a lump under the skin in the umbilical or inguinal area

On the day of the assessment, ambient temperatures and relative humidity were recorded inside each farm to minimize differences due to ambient variation and standardize records. The ambient quality was evaluated by recording ammonia levels using an ammonia detector (Gasalert NH3 Extreme, Model Gaxt-A-DL, BW Technologies, Calgary, Canada) and lighting intensity inside the barn subjectively following the ACA guidelines (CPC, 2011), which suggest at least 40 lux light intensity for at least 8 h/day.

Table 5. Scoring scale for good feeding, good housing and good health animal-based measures on farm using the Welfare Quality audit protocol (WQ®, 2009a)

Animal-based measures	Score	Description
Body condition	0	Animal with a good body condition
	2	Animal with visible spine, hip, and pin bones
Bursitis	0	No evidence of bursae/swelling
	1	One or several small bursae on the same leg or one large bursa
	2	Several large bursae on the same leg or one extremely large bursa or any eroded bursae
Manure on the body	0	Less than 20% of the body surface is soiled
	1	More than 20% but, 50% of the body surface is soiled with feces
	2	Over 50% of the body surface is soiled with feces
Shivering	0	No vibration of any body part
	2	Slow and irregular vibration of any body part, or the body as a whole
Panting	0	Normal breathing
	2	Rapid breath in short gasp
Huddling	0	Pig lying with less than half of its body on top of another pig
	2	Pig lying with more than half of its body on top of another pig
Lameness	0	No weight-bearing on the affected limb, or not able to walk
	1	Normal gait or difficulty in walking, but still using all legs; swagger of caudal body while walking; Shortened stride
	2	Severely lame, minimum weight-bearing on the affected limb
Wounds on body	0	If all regions of its body have a maximum of 4 lesions
	2	When >10 lesions are observed on a minimum of 2 zones of the body or if any zone has >15 lesions
Tail biting	0	No evidence of tail biting; superficial biting but no evidence of fresh blood or of any swelling
	2	Bleeding tail and/or swollen infected tail lesion, and/or part of tail tissue missing and presence
Pumping	0	No evidence of labored breathing
	2	Evidence of labored breathing
Twisted snouts	0	No evidence of twisted snouts
	2	Evidence of twisted snouts
Rectal prolapse	0	No evidence of rectal prolapse
	2	Evidence of rectal prolapse
Scouring	0	No liquid manure visible in the pen
	1	Areas in the pen with some liquid manure visible
	2	All feces visible inside the pen is liquid manure
Skin condiction	0	No evidence of skin inflammation or discolouration
	1	More than zero, but less than 10% of the skin is inflamed, discoloured, or spotted
	2	More than 10% of the skin has an abnormal colour or texture
Hernias	0	No hernia/rupture
	2	Bleeding lesions, hernias/ruptures and/or hernias/ruptures touching the floor

Audit Protocols at Loading and Transport

Each load was audited for the quality of loading facilities, handler or trucker skills and animal behaviour during handling. The quality of loading facilities were evaluated by filling out a questionnaire reporting the type of flooring and the slope of the loading ramp, and the presence of sharp edges in the alleys and loading ramp according to the AMI protocol (AMI, 2012). The questionnaire also included an evaluation of farm handler and trucker skills, and a note on the handling devices used during loading. The number of falls and slips, indicators of the quality of the ramp design (slippery floor and steep slope), and turning-back and reluctance to move, indicators of general fear, were noted according to the WQ® protocol (Table 6).

The trucks were audited upon arrival at the plant by filling out a questionnaire including criteria of the AMI (2012) protocol in order to evaluate general transport conditions and truck design (Table 7). Loading density was assessed by recording the truck size and the number and average weight of pigs (expressed as m^2/100 kg of pig).

Audit Protocols at the Slaughter Plant

Unloading at the plant. In the unloading area, consisting of the external truck ramp and the unloading dock, the proportion of pigs slipping, falling, turning back and reluctant to move was noted using the WQ® protocol (WQ®, 2009a; Table 8). The electrical prod use was not allowed during unloading as required by the internal guidelines of the abattoir. Thermal behaviours, such as shivering and panting, and lameness and sickness (rectal prolapse and hernias) were scored using the WQ® protocol (WQ®, 2009a) as described in Table 5. Lameness was scored while pigs were moved through the lairage alley to the rest pens, with observations starting from a pre-selected site on the unloading dock up to the end of the alley (9.1 m walking distance). The number of dead-on-arrival (DOA) and non-ambulatory (NA) pigs was also noted.

Resting conditions and handling in lairage. The assessment of the welfare conditions of pigs while resting before slaughter was done in 6-7 lairage pens/truck/farm using the WQ® principles and criteria (WQ®, 2009a; Table 8). The audit of handling at the plant was completed by the observation of animal behaviour and HAR at the exit of the lairage pen and along the alley leading to the stunning

Table 6. Ethogram of pig behaviour during loading, unloading and in the stunning chute area using a modified Welfare Quality audit protocol (WQ, 2009a)

Behaviour	Description
Slip	Loss of balance without the body touching the floor
Fall	Loss of balance in which a part of the body other than the legs are in contact with the floor
Overlap	Pig mounts another pig, with its 2 front legs on the back of the other pig
Turn back	Pig makes a 180º turn, ending with its rear extended in the direction of intended movement
Reluctance to move	Pig showing reluctance to move when it stopped walking, without moving its head and body, and failed to explore for at least 2 s

Table 7. Description of the measures for the welfare assessment of pigs during transport and on arrival at the slaughter plant using the American Meat Institute Audit Guide (AMI, 2012)

Criteria	Score definition
Internal ramps	0 = Presence
	1 = Absence
Presence of sharp or protruding objects	0 = Presence
	1 = Absence
Bedding	0 = Presence
	1 = Absence
Waiting time for unloading	0 = More than 30 min
	1 = Less than 30 min
Alignment between truck and ramp	0 = More than 5 cm to the ramp
	1 = Less than 5 cm to the ramp

chute area (SCA) using a modified audit protocol, where some criteria of the WQ® protocol (WQ®, 2009a; Table 8) and AMI audit guide (AMI, 2012) were merged. This protocol included the observation of behaviours, such as falls, slips, turning back, reluctance to move and high-pitched vocalizations (HPV), and the frequency of electric prod (EP) use.

The HAR was assessed by recording animal high-pitched vocalization, which is defined as squealing or screaming at group level while pigs are moved through the SCA (WQ®, 2009a). Two types of measures were taken: 1) one-zero sampling, which consists of assessing whether any animal is showing any vocalization during a 20 s period and 2) instantaneous sampling, assessed if any animal is vocalizing at the end of each period of 20 s. Additionally, when at the 20th s only one animal was vocalizing it was considered as a "single vocalisation". However, if more than one animal was vocalizing was considered "multi-vocalisation". The HPV assessment was carried out using 4 scans of 4 min each with an interval of 4 min between scans in every group of pigs.

Stunning effectiveness. The assessment of the stunning effectiveness, which is a GHE criterion of the WQ® protocol (Table 8), was done by observing the absence of rhythmic breathing (as indicated by the lack of movements of the flanks), corneal reflex (through physical stimulation of the cornea), vocalisation and righting reflex (determined when the animal was not able to hold its head) in 120 pigs per day (60 pigs/truck load or farm) following the WQ® protocol (2009a).

Post-Slaughter Measurements

Exsanguination Blood Lactate

Blood samples were collected from the bleeding wound 120 pigs (60 pigs from each truck/day) in a plastic cup and lactate level was immediately assessed in duplicate using a hand-held Lactate Scout Analyzer (EKF Diagnostic GmbH, Magdeburg, Germany) by dipping the test strips into a sample blood (two strips or replicate/animal). The results were obtained in approximately 15 s and are expressed in mM.

Table 8. Description of the measures for the welfare assessment of pigs at the slaughter plant using the Welfare Quality audit protocol (WQ®, 2009a)

Welfare criteria	Measures
Good feeding	
Absence of prolonged thirst	Water supply (number of drinking points, state)
Good housing	
Comfort around resting	Density and flooring of lorries, density of lairage pens
Thermal comfort	Percentage of animals shivering or panting, degree of social thermoregulation/huddling
Ease of movement	Percentage of animals that slip and/or fall during unloading
Good health	
Absence of injuries	Skin lesions, lameness score
Absence of disease	Percentage of sick and dead animals on arrival and in the lairage pens, slaughter checks (pneumonia, pleurisy, pericarditis, white spots in the liver)
Absence of pain induced by management procedures	Stunning effectiveness (presence of corneal reflex, righting reflex, rhythmic breathing, vocalisations)
Appropriate behaviour	
Absence of general fear	Reluctance to move and turning back during unloading
Good human-animal relationship	High-pitched vocalisations when driven to stunning area

Carcass Quality Measurements

After slaughter, carcasses were eviscerated, split and transferred to standard chilling rooms (4˚C), where they were kept until the next day. Hot carcass weight (HCW) was recorded and lean yield was obtained by measuring carcass fat and lean depth lean depth at the third/fourth last rib level by a Destron optical probe (PG-100 model, Anitech Enterprises Inc., Markam, Canada).

The number of skin lesions was counted on 120 carcasses/slaughter day in the cooler at 5 anatomical locations: ears, front (from the head to the back of shoulders), middle (from the back of shoulders to the hind-quarters), hind-quarters and legs as suggested by the WQ protocol (WQ®, 2009a). Each location was scored regardless to the side of the carcass as follows: 0) no visible skin damage or only one lesion greater than 2 cm or lesions smaller than 2 cm; 1) between two and 10 lesions greater than 2 cm; 2) any wound which penetrates the muscle tissue, or more than 10 lesions greater than 2 cm as suggested by the WQ protocol (WQ®, 2009a). The final score took into account the 5 carcass sites scores and one of the possible scores was assigned: 0) all body parts received a zero score; 1) when at least one body part was scored as 1; 2) when any body part was scored as 2.

Internal Organs Conditions

After slaughter, the presence of pleurisy and pneumonia in the lungs, pericarditis in the heart and white spots in the liver, indicators of the GHE principle, were assessed by veterinary inspection in all carcasses along the slaughter line following the WQ protocol (WQ®, 2009a).

Meat Quality Measurements

Meat quality was assessed at 24 h *post-mortem* in the *longissimus lumborum* (LL) muscle (between the 2nd and 3rd last lumbar vertebra) of the 120 pigs that were previously blood sampled by measuring pH (pHu) by means of a portable pHmeter (Oakton Instruments Model pH 100 Series, Vernon Hills, IL) fitted with a Cole Parmer spear tip electrode (Cole Palmer Instrument Company, Vernon Hills, IL) and an automatic temperature compensation (ATC) probe. At the same anatomical location, visual colour was evaluated using the Japanese colour standards (Nakai et al., 1975), whereas instrumental colour (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 after exposing the muscle surface to 15 min blooming time. Drip loss was also evaluated using the filter paper wetness (FPW) test as described by Kaufmann et al. (1986). Briefly, a filter paper (Whatmann PK100, VWR International Co., Mont Royal, Canada) was placed on the LL muscle cut surface after 10 min of air exposure and weighed using an analytical scale (Sartorius model 1419MP8, Fisher Scientific, Ottawa, Canada) after 3 sec of fluid accumulation on the paper. Percentage of drip loss was calculated by the following equation: [% drip loss = - 0.1 + (0.06 × mg fluid)]. Loins were classified into 5 pork quality categories, namely PSE (pale, soft, exudative), PFN (pale, firm, non-exudative), RSE (red, soft and exudative), RFN (red, firm, non-exudative) and DFD (dark, firm, dry) according to pHu, light reflectance (L*) and drip loss variations (Table 9; Correa et al., 2007).

Table 9. Pork quality classification upon ultimate pH (pHu), drip loss (DL) and light reflectance (L*) as assessed in the LL muscle (modified from Correa et al., 2007)

Quality class[1]	pHu	DL	L*
PSE	<5.5	>5%	>50
PFN	5.5 – 5.8	<5%	>50
RSE	5.6 – 5.8	>5%	42-50
RFN	5.6 – 5.8	<5%	42-50
DFD	> 6.1	<2%	≤42

[1]PSE = pale, soft and exudative; PFN = Pale, firm, non-exudative; RSE = reddish-pink, firm, exudative; RFN = reddish-pink, firm, non-exudative and DFD = dark, firm, dry

Statistical Analyses

The results of the on-farm audits were obtained using the Welfare Quality scoring system (WQ, 2009b), where the overall results for the 12 farms are given on three levels (criteria, principle and overall result). In brief, 32 welfare measures on-farm were aggregated into 12 criteria, these 12 criteria were aggregated into four main animal welfare principles, which, in turn, were aggregated into one classification (four levels). Different types of algorithmic operators were used in this aggregation process: decision tree, weighted sum, linear combination, conversion to ordinal score, least

squares spline curve fitting and Choquet integral (WQ®, 2009a). The Choquet integral was used to aggregate the 12 criteria into four principles using weights to combine the different criteria scores into 1 principle score (expressed on the 0 or worst to 100 or best scale), while limiting the possibility that a poor score of one criterion is compensated for by excellent scores of others. As the WQ protocol was designed to be used universally, considering all kinds of pigs' raising systems around the world, some criteria known to be largely variable between systems (*e.g.* age, weights, pen dimension, etc.) had their influence lowered by the use of different weights per criterion in the welfare quality calculation of scores (WQ®, 2009a). Hence, this approach allowed the final scores given by the WQ®, which is expressed by a 0 (worst) to 100 (best) scale, to be compared between the farms assessed through this protocol, since the assessments are focused more on animal-based rather than the resource and management-based measures. Farms were then assigned one of four possible assessments ("Not Classified", "Acceptable", "Enhanced" or "Excellent"), based on reference profiles for the four principles. All statistical analyses were carried out using the Statistical Analysis Software (SAS Institute Inc., Cary, NC, 2002). Differences in the Welfare Index (WI), welfare principles data and animal based-measurements, such as presence of manure on body, bursitis, hernia, mortality rate and all appropriate behaviour measures, between farm systems, were analysed using the MIXED procedure of SAS in a one-way analysis of variance including the farm system as a fixed effect. For the measures that did not show a normal distribution of residuals, such as wound on body, tail biting and scouring, the comparison between farm systems was performed using a Wilcoxon Mann-Whitney test with the NPAR1WAY procedure and the WILCOXON option.

Handler's intervention and animal behaviour data, such as electric prod use, falls and slips, reluctance to move, and turning back during loading and turn back and falls during unloading were transformed into percentages, and data analysis was done through an adjustment to the negative binomial distribution using the GLIMMIX procedure of SAS.

The analysis of other observed variables, such as hernias, DOA and NA pigs on arrival at the slaughter plant, showing a non-normal distribution of residuals was performed with the non-parametric Wilcoxon Mann-Whitney test using the NPAR1WAY as described above. Finally, handler's and animal's behaviours data,

such as reluctance to move and slips during unloading and turn back, reluctance to move, slip, overlap, EP use and HPV with normal distribution were analysed by the MIXED procedure of SAS in a one-way analysis of variance including the farm system and the driver within farm system as fixed effects.

Skin bruises scores, post-stunning consciousness signs data and the frequency of meat quality classes were analyzed by the FREQ procedure of SAS. More in particular, skin bruise scores were tested using a Cochran-Mantel-Haenszel Row Mean Score statistics, while consciousness signs and meat quality classes were tested using a Chi-square to determine differences between frequencies of distribution. Percentages of meat quality classes, bruises and consciousness signs data, such as corneal reflex and rhythmic breathing reflex, were also analysed by MIXED procedure of SAS in order to assess the effects of the farm type and driver within farm type.

Blood lactate, meat quality and carcass data were analyzed using the MIXED procedure of SAS in a one-way analysis of variance including the farm system and the driver within farm system as fixed effects with the animal as the experimental unit and the week as random effect. Multiple comparisons between means were adjusted with a Tukey-Kramer correction. Spearman correlations were performed using SAS to determine relationships between the on-farm animal welfare audit scores for each assessed audit criterion, animal behaviour, skin bruises, blood lactate level and meat quality traits. A principal component analysis (PCA) was also performed to study the relationships between animal behaviours, blood lactate level and meat quality variation within farm type.

A probability level of $P \leq 0.05$ was chosen as the limit for statistical significance in all tests, whereas probability levels of P between > 0.05 and < 0.10 were considered to be a tendency.

RESULTS AND DISCUSSION

On-farm Audit Results

Based on the outputs of the Welfare Quality scoring system (WQ, 2009b), four AWIRS farms (B, C, D, E) and three CON farms (F, H, and I) were assessed as “Enhanced”, which means that these farms had good animal welfare conditions, while one AWIRS farm (A) and four CON farms (G, J, K, and L) were assessed as “Acceptable”, suggesting that the animal welfare conditions at these farms were

slightly above or only reached minimal requirements. No farm was assessed as "Excellent" or "Not-classified" in this study.

Animal Welfare Principles

In this study, no significant difference in GF and AB final scores was found between AWIRS and CON raising systems ($P > 0.10$; Table 10). The lack of difference in GF final scores is not surprising as pigs raised under North American intensive finishing conditions are usually fed and provided with water *ad libitum*. Whereas, the low levels of social behaviour and poor HAR test results at all farms may explain the lack of difference in AB scores between farm systems. However, a difference was found between farm types for GHO and GHE principle final scores, with AWIRS farms presenting greater final scores (expressed by a scale from 0 = worst to 100 = best) for GHO ($P = 0.001$) and GHE ($P = 0.006$) principles compared with CON farms (Table 10).

Table 10. Welfare Quality principles scores for AWIRS and CON farm types[1]

WQ® principles	**AWIRS**	**CON**	**SEM**	***P value*[2]**
Good Housing (GHO)	67.9	39.4	4.5	0.001
Good Feeding (GF)	66.8	66.8	10.8	NS
Good Health (GHE)	62.9	50.7	2.5	0.006
Appropriate Behaviour (AB)	42.4	32.9	4.6	NS

[1]AWIRS = Animal welfare improved raising system; CON = Conventional raising system

[2] NS = Non significant ($P > 0.10$)

The differences in animal welfare principles final scores between farm units were only numerical in this study (Fig. 1). Farm C received the highest score for the GHO (86.9) and GHE (71.9) principles, meaning better housing and pig health conditions at this farm. Whereas, the lowest score for the GHO principle was found at the farms H and K (39.4 for both farms), while the poorest GHE score was recorded at farms K and J (43.3 and 46.5, respectively), meaning that these farms only met the minimum standards for animal welfare required by the WQ® protocol (2009a). Interestingly,

among CON farms, farm I received a high score for GHO (71.9) and GHE (55.1) principles. The high GHO score may be attributed to the low bursitis incidence (37.3 % with scores 1 and 2; data not shown) and the low percentage of pigs showing manure on body (8.0 % with score 1 and 2; data not shown) observed at C farm. While the better GHE final score at C farm may be related to the numerically lower mortality rate (1.7 %) and pneumonia cases (2.1 %) compared to the others CON farms (data not shown).

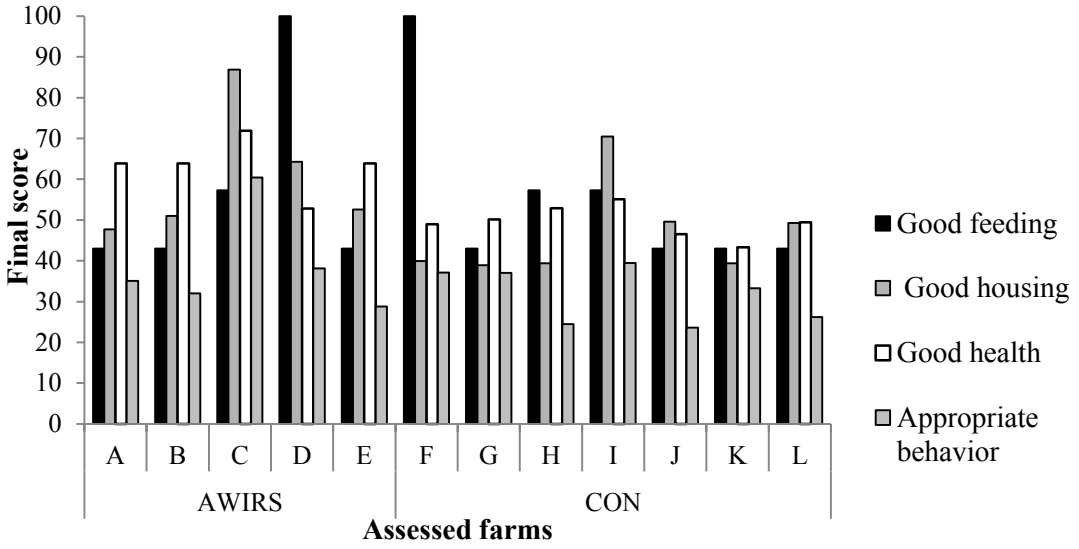

Figure 1. WQ® principles scores (scale from 0 = worst to 100 = best) by farm unit

Good Housing Criteria

Within the GHO principle, the proportion of pigs showing bursitis tended to be greater (P = 0.07) at CON farms compared with AWIRS farms (Table 11). Although bursitis does not produce pain in pigs, its occurrence is an indicator of poor comfort around the resting area and difficult environmental conditions (Courboulay, 2007). The most likely explanation for the more frequent occurrence of bursitis at CON farms when compared with AWIRS farms may be the greater percentage of slatted flooring (61.1 % ± 7.2 *vs.* 33.0 % ± 8.5; P = 0 .03; data not shown) and the lack of bedding (Table 2). The effect of raising pigs on slatted floor on the incidence of bursitis is well documented (Smith, 1993; Lyons et al., 1995; Mouttotou et al., 1999; Guy et al., 2002) and has been associated to the pig effort to support its weight with the legs on the slat, which is a smaller area compared with a full solid floor (Mouttotou et al., 1997). This physical effort increases the risk of trauma of the

superficial lymphatic vessels and capillaries which eventually results in bursae development (Mouttotou et al., 1997). The presence of bedding also prevents the risk of bursitis as it is resilient and nonabrasive substrate for the pigs to rest, walk and play upon (Mouttotou et al., 1997). These results validate the efficiency of bursitis as a discriminating criterion when auditing farms for the GHO principle as already reported by Temple et al. (2011) at Spanish farms audited using the WQ® protocol.

As the room temperature at all farms was within the thermoneutral zone for growing pigs (20.0 ˚C, ranging from 17.2 to 22.0˚C), no thermal behaviours, such as shivering, panting or huddling, were observed at any farm and, thus, the scores did not differ between farm types ($P > 0.10$).

Good Health Criteria. No difference in lesions on the body, tail biting, scouring, skin condition and rectal prolapses as assessed on-farm was observed in pigs raised at the audited farms in this study (Table 11). Overall, the better final scores for GHE principle observed in AWIRS system are likely due to the absence of pain induced by management procedures, such as tail docking, and the low frequency of pneumonia and pleurisy occurrences observed *post-mortem* (Table 12).

Although AWIRS farms received a higher score for the GHE principle (Table 10), a greater incidence of umbilical hernias ($P = 0.01$) and mortality rate ($P < 0.001$), and a trend for a higher ($P = 0.06$) proportion of lame pigs were reported at these farms than at CON farms (Table 11). The hernias observed at AWIRS farms may be associated to events occurring during the farrowing phase, such as abnormal stretching of the umbilical cord, placing navel clips too close to the skin and any infection of the umbilical stump, that interfere with the closure of the umbilical cord resulting in the development of hernias (Straw et al., 2009). Umbilical hernia may be also related to traumas in the early stages of the post-natal period (9 to 14 wk of age) or may result from hereditary predisposition (Searcy-Bernal et al., 1994; Done et al., 2012).

The reason for the higher mortality rate at AWIRS farms is hard to explain. The likely explanation may be either the lighter starting weight of AWIRS pigs (7.8 *vs.* 31.0 kg for the CON farms) as higher mortality rates are usually recorded in younger pigs or the longer time that AWIRS pigs spent at the farm (153 *vs.* 103 d). The overall proportion of lame pigs was small at AWIRS farms (Table 11).

Table 11. Percentage and number of pigs affected for each animal-based measure assessed within the Welfare Quality principles at each farm type[1]

Animal-based measure	AWIRS	CON	SEM[2]	*P value*[4]
Good housing				
Manure on body,%	34.02	24.33	11.77	NS
Bursitis,%	25.02	38.60	5.09	0.07
Floor space allowance, m^2/100 kg	0.78	0.58	-	NS
Good health				
Wounds on the body, %	0.67	0.68	-	NS
Tail biting, %	0.67	0.00	-	NS
Scouring	0.00	0.00	-	NS
Skin condition	0.00	0.00	-	NS
Coughs, n	3.00	1.00	-	NS
Sneezes, n	2.00	3.00	-	NS
Hernia, %	1.87	0.67	0.29	0.01
Lameness, %	0.67	0.00	-	0.06
Rectal prolapses, %	0.00	0.00	-	NS
Mortality rate, %	6.32	2.85	0.45	< 0.001
Appropriate behaviour				
Active (AB), %	76.62	69.20	4.87	NS
Positive social (PSB),%	6.97	9.29	01.16	NS
Negative social (NSB), %	1.33	2.00	00.36	NS
Exploration (EB), %	15.52	17.08	2.40	NS
Other, %	55.54	50.65	5.09	NS
HAR[3], %	26.00	37.14	15.30	NS

[1]AWIRS = Animal welfare improved raising system; CON = Conventional raising system

[2]Missing SEM values means that data were analysed using the Wilcoxon T-test.

[3] HAR = Human-Animal Relationship

[4]NS = Non significant ($P > 0.10$)

Table 12. Differences between farm types for the percentage of carcasses showing pneumonia, pleurisy, pericarditis and white spots in the liver[1]

Parameter	AWIRS	CON	SEM[2]	*P value*[3]
Pneumonia, %	2.76	16.50	5.54	0.09
White spots on liver, %	0	0	-	NS
Pericarditis, %	0	0	-	NS
Pleurisy, %	4.07	6.43	1.40	NS

[1]AWIRS = Animal welfare improved raising system; CON = Conventional raising system

[2] Missing SEM values means that data were analysed using the Wilcoxon T-test

[3] NS = Non significant ($P > 0.10$)

The difference in the incidence of lameness between farm systems may be attributed to the presence of wet bedding observed at some AWIRS farms (L.M. Rocha, personal observation). Indeed, increased risk of lameness due to hoof softening and damage has been reported in pigs raised on wet and slippery floor (Smith and Robertson, 1971; Grandin, 2010). However, as a *post-mortem* assessment of foot lesions was not performed in this study, this interpretation cannot be confirmed.

Appropriate Behaviour Criteria. Within the AB criteria, the frequency of pigs showing EB, PSB and NSB did not differ between raising systems ($P > 0.10$; Table 10). The human-animal relationship test did not identify differences in animal behaviours related to panic between raising systems either (Table 11). However, qualitative behaviour assessment results showed that CON pigs tended to be more fearful ($P = 0.08$) and bored ($P = 0.06$) compared with AWIRS pigs (Fig. 2). The lack of environmental enrichment at CON farms may explain the different qualitative behaviour assessment results in this study. According to Wemelsfelder (2005), boredom is envisaged as resulting from a chronic lack of opportunity for active interaction between animal and the environment.

Animal Behaviour

Loading. The Welfare Index (expressed by the scale from 0 = worst to 100 = best) at loading tended to be higher ($P = 0.10$) at CON farms compared with AWIRS farms (0.72 ± 0.04 *vs.* 0.61 ± 0.05; data not shown). No difference in slips, falls, reluctance to move, turning back or electric prod use as single factor was found in pigs loaded at either farm system. However, when loading was performed by driver A, a greater ($P = 0.02$) proportion of reluctant to move pigs was observed in both type of farms (Fig. 3). This result may be explained by the difference in handling skills between drivers, with driver A standing at the truck gate while pigs were moving forward through the loading dock (L.M. Rocha, personal observation). It has been reported that fear behaviour in pigs is strongly influenced by the attitudes the stockperson towards pigs and by his posture and positioning during handling (Hemsworth et al., 1989; Miura et al., 1996; Gonyou, 2000).

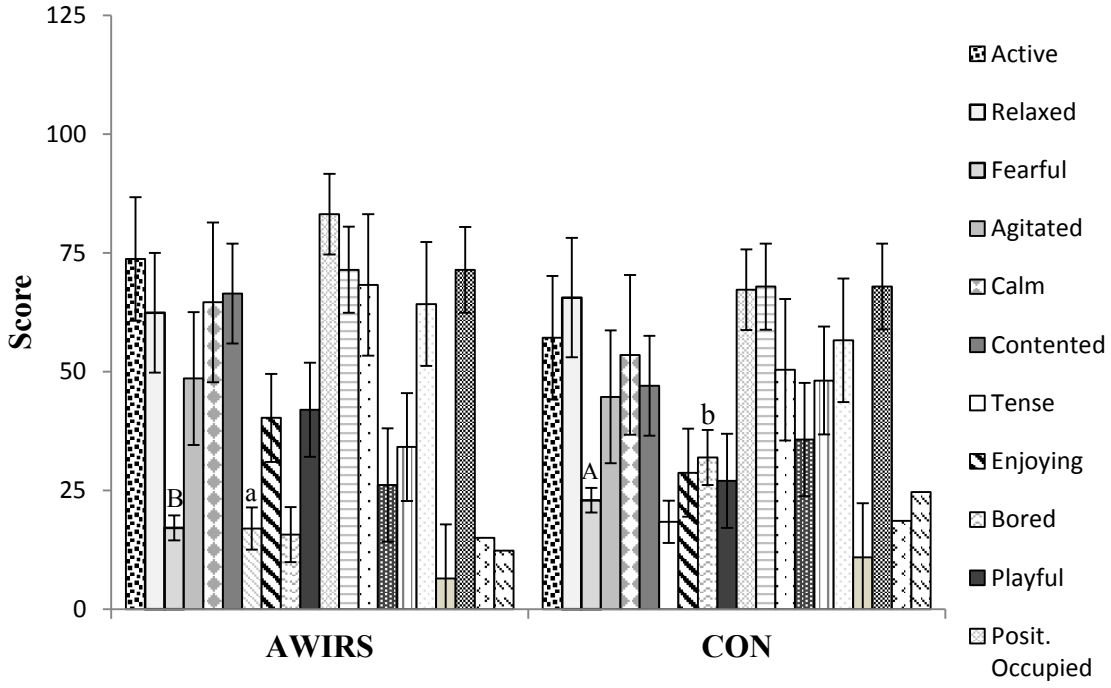

Figure 2. Least squares means (± SEM) of the QBA scores by farm type. CON pigs tended to be more fearful (P = 0.08) and more bored (P = 0.06) than AWIRS pigs when approached by humans. QBA = Qualitative Behaviour Assessment; AWIRS = Animal welfare improved raising system; CON = Conventional raising system. Superscript letters mean that treatments tend to be significantly different (P < 0.10). Posit. Occupied = Positively occupied.

Transport Conditions. Pigs from CON farms travelled longer than AWIRS pigs (4.13 *vs.* 2.47 h; $P = 0.02$; data not shown). However, no difference in the Welfare Index during transport was found between transports from AWIRS and CON farm systems (0.96 ± 0.02 vs. 0.98 ± 0.02, respectively; $P = 0.72$; data not shown). Therefore, travel time is not expected to have had an impact on the pig welfare in this study as transport conditions were good. Indeed, loading densities were in compliance with the AMI guide recommendations (0.49 and 0.46 m^2/pig for AWIRS and CON pigs, respectively). This result confirms that when pigs are transported in comfortable conditions, travel time has no effect on their welfare (Weschenfelder et al., 2012).

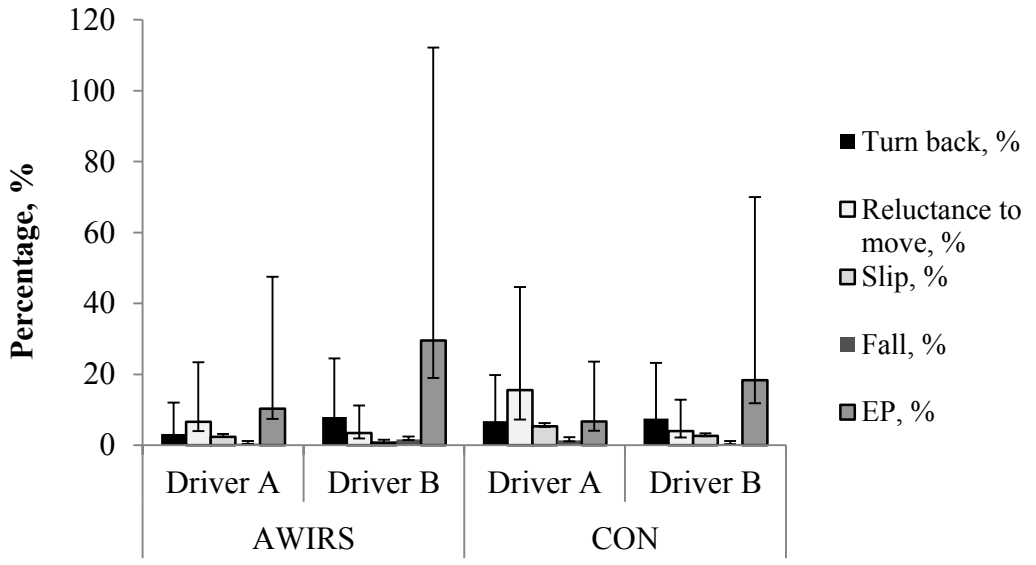

Figure 3. Back-transformed least square means ± confidence limits of the effects of the interaction raising system[1] x truck driver on behaviour of pigs during loading. Pigs from both systems, AWIRS and CON, were more reluctant to move when handled by driver A at loading ($P = 0.02$). [1]EP = Electric prod.

Unloading. When compared to driver A, AWIRS pigs unloaded by driver B slipped and turned back more ($P < 0.001$ and $P = 0.01$, respectively), while CON pigs were more reluctant to move ($P < 0.001$; Fig. 4). As unloading facilities are the same for all audited trucks, slips and turning back, both indicators of fear response ($r = 0.52$; $P = 0.009$; data not shown), may be explained by the poor handling skills of the driver rather than by the effect of the farm system. According to Dalmau et al. (2009), the

unskilful handler is a major cause of turning back in pigs at unloading from the truck. Furthermore, although unloading duration did not differ between AWIRS and CON loads (24 *vs.* 22.5 ± 2.11 min; $P > 0.10$) in this study, driver B tended to unload slightly quicker than driver A (21 *vs.* 24 ± 1.49 min; $P = 0.09$) and vocalized more (L.M. Rocha, personal observation) than driver A while handling pigs.

Moreover, a trend for a greater ($P = 0.06$) percentage of panting pigs from CON farms was observed at unloading at the plant compared with AWIRS pigs (1.45 *vs.* 0.49 %). No effect of driver was found on the number of panting pigs. Overall, 83 % ($n = 63$) of pigs panting at unloading were from CON farms and of these 68 % ($n = 43$) were unloaded from the upper deck of the pot-belly trailer.

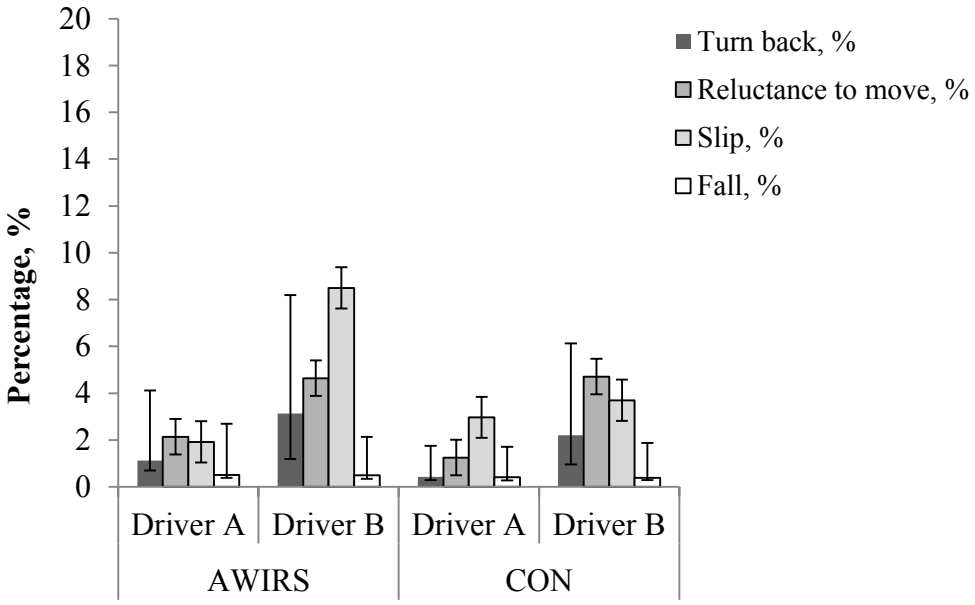

Figure 4. Back-transformed least square means of fall and turn back behaviours ± confidence limits. Least squares means (± SEM) of the effects of the interaction raising system[1] x truck driver on behaviour of pigs during unloading. When pigs from AWIRS system were handled by driver B greater turning back ($P = 0.01$), slips ($P < 0.001$) and reluctance to move ($P < 0.001$) were observed. [1]AWIRS = Animal welfare improved raising system; CON = Conventional raising system.

Conte et al. (2015) reported increased body temperature in pigs transported in this truck location as a consequence of pigs' physical effort to negotiate the ramp feeding this deck. Panting observed at unloading in CON pigs is thus a behavioural sign of their poor physical fitness combined with the increased frequency of pneumonia in

these pigs (Table 12) resulting in lower respiration rate and difficulty in meeting oxygen demand after a physical effort.

Neither dead-on-arrival nor non-ambulatory pigs or hernias were reported at unloading and no effect of farm system was found on the number of pigs presenting prolapses (0.25 ± 0.17 for AWIRS *vs.* 0.39 ± 0.14 for CON; $P > 0.10$; data not shown) and lameness (0.75 ± 0.38 for AWIRS *vs.* 1.28 ± 0.32 for CON; $P > 0.10$; data not shown) at this stage.

Thermal Comfort in Lairage Pens. In this study the average ambient temperature during lairage was of 15˚C (ranging from 9.5˚ to 19.5˚C). During resting time, AWIRS and CON pigs did not differ in the expression of thermal behaviours, such as shivering (0.5 *vs.* 1.5; $P > 0.10$; data not shown), panting (1.0 *vs.* 0; $P > 0.10$; data not shown) and huddling (3.25 ± 0.68 *vs.* 4.60 ± 0.57; $P > 0.10$; data not shown).

Moving From Lairage to Restrainer. At the end of 82 min lairage, pigs were driven in small groups to the electrical stunner by the slaughter plant staff. Overall, in this study, 11.9 % of pigs were prodded while entering in a single line into the restrainer chute (SCA). This EP use is considered as acceptable according to the threshold of EP use (25 %) set by the AMI protocol (AMI, 2012). However, compared with CON pigs, a trend for a greater ($P = 0.08$) EP use was observed on AWIRS pigs in the SCA (Fig. 5). As no differences were observed for human-animal relationship test in this study, the lower easiness to handling these pigs may be either explained by the difference in pigs' previous experience of handling between the two farm systems or difference in liveweight between AWIRS and CON pigs (Table 2), with the heavier AWIRS pigs being more difficult to handle.

The greater easiness to handle CON pigs in this study is likely related to their previous experience with the handling (including loading and unloading) and transport procedures compared with AWIRS pigs that had none. Following the normal practices of conventional raising conditions, CON pigs were, in fact, moved twice during their life, *i.e.* from farrowing to nursery and from nursery to the growing unit, compared with AWIRS pigs that were transported only once (from the farrowing unit to nursery). Abbott et al. (1997) also reported a greater willingness to move forward preslaughter in pigs being accustomed to walk through the farm alley before transport to slaughter. Studies of pigs' cognitive abilities have reported that pigs may remember

a previous handling experience for at least 4 to 5 wk (Abbott et al., 1997; Brajon et al., 2015). However, the results of this study suggest that pigs may be able to remember previous handling experiences for as long as 15 wk.

The difference in liveweight between AWIRS and CON pigs (Table 2) may be also a source of variation in the easiness to handle between these pigs. Bertol et al. (2011) also reported that heavier pigs needed more handler interventions during handling than lighter pigs.

Similarly to what was observed at loading and at unloading, the interaction farm system × driver influenced the behaviour of pigs in the lairage alley between the pen and the stunning chute. A greater proportion of falls and of turning back ($P = 0.02$ for both) was observed in AWIRS pigs transported by driver B and driver A, respectively (Fig. 5). A greater proportion of AWIRS pigs transported and handled by driver A also displayed a greater percentage of high-pitched vocalizations ($P = 0.01$) than CON pigs at this stage (Fig. 5).

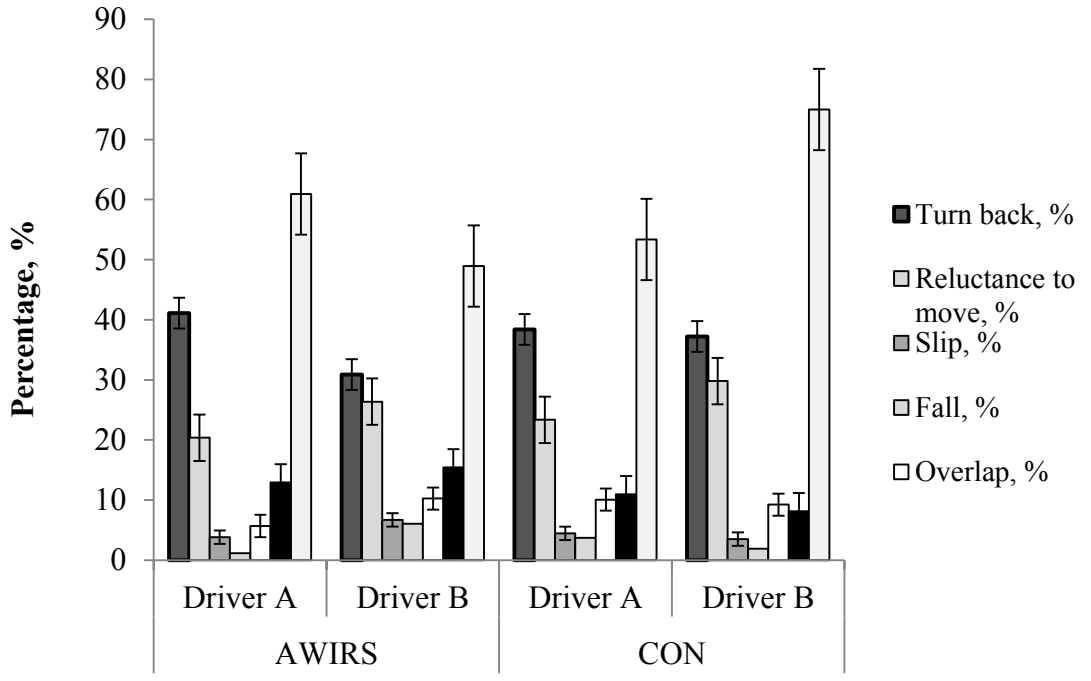

Figure 5. Least squares means (± SEM) of the effects of the interaction raising system1 x truck driver on behaviour of pigs being handled from the lairage pen to the stunning chute area. AWIRS pigs handled by driver B showed more falls (P = 0.02),

whereas pigs driven by driver A showed greater turn back (P = 0.02) and HPV (P = 0.01). 1AWIRS = Animal welfare improved raising system; CON = Conventional raising system; EP = Electric prod; HPV = High pitch vocalization.

Additionally, the close correlation found between turning back and high-pitched vocalizations (r = 0.92; $P < 0.001$; data not shown) for AWIRS pigs confirms that high-pitched vocalizations increases as this negative behaviour increases, suggesting that both behaviours are associated to fear response as previously reported by Von Borell and Ladewig (1992) and Warriss et al. (1994).

Stunning Effectiveness. Overall, the signs of consciousness observed after stunning were of low frequency which indicates the good effectiveness of the stunning system assessed in this study. When comparing the behaviour response of AWIRS and CON pigs after stunning, neither difference ($P > 0.10$) in the number of pigs presenting corneal reflex (6.17 *vs.* 4.67 ± 0.10; data not shown), rhythmic breathing (1.5 *vs.* 1.0; data not shown) was found nor in the percent of pigs showing righting reflex (0.42 *vs.* 0.27 ± 0. 26 %; data not shown) and vocalisation (0.07 *vs.* 0.21 %; data not shown).

Blood Lactate. Exsanguination blood lactate levels were greater (P = 0.03) in CON pigs than in AWIRS pigs (14.3 *vs.* 13.1 mM ± 0.40; data not shown) which may indicate a greater resistance to physical exercise and handling in AWIRS pigs. The presence of bedding at AWIRS farms stimulating walking and exploring (Guy et al., 2002; Gondret et al., 2005; Morrison et al., 2007) may have resulted in greater physical fitness and improved the muscle oxidative capacity reducing lactate production after a physical effort in these pigs (Jorgensen and Hyldegaard-Jensen, 1975; Foury et al., 2005).

Carcass Quality Traits

When compared with CON carcasses, AWIRS carcasses were heavier (111.08 ± 1.58 vs. 101.7 ± 4.19 Kg; $P < 0.001$) and slightly fatter as showed by the slightly thicker backfat (18.54 ± 0.42 *vs.* 16.54 ± 0.42 mm; $P < 0.001$) and lower lean percentage (61.09 ± 0.19 *vs.* 61.98 ± 0.19 %; P = 0.002). These results can be explained by the age of pigs at slaughter, with AWIRS pigs being 50 d older than CON (Table 2). The increase in carcass weight and fat content with age is well known (Candek-Potokar et al., 1999; Virgili et al., 2003; Correa et al., 2006).

Carcass Bruises

Overall, most carcasses (70 %) received a bruise score of 1 and 2, meaning that any region of the body presented from two to 10 lesions (score 1) or more than 10 lesions (score 2), respectively. However, bruise score frequencies on the carcass were not different between AWIRS and CON pigs (score 0 = 29.7 *vs.* 30.1 ± 3.2 %; score 1 = 57.9 *vs.* 60.9 ± 2.5 %; score 2 = 12.3 *vs*. 8.9 ± 1.6 %, respectively; $P > 0.10$ for all scores; data not shown). Bruise score frequency on the carcass was not influenced by driver A or B either (score 0 = 28.9 *vs.* 30.2 ± 2.92 %; score 1 = 60.1 *vs.* 60.2 ± 2.32 %; score 2 = 11.5 *vs*. 9.6 ± 1.52 %, respectively; $P > 0.10$; data not shown).

Health Conditions

No difference in the frequency of white spots on liver and pleurisy as assessed after slaughter was observed in pigs raised at the audited farms in this study (Table 12).

In CON pigs the frequency of pneumonia occurrences tended to be greater ($P = 0.09$) than in AWIRS pigs (Table 12) and exceeded the threshold set for this health criterion by the WQ audit protocol (6 %; WQ®, 2009a). Pneumonia is a disease of the lower respiratory tract, which impairs animal health and lowers individual and herd performance (Lawhorn, 1998). Although the greater pneumonia frequency contributes to explain the lower score for the GHE principle at CON farms compared with AWIRS farms, this difference is hard to explain as the frequency of coughs and sneezing and ammonia levels were similar between farm types or between farms within farm type in this study (Table 11).

Meat Quality Traits

In this study, a trend for a lower ($P = 0.07$) pHu and greater ($P = 0.003$) drip loss was found in the LL muscle of AWIRS pigs compared with CON pigs (Table 13). No effect of the raising system was found on any colour coordinates, including L* value ($P > 0.10$).

A greater proportion of PSE (pale, soft, exudative) and RSE (red, soft, exudative) pork (P = 0.006 and P = 0.01, respectively) was observed in AWIRS than in CON loins (Fig. 6). The greater acidification and exudation rate of AWIRS loins may be related to the higher residual glycogen level in the LL muscle that may be associated to the greater resistance to physical activity of these pigs.

Table 13. Effects of on-farm raising system on meat quality traits as assessed in the *Longissimus lumborum* muscle

Variable	**AWIRS**	**CON**	**SEM**	***P value*[2]**
pHu	5.64	5.67	0.01	0.07
L*	49.84	49.23	0.32	NS
a*	7.49	7.34	0.19	NS
b*	4.08	3.94	0.16	NS
Drip loss, %	4.54	3.41	0.25	0.003

[1]AWIRS = Animal welfare improved raising system; CON = Conventional raising system

[2] NS = $P > 0.10$

Fit muscles generate relatively less adenosine triphosphate (ATP) through anaerobic pyruvate catabolism when they are submitted to a given work load which results in lower lactate production and higher residual glycogen in the muscle at the time of slaughter (Petersen et al., 1997). The higher availability of glycogen for the *post-mortem* glycolysis leads to greater accumulation of lactate in the muscle finally resulting in extended muscle acidification (lower pHu) and greater exudation. A greater residual glycogen in the muscle resulting in lower meat pHu was reported by Chevillon et al. (2005) and Foury et al. (2005) in pigs raised in enriched housing (*i.e.* straw-bedding) conditions. Klont et al. (2001) also hypothesized the effects of different muscle glycolytic potential when interpreting meat quality variation between pigs kept under enriched environmental conditions compared to conventional ones. However, contrarily to our study, these authors reported greater pHu and water-holding capacity values in the loin muscle of pigs raised in enriched housing conditions. Anyway, as the residual muscle glycogen at slaughter was neither analysed in our study nor in the Klont et al. (2001) one, further research is needed to validate the different interpretations.

The driver also had an impact on pork quality variation in this study, regardless of the farm system of origin, with the proportion of PFN (pale, firm, non-exudative) pork increasing in pigs handled by driver B compared with driver A (25.21 ± 4.19 and 38.66 ± 4.19 % vs. 17.5 ± 4.19 and 17.78 ± 4.19 %, for AWIRS and CON farms, respectively; $P < 0.001$; data not shown).

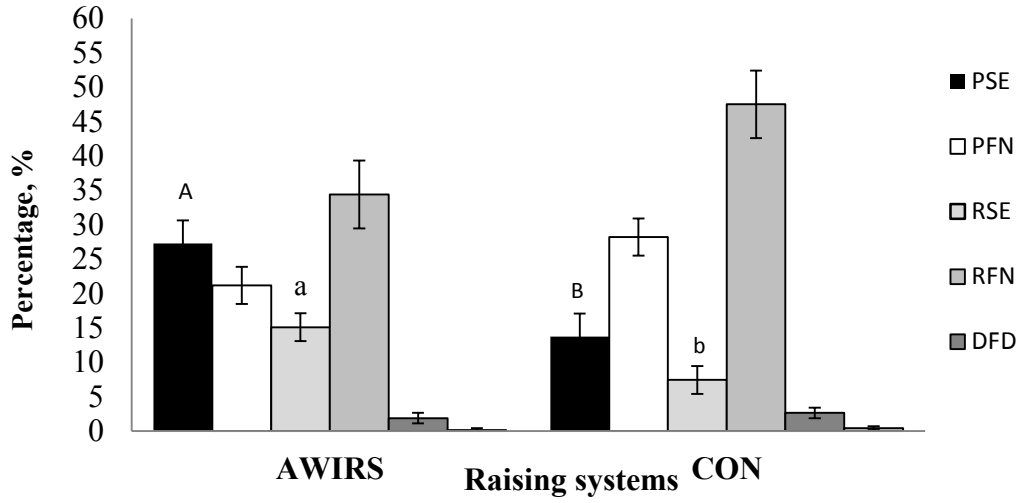

Figure 6. Meat quality classes frequencies between farm types. A greater percentage of PSE and RSE pork was found in AWIRS than in CON carcasses. [1]AWIRS = Animal welfare improved raising system; CON = Conventional raising system. Superscript letters mean that treatments are significantly different ($P < 0.001$). PSE = pale, soft, exudative; PFN = pale, firm, and nonexudative; RSE = red, soft, exudative; RFN = red, firm, and nonexudative; DFD = dark, firm, and dry.

This result shows the long lasting effects of poor previous handling experience on pork quality variation. Correa et al. (2010) and Edwards et al. (2011) also reported poor pork quality in pigs handled with electric prods at loading.

Correlations between on-farm audits scores and animal behaviour at plant

Except for ease of movement, no significant correlation was found between on-farm audit results and animal behaviour at the slaughter plant in this study. Better ease of movement recorded by for the assessment of the GHO principle at the farm showed a moderate correlation with reluctance to move at the plant ($r = 0.50$; $P = 0.01$; data not shown) suggesting that a greater freedom of movement in the farm pen, such as that observed by the greater final scores obtained by the Welfare Quality scoring system (WQ, 2009b) at AWIRS farms in this study, may result in greater reluctance to move during handling at the plant. Additionally, this result may also be explained by the lower habituation to harsh handling and from a greater adaptation to human interactions due to the frequent contacts with humans during the management operations at the farm (*e.g.* change of bedding; Grandin, 1987).

Correlations between on-farm audits scores and meat quality traits

Among the WQ® on-farm audit principles, only the GHO principle was significantly correlated with meat quality traits variation in this study, with the greatest relationship being found between GHO and pHu (r = - 0.75; P = 0.01; data not shown) and L* value (r = 0.87; P < 0.001; data not shown). Among the GHO principle criteria, ease of movement showed the greatest correlations with pHu (r = - 0.82; P = 0.001; data not shown) and L* value (r = 0.92; P < 0.001; data not shown). Although the muscle glycolytic potential at slaughter was not measured in this study, the greater incidence of PSE loins recorded in pigs having experienced better housing conditions (*e.g.* straw bedding, greater space allowance, etc.) at the farm may be explained by their greater energy reserves in the muscle at slaughter.

Correlations between slaughter plant audit criteria and meat quality traits

The Principal Component Analysis (PCA) results are shown in Fig. 7 for the first two principal components (PC), where PC1 represents 20 % and PC2 represents 14 % of the total variation within the dataset, which includes animal behaviour assessed during loading (LO), unloading (UN) and in the stunning chute area (SCA), and exsanguination blood lactate content and meat quality traits. In this plot, drip loss, L* value, slips during UN and at SCA are located far from the origin and on the right of the first PC showing their contribution in defining this PC in contrast with slips, electric prod use and reluctance to move at LO and SCA. The second PC is characterised by the contrast of falls at LO and SCA with high pitch vocalization and reluctance to move at UN and at SCA. Overall, the PCA results showed that slips at UN and SCA are correlated with the variability in drip loss and L* values. Moreover, these results support the above-mentioned correlations found between audit criteria at the plant and drip loss in the LL muscle, with muscle exudation being mostly related to the percentage of slips at unloading (r = 0.63; P = 0.001) and in the SCA (r = 0.74; P < 0.001) combined with the EP use in the SCA (r = 0.69; P = 0.002). A correlation of smaller magnitude was also found between the EP use in the SCA and L* value (r = 0.41; P = 0.05) of the LL muscle. Short-term stressors immediately prior to slaughter may hasten muscle glycogen degradation and often results in a fast pH decline and in an increased muscle temperature by the activation of the glycolytic system just prior to slaughter (Van der Wal et al., 1999; Hambrecht, 2004). In addition to these factors, the physical effort performed by the pigs may have caused

skeletal muscle damages and breakdown of muscle proteins affecting the protein's ability to bind water after slaughter, which results in exudative meat and tends to increase light scattering, giving higher L* values to the meat (Offer and Knight, 1988).

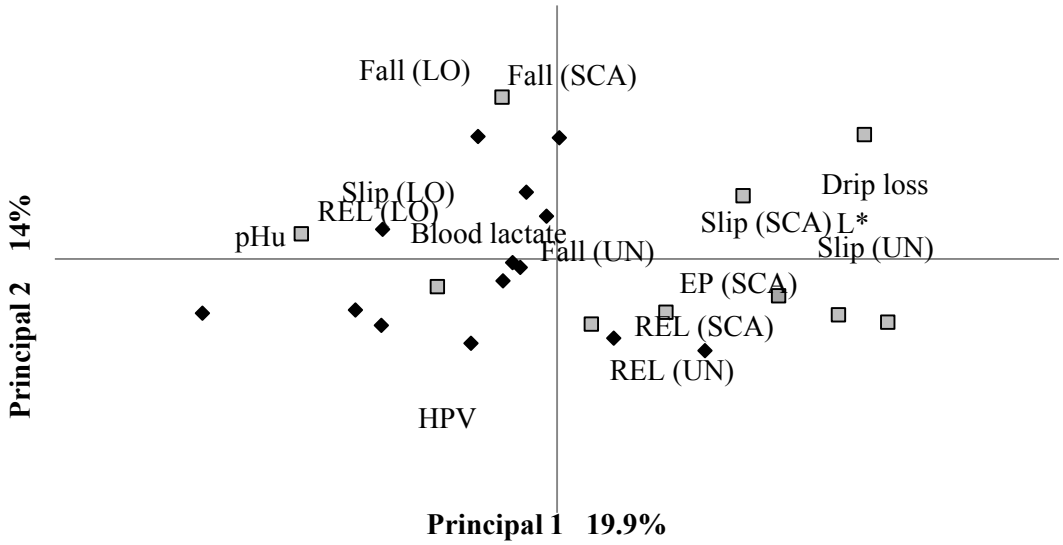

Figure 7. Principal component analysis results for animal behaviour, blood lactate and meat quality traits by farm type.[1,2]. [1]AWIRS = animal welfare improved raising system; CON = Conventional raising system; [2]LO = Loading; UN = Unloading; SCA = Stunning chute area; REL= Reluctance to move; EP= Electric prod use; HPV = High Pitch Vocalization.

CONCLUSIONS

On-farm animal welfare audit scores could not explain the variation of pig behaviour at the slaughter plant in this study, likely because pig behaviour was biased by the handler skills. The results of this study, in fact, evidenced the impact of the truck driver handling skills on pig behaviour at loading and unloading and highlighted the importance of handler training to improve the easiness of moving pig forward.

Furthermore, on-farm housing conditions (ease of movement) and preslaughter pig behaviour (slips) and handler interventions (EP use) during handling in the SCA at the plant, as assessed by the audit protocols used in this study, also contributed to pork quality variation.

The results of this study also showed that while pigs raised in welfare-friendly housing conditions may be more resistant to physical exercise, as showed by the lower lactate levels in exsanguination blood, they are also at greater risk of producing pale and/or exudative pork, likely because of the greater residual glycogen content in the muscle at slaughter, providing the favorable conditions for an extended *post-mortem* muscle acidification. Among the various factors affecting meat quality, the effects of housing system may have been biased by the differences in liveweight or carcass fatness affecting carcass cooling rate between the farm systems in this study, although the effects of these two variables on fresh pork quality reported in the literature are inconsistent. To accommodate the animal welfare needs at the farm and preslaughter and to optimize meat quality, specific on-farm management strategies regulating the glycogen level in the muscle at slaughter, should be applied on pigs raised in enriched raising conditions.

Chapter 5: Identification of pig anatomical location ensuring effective assessment of body temperature variation in response to physical stress by infrared thermography

The stimulation of the autonomic nervous system in response to stress induces changes in the peripheral vascular tone and blood flow in animals, resulting in increased body temperature. Changes in body surface temperature have been associated with emotional responses to stress and used as an indicator of farm animal welfare in a variety of species. However, despite the importance of body temperature as stress indicator, this measure is difficult to take under commercial conditions because the most common techniques, such as rectal, bladder and gastro-intestinal tract temperature are invasive and imply animal restraint, which is a stress on its own. Recently, infrared thermography has demonstrated potential as a non-invasive technology to assess stress through alterations on skin surface temperature. However, the literature results are inconsistent and no clear indication of the best anatomical location for reliable assessment of stress by the measure of infrared body temperature exists. Therefore, this study was done to identify the most adequate anatomical location for infrared body temperature assessment that could be used to explain variations in the physiological status of pigs in response to physical stress during handling and transport.

The rationale for this study is based on the results from a previous study (Weschenfelder et al. 2013. Meat Science 95:616-620) in which Luiene participated as a collaborator. The preliminary results of the following chapter were presented as poster communication at the 2015 Canadian Society of Animal Science (CSAS)-Canadian Meat Science Association (CMSA) joint meeting in Ottawa (ON). The abstract was published in the Canadian Journal of Animal Science. Rocha, L.M., Saucier, L., Maldague, X., Fleuret, J., and Faucitano, L. (2015). Identification of the anatomical location on the pig body ensuring the most efficient use of infrared thermography for the assessment of body temperature variation in response to physical stress. Canadian Journal of Animal Science. (Abstr.). A Master's student is currently using the results generated by this study to further our investigation on the potential use of thermography to monitor welfare. The results of this latest investigation will provide valuable information, allowing us to set up new studies which can be applied under commercial conditions.

Contributing authors:

Luiene Moura Rocha (Ph.D. candidate): Study design, discussion and development of protocol, personnel coordination and administration, planning and data collection, research and selection of a suitable software for image analysis, data analysis and manuscript preparation.

Linda Saucier and Luigi Faucitano (Research director and, co-director, respectively): student supervision, revision and correction of the manuscript.

Xavier Maldague (Ph.D., Infrared thermography approaches and image processing expert): Professor at electrical and computer engineering department of Université Laval, principal investigator for the grant obtained from the Fonds de recherche du Québec – Nature et technologies (FRQNT), he provided student supervision on the infrared technology.

Julien Fleuret (Collaborator): Ph. D. Candidate at electrical and computer engineering department of Université Laval, under supervision of Dr. Maldague, responsible by IR image capture and handling.

Other contributors:

Steve Méthot (Statistician**)**: support for statistical analysis, result interpretation and manuscript revision.

Identification of pig anatomical location ensuring effective assessment of body temperature variation in response to physical stress by infrared thermography

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ABSTRACT: The objective of this study was to identify the best anatomical location for the measure of body temperature by infrared (IR) thermography that could be used to monitor the variation in the physiological status of pigs in response to stress. The study was conducted using a total of 63 crossbred gilts (104 ± 5 kg BW). Pigs were subjected to a handling stress test (HST), which consisted in driving cohorts of 3-4 pigs out of the pen and through the farm alley in 3 consecutive laps. : Blood lactate level, rectal temperature (RT) and IR skin temperature were taken when pigs were resting in the pen (RE) and right after HST at three different anatomical locations: behind the ear (BE) and on the neck and rump surface (NS and RS). The IR thermograms were analysed using the IR thermography Cronista Professional software. Blood lactate, RT and IR temperature data were analysed using a MIXED procedures of SAS. Correlations were calculated using SAS to determine the relationship between IR anatomical sites temperatures, RT and blood lactate. Blood lactate levels and RT increased after HST ($P < 0.001$). Likewise, IR skin temperature increased after HST ($P < 0.001$) at all anatomical locations, with greater temperatures being found at BE followed by NS and RS sites. Blood lactate concentration after HST was correlated with IR temperature at the RS ($r = 0.37$; $P = 0.004$) and NS ($r = 0.27$; $P = 0.04$), whereas RT was only correlated with IR temperature at BE ($r = 0.29$; $P = 0.02$) and RS ($r = 0.35$; $P = 0.005$) sites at rest. The results of this study show that IR thermography may be a potentially useful technique for the assessment of body temperature variation after physical stress, especially when this measure is taken behind the ears.

Keywords: infrared temperature, stress, blood lactate, rectal temperature, pigs.

1. Introduction

The stimulation of the autonomic nervous system in response to stress induces changes in the peripheral vascular tone and blood flow in animals, resulting in increased body temperature (Blessing, 2003; Mitchell, 2013). Previous studies of Abrahams et al. (1964) observed changes in temperature surface as an index of muscle vasodilatation occurring during defence reactions. However, despite the importance of body temperature as stress indicator its measure is difficult to take under commercial conditions as most common techniques are invasive, implies animal restraint and need of trained manpower.

IR has great potential to measure effectively changes in radiant body surface temperature.Small changes in temperature results in substantial amounts of emitted photons that can be accurately detected by IR. There are numbers of anatomical locations, alone or in combination, which can be used for IR assessment of body temperature in response to a wide range of stressors or ambient conditions. However, the results reported in recent studies are conflicting and no clear indication of the best anatomical location for reliable assessment of body temperature using IR exists. For example, positive correlations, although rather weak, were either reported between IR inner ear temperature and exsanguination blood creatine kinase level (r = 0.55; P < 0.05; Warriss et al., 2006) or between IR orbital temperature and exsanguination blood lactate level (r = 0.20; P = 0.001; Weschenfelder et al., 2013) in pigs. The inconsistency in the results and the low magnitude of correlations may be explained either by the accuracy difference of the IR camera or the inadequate anatomical location for IR measurement used in these studies.

Therefore, the objective of this study was to identify the most adequate anatomical location for IR body temperature assessment that could be used to explain variation in the physiological status of pigs in response to stress.

2. 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 (2009).

2.1. Animals and housing

In December 2013, a total of 63 crossbred gilts (104 ± 5 kg BW; G-Performer 8.0 × Fertilis 25 developed by Genetiporc Inc., St.-Bernard, Canada) were delivered at the AAFC experimental swine growing-finishing unit in Sherbrooke (QC). On arrival at the farm, pigs were identified by a numbered plastic ear tag and the day of test with a number drawn on their back to facilitate their identification during handling trials. Pigs were housed in groups from October to December of 2013 in terms of 62 pigs/ finishing pen, at density ranging between 0.72 to 0.74 m^2/ pig.

To minimize the effects of ambient conditions on the physiological response to handling, from the first day on the farm and to the day of handling trials, the ambient temperature inside the farm was set and maintained around 20˚C.

2.2. Handling stress test

Pigs were randomly sorted out from the home pen in cohorts of 3- 4 pigs and driven through the alley in 3 consecutive laps (120 m total distance) on the same day. As the objective of the handling test was to impose acute physical stress, pigs were pushed by a trained handler to run or walk at a fast pace using vocalisation, rattle noise and physical interaction, using a rattle noise when needed. To the same end, the alley design was modified and consisted of funnel-type passages, narrowed path (0.96 m) including two 90˚corners and a 12 cm step to be climbed (Fig. 1). The total time taken to move a group of pigs through each lap of the alley was noted.

2.3. Physiological variables

Physiological data were collected at rest in the pen (RE) before the start of the handling test and immediately after the return at the pen from the handling test (HST).

2.4. Blood lactate analysis

Blood lactate was measured as reported by Rocha et al. (2014). Briefly, pigs were individually restrained by an operator in the corner of the resting pen using a plastic board and blood samples were collected from each pig by pricking one of the animal's distal ear veins with a retractable gauge needle. A drop of blood, approximately 0.5 µl of blood, 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 sec.

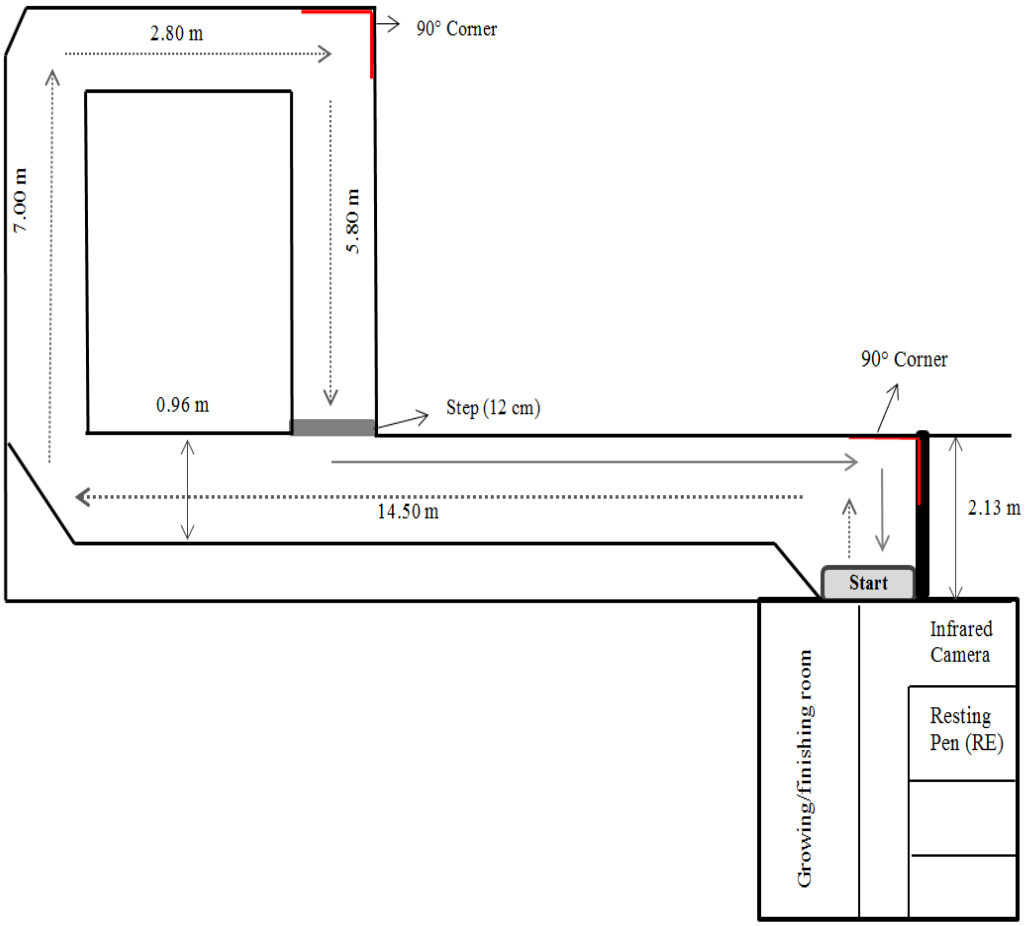

Figure 1. Diagram of the handling stress test course

Overall, the blood test took up to 60 seconds/ pig, including restrainting the pigs and blood samplings.

2.5. Rectal temperature recording

Rectal temperature (RT) was assessed using a regular digital thermometer (Formedica®, Model 8086, Montreal, Canada) inserted at a depth of approximately 10 cm into the rectum of each pig. The digital thermometer was disinfected after each measurement and lubricated with gel for rectal examination in order to decrease the stress to the animals.

2.6. Capture of infrared thermography images

One IR camera (JenOptik IR-TCM 384, Jena, Germany) connected to a laptop (Toshiba, W530, Markham, ON) was installed in the right side of the resting pen, allowing a perfect view of pigs at the same time than the physiological measures were taken (Fig. 1). The camera was operated by a trained technician and the emissivity

was set at 0.95, based on the emissivity values range (0.95-0.98) proposed in the literature (Gariépy et al., 1989; Jones and Plassmann, 2002; Weschenfelder et al., 2013). A total of 7-9 images/sec were taken from each pig at a distance of 1.20 m to detect skin temperature variation at four different anatomical locations: right eye (EY), behind left ear (BE), neck surface (NS) and rump surface (RS).

2.7. Statistical analysis

The IR thermograms were analysed using the IRT Cronista Professional software (Grayess®, version 3.6, Bradenton, FL) for the determination of the maximum, minimum and average temperature (ºC) at the four different anatomical locations.

All statistical procedures were carried out using the Statistical Analysis Software (SAS Institute Inc., Cary, NC, 2002). Blood lactate data were transformed (log10) for data normalization before analysis. Log values, rectal temperature and IR data were analysed using the MIXED procedure of SAS in a one-way analysis of variance for repeated measures with the animal as the experimental unit for each assessment point. The resulting adjusted means and confidence limits of lactate data were back transformed to the original scale (mM). As for IR data, thermogram means for maximum temperature at each body location were used and delta temperatures (ΔT) were calculated in order to provide the variation in body temperature between RE and HST. The differences between anatomical locations were analysed using a MIXED procedure of SAS in a one-way analysis of variance for repeated measures with the animal as the experimental unit.

The CORR procedure of SAS was used to calculate Spearman and Pearson correlations coefficients between blood lactate, rectal temperature and IR at each anatomical location at RE and after HST. Person correlation was used only for blood lactate data because model appeared to be more adequate. Kendall's coefficient of concordance was also calculated for measuring the agreement between temperatures measured in each anatomical location. As, due to technical reasons, only a few IR temperature data could be captured at the EY location (n = 24 at RE and n = 17 at HST). The reason for the low number of IR images of the eye location was the hardness to capture good images of that region in a free-pen situation. The natural anatomy of the pigs, with drooping ears and slant forward nearly parallel to the bridge of a straight nose, reduced the exposing area and has hid the pigs' eyes.

3. Results and discussion

3.1. Blood Lactate and rectal temperature variation

The stimulation of the sympathoadrenal axis during physical stress response has been related to increased metabolic activity resulting in greater heart rate (Correa et al., 2010), blood lactate levels (Edwards et al., 2010a; Rocha et al., 2015) and body temperature (Blessing, 2003).

The average levels of blood lactate increased from RE to HST ($P < 0.001$; Table 1). Likewise, a 0.51ºC increase in the rectal temperature recording was found between RE and HST ($P < 0.001$; Table 1). The average normal resting level of blood lactate (Edwards et al., 2011; Rocha et al., 2015) and rectal temperature (Hannon et al., 1990; Yoshioka et al., 2004; Asala et al., 2010) found in this study confirm those reported in the literature and their variation after physical stress followed the same pattern showed in a number of previous studies (Ritter et al., 2008, 2009; Edwards et al., 2011; Rocha et al., 2015).

3.2. Variation of Infrared Temperature between Anatomical Locations

Infrared temperatures increased from RE to HST at all anatomical locations ($P < 0.001$; Table 1), with greater variation being found at BE ($\Delta T = 1.90$ºC) followed by NS ($\Delta T = 0.89$ ºC) and RS ($\Delta T = 0.77$ ºC) sites. Additionally, BE ($P < 0.001$; Fig. 2) showed greater IR values compared to NS and RS within RE and HST events. Variation in skin temperature between these anatomical locations may be either explained by the difference in blood supply and/or thickness, density and quality of hair covering the different parts of the body (McCafferty, 2007; Banhazi et al., 2009). The greater skin temperature variation at BE may be explained by a better vascularization and lower hair coverage of this body location, resulting in a greater level of emitted radiation, compared to the other anatomical sites assessed in this study (Niiyama et al., 1985).

In the present study, a smaller range of variation in the maximum IR values within each anatomical location was observed, compared to the maximum IR results obtained in previous studies using the fore-back skin and in the inner ear body sites of pigs (Gariépy et al., 1989; Warriss et al., 2006; Weschenfelder, 2012).

Table 1. Variation of blood lactate, rectal temperature and infrared temperature between anatomical locations measured at rest and after handling stress (HST)

Variables	*n*	At rest	*n*	HST	SEM[1]	*P values*
Blood lactate, mM	60	2.29	58	6.50	-	< 0.001
Rectal temperature, °C	60	38.85	61	39.35	0.05	< 0.001
Behind ear, °C	59	35.90	60	37.80	0.09	< 0.001
Neck surface, °C	60	35.52	61	36.41	0.12	< 0.001
Rump surface, °C	60	35.62	61	36.39	0.09	< 0.001
Eye, °C	24	35.99	17	36.62	0.20	0.02

[1]Missing SEM values means that data were analyzed in Log values and back transformed to the original scale (mM)

These results may indicate a better accuracy of the IR camera and software for thermogram analysis used in this study.

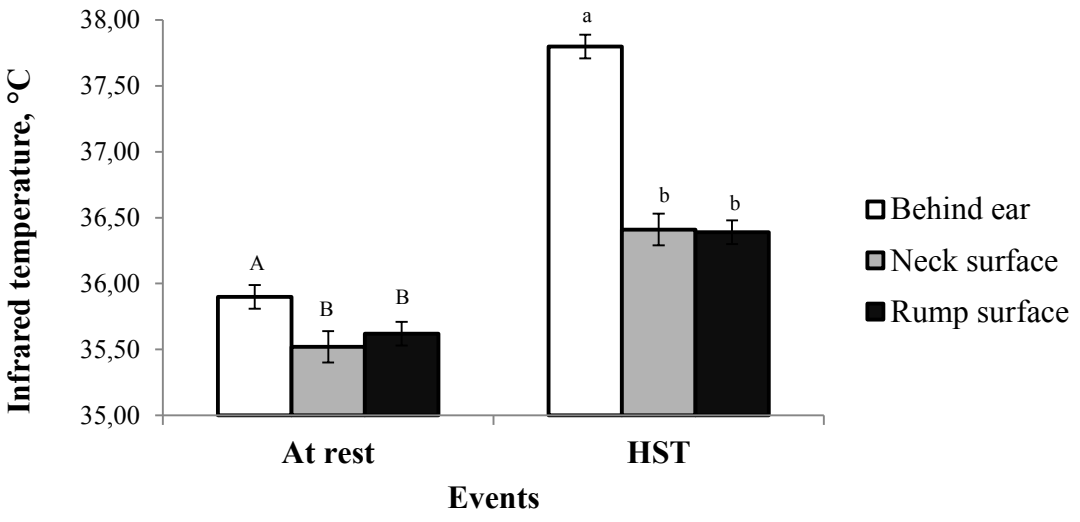

Figure 2. Differences between infrared temperatures measured at different anatomical locations within the same event[1]. [1]RE = At rest; HST = After handling stress test; Superscript letters mean that infrared temperature values are significantly different (*P* < 0.001)

3.3. Correlations between rectal temperature, blood lactate and IR at different Anatomical Locations

No significant correlation was found between ΔT of IR temperatures in each anatomical location and blood lactate and RT ($P > 0.10$; data not shown). Pearson and Spearman correlation coefficients between anatomical locations of IR temperatures, blood lactate and RT are shown in Table 2. The greatest correlation was found at RE and after HST between IR temperatures at BE and NS ($r = 0.42$ and $r = 0.62$, respectively; $P < 0.001$ for both) and between those at NS and RS ($r = 0.51$ and $r = 0.50$, respectively; $P < 0.001$ for both). As confirmed by the analysis of Kendall's coefficient of concordance between IR temperatures at BE and NS ($r = 0.45$) and at NS and RS ($r = 0.52$) after the handling trial, the pattern of variation in IR skin temperatures following physical stress is similar between these anatomical locations.

Significant correlations, although of low magnitude, were found between RT and IR temperatures at RS ($r = 0.35$; $P = 0.005$) and at BE ($r = 0.29$; $P = 0.02$) at RE. However, unlike Loughmiller et al. (1999) who reported a moderate correlation ($r = 0.52$; $P < 0.01$) between RT and IR temperature variation in response to heat stress, and Lonardi et al. (2015) who reported a correlation ($r = 0.31$; $P < 0.01$) between RT and IR orbital temperature in piglets 4 h following castration, in this study, RT was not correlated with IR temperatures at any anatomical site after HST. This lack of correlation may be explained by the shorter time lapse for the skin temperature increase after a physical stress compared with core temperature. Indeed in this study physical exercise lasted for 01:55 min. (ranging from 01:36 to 02.30 min.) and it has been reported that rectal temperature is very slow to respond to changes in blood flow taking as long as 5.3 min to increase after stress (Molnar and Read, 1974).

No significant correlation was found between blood lactate levels and IR temperatures at RE. After HST, correlations between these physiological variables were significant, but weak, with blood lactate levels being correlated with IR temperatures at RS ($r = 0.37$; $P = 0.004$) and NS ($r = 0.27$; $P = 0.04$), and tended to be related with IR temperatures variation at BE ($r = 0.25$; $P = 0.06$). Weschenfelder et al. (2013) also reported a weak correlation ($r = 0.20$) between blood lactate and IR temperature variation. The reason for the poor correlation between these variables may be two-fold. It may be either explained by the short physical effort (approx. 2 min), which

may have not allowed blood lactate levels to reach the highest concentration (peak reached at 4 min; Anderson, 2010) or by the analysis technique as LSA measuring lactate level in whole blood underestimates blood lactate concentrations compared with plasma analysis, especially when blood is sampled from the distal ear veins (Rocha et al., 2015).

4. Conclusions

This study indicates that IR technology applied behind the ears is capable of detecting changes in body temperature associated with physical stress more precisely. Therefore, measurements of surface temperature using IR may constitute a useful non-invasive procedure that might readily be applied in a commercial setting for purposes of animal welfare monitoring. However, to validate the efficiency of infrared thermography as useful tool to monitor the overall physiological response to stress in pigs, further studies including objective animal welfare measures, such as behaviour, hear rate and blood indicators other than LSA lactate, are necessary.

Table 2. Spearman and Pearson correlations between IR body temperatures assessed at three different anatomical locations and blood lactate and rectal temperature measured at rest and after handling stress test (HST)

Events			**At rest**					**After HST**		
Parameters	**Behind ear**	**Neck surface**	**Rump surface**	**Blood lactate[1]**	**Rectal temp.**	**Behind ear**	**Neck surface**	**Rump surface**	**Blood lactate[1]**	**Rectal temp.**
IR measure										
Behind ear	1	0.42***	0.23+	-0.02	0.29*	1	0.62***	0.55***	0.25+	0.07
Neck surface		1	0.51***	-0.06	0.20*		1	0.50***	0.27*	0.12
Rump surface			1	0.20	0.35*			1	0.37*	0.01
Blood lactate				1	0.02				1	0.01
Rectal temperature					1					1

*$P < 0.05$; *** $P < 0.001$; +$P < 0.10$

[1] Coefficients of Pearson correlations;

Chapter 6: Composition of exudates from meat drip loss and microbial spoilage differences between various pork quality classes

The PFN and RSE quality classes have been recently recognized as major quality defects in Canada, representing more than 13 % of all defects compared to PSE (13%) and DFD (10%) meat. DFD meat conditions are well known to provide a favourable substrate for microbial growth due to its high pH (> 6). Pork of the RSE quality class was reported to have shorter shelf-life compared with the other quality classes. The quality and composition of the drip may be responsible for bacterial proliferation in RSE pork during storage. However, the chemical nature and composition of drip from the various pork classes and their contribution to microorganism's growth are unknown. Hence, this chapter presents the results of the study conducted to give evidence of the difference in composition of the exudate obtained from pork of each quality class.

This study was developed as part of the course SAN 7001-A (3 cr; Sujets spéciaux - Sciences animales). It allowed the candidate to get familiar with meat microbiology. Additionally, this short study was considered an important contribution to the candidate training in meat science and technology. A short paper including these results was submitted to the 61st International Congress of Meat Science and Technology (ICOMST), August 23-28, 2015, Clermont-Ferrand, France and accepted as it is by the congress scientific committee. The communication was in the poster format.

Contributing authors:

Luiene Moura Rocha (Ph.D. candidate): personnel coordination and administration for laboratory analysis, pre-analytical handling and preparation of samples, bibliographic research and set-up of the experimental design, data analysis and manuscript preparation.

Linda Saucier and Luigi Faucitano (Research's director and corresponding author, and co-director, respectively): support for the set-up of the experimental design, student supervision, revision and correction of the manuscript. The poster was prepared and presented by Prof. Linda Saucier.

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Composition of exudates from meat drip loss and microbial spoilage differences between various pork quality classes

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Abstract – This study aimed at elucidating the differences in the composition of meat exudates and determining which constituents contribute the most to microbial growth between five pork quality classes (DFD=Dark, Firm, Dry; RFN=Reddish-pink, Firm and Non-exudative; RSE=Red, Soft and Exudative; PFN=Pale, Firm and Non-exudative and PSE=Pale, Soft and Exudative). A total of 65 L*ongissimus* muscle samples (*n*=15/pork quality class; *n*=5 for DFD meat class) were analyzed in triplicate for glucose, glucose-6-phosphate, lactate and protein content, and microbial growth. Differences between pork quality classes were assessed using the MIXED procedure of SAS. Surprisingly, after storage at -80°C, the greatest pH value was observed in the purge of RFN pork ($P<0.001$), while lactate content of DFD pork tended to be lower ($P=0.08$) than the other pork quality classes. No differences were observed for glucose, glucose-6-phosphate and total protein values between pork quality classes ($P>0.05$). Volume of drip loss was a major limit with the methods used. High throughput mass spectroscopy is currently under investigation as a more effective tool to study drip loss composition and effect on microbial growth.

Key Words: microbial growth, purge composition, shelf life.

I. INTRODUCTION

Traditionally, raw pork has been classified into three quality categories, namely reddish-pink, firm and non-exudative (RFN), pale, very soft and exudative (PSE) and dark, firm and dry (DFD) meat, according to three main technological parameters, namely ultimate pH (pHu), colour (L* value) and water-holding capacity (WHC) or drip loss. Over the past few years, for a more reliable quality assessment taking into account the variation in either colour or exudate, additional quality categories, such as reddish-pink, firm and exudative (RSE) and pale, firm and non-exudative (PFN) meat, have been described (Kauffman et al., 1992; Kauffman et al., 1993; Van Laack and Kauffman, 1999).

RSE pork is a quality category with an acceptable colour, but with soft texture and exudation rate similar to PSE meat (Kauffman et al., 1992). Due to its similarity in the exudation rate with PSE meat and its greater pHu (Warner et al., 1997; Joo et al., 1999), RSE meat has been defined as a mild form of PSE (Van Laack and Kauffman, 1999; Faucitano et al., 2010a). Additionally, Faucitano et al. (2010a) observed that among the meat quality classes with a pHu value lower than 6, RSE meat showed a greater microbial load after 35 days of storage under vacuum and refrigerated conditions when compared with the other meat quality classes. A higher pHu promotes microbial growth and shorter shelf-life due to a pHu higher than 6 has been well demonstrated in DFD meat (Gill et al., 1976), but the reasons for the greater susceptibility to bacterial spoilage in RSE meat during storage are still unknown. Previous studies have reported that the meat juice exuding from the meat during *post-mortem* storage is mainly composed of low molecular weight compounds readily available for bacterial growth (Savage et al., 1990; Faucitano et al., 2010a; Kim et al., 2013). However, the chemical nature and proportion in drip composition from the various pork quality classes and their contribution to microbial growth is still unclear. Therefore, the objectives of this study were to elucidate the composition of meat exudates obtained from all five pork meat quality classes and their contribution towards microbial growth and, consequently, to fresh meat shelf-life.

II. MATERIALS AND METHODS

Meat exudate

A total of 65 *Longissimus* muscle samples (*n*=15, for RFN, PFN, RSE and PSE; *n*=5 for DFD) were analyzed for the composition of frozen meat exudate collected from loins of five quality classes during drip loss measurement in a previous study (Weschenfelder et al., 2012). As the volume of meat exudate samples was very small for some quality classes (*e.g.,* PFN = ± 0.4 and DFD = ± 0.2 mL/sample) samples were pooled by meat class in order to have sufficient volume of material to run the analysis. The pools were done by blending 4-5 samples of same meat class in order to have a similar pH for each pool. Thus, a total of 14 pools (PSE = 4, RSE = 4, DFD = 1, RFN = 2, and PFN = 3) were used for the following analyses: exudate pH, enzymatic determination of glucose, glucose-6-phosphate, lactate, total protein and microbial growth. Even after pooling, the number of DFD exudates was so low that only one pool could be obtained.

Exudate pH and composition

The pH of the meat exudate was assessed in each sample before blending and in each pool, by meat quality class, using a portable pHmeter (Oakton Instruments Model pH 100 Series, Vernon Hills, IL) fitted with a MicroProbe™ (Accumet, model: CP-620-96, Montreal, Canada).

Lactate content in the meat exudate was analyzed according to the method described by Monin and Selier (1985). Briefly, for the enzymatic determination of glucose and glucose-6-phosphate (G6P), 500 µl of meat exudate was transferred to a glass tubes and another 500 µl was transferred to regular eppendorf tube and kept at 4°C for the enzymatic determination of lactate. The 500 µl of meat exudate was then homogenized in acetate buffer containing *Rhizopus* amyloglucosidase to decompose glycogen to glucose and G6P. Then, glucose concentration was determined using a nicotinamide adenine dinucleotide (NAD), G6P, adenosine triphosphate (ATP) and enzymatic solution of hexokinase. Lactate concentration was determined with 100 µl of meat exudate using NAD and lactate dehydrogenase. Finally, total protein concentration was determined on 100 µl of meat exudate using the bicinchoninic acid protein assay kit (Pierce^{TM}, Rockford, IL).

Meat microbiology

Microbial growth was performed by plating 100 µl of a 1:10 dilution (Phosphate buffered saline solution) onto growth medium (Lysogeny Broth) in duplicate for RSE, PSE and DFD meat classes. DFD and PSE classes are known to be permissive and non-permissive for microbial growth (Gill and Newton, 1979; Knox et al., 2008), respectively, and were compared with RSE meat exudate. For the DFD meat exudate, an additional serial dilution (1:100) was necessary. Plates, including a negative control, were incubated at 37°C overnight and colonies were enumerated after 48 h. The mean of the duplicate plating was calculated and the data were tabulated as colony forming units (CFU) per ml.

Statistical Analyses

Differences in glucose, G6P, lactate and total protein data were analyzed using the MIXED procedure of SAS (2002) in a one-way analysis of variance for the pork class effect and with the pool per meat quality class as the experimental unit. Spearman correlations were performed using SAS to determine relationships between the meat exudate components for each pork class. The meat microbiology results were transformed in Log CFU/ml and numerically compared between quality classes. A probability level of $P < 0.05$ was chosen as the limit for statistical significance, whereas $P \leq 0.10$ were considered to be a tendency.

III. RESULTS AND DISCUSSION

The microbial analysis showed that counts with PSE exudate (1.85 Log CFU/ml) were 2.45 and 0.45 Log below the results for DFD (4.30 Log CFU/ml and RSE (2.30 Log CFU/ml) exudates, respectively. Hence, DFD was the most spoiled samples whereas RSE was classified as intermediate compared to PSE. Greater counts in DFD exudates is not surprising based on its low concentration of lactic acid/lactate and the higher pHu (Gill and Newton, 1979; Grau, 1981), both known to promote microbial growth at a faster rate. The greater availability of glucose on the PSE meat exudates delays the use of amino acid as a carbon source and thereby delays spoilage (Gill and Newton, 1979). These results confirm the greater predisposition for bacterial spoilage of RSE pork, which is only second to DFD pork, as reported in a previous study (Faucitano et al., 2010a).

Faucitano et al. (2010a) suggested that the meat exudate composition or quantity may contribute to meat spoilage in pork presenting a pHu<6. Kim et al. (2013) also highlighted the role of meat exudate as a suitable substrate for bacterial growth in meat during cold storage. However, in this study, the differences in the exudate components among the five meat quality classes were mostly numerical (Table 1). The small sample size may explain the lack of significant differences.

Table 1. Variation of lactate, glucose, glucose-6-phosphate, total protein content and pH in exudate by pork quality class

Variables	PFN	PSE	RSE	RFN	DFD[1]	SEM	*P* value[2]
G6P[3]	2.45	2.47	1.78	2.01	-	0.63	NS
Glucose[4]	11.54	13.73	11.55	8.46	-	1.70	NS
Lactate[5]	141^{AB}	150^{A}	150^{A}	142^{AB}	128^{B}	6.9	0.08
Protein[6]	99.7	98.4	97.6	106.0	115.0	7.0	NS
Exudate pH	5.28^{c}	5.29^{c}	5.90^{b}	6.45^{a}	-	0.11	< 0.001

[A,B] Different capital letters mean that values tend to be different ($P < 0.10$).

[a,b] Different lower cap letters mean that values are significantly different ($P < 0.05$).

[1]Missing values are due to lack of DFD samples to run these analyses.

[2] NS = $P>0.10$.

[3] Glucose-6–phosphate (µmole/mL).

[4] Glucose (µmole/mL).

[5] Lactate (µmole/mL).

[6] Total protein (mg/mL).

Unsurprisingly, the lactate content of DFD meat exudate tended to be lower ($P = 0.08$) compared to PSE and RSE meat exudates (Table 1). These results are consistent with the findings of Traore et al. (2012), who reported greater lactate content in the exudate from exudative compared to non-exudative meats. This difference in lactate concentration likely led to differences in the pH of meat exudates ($P < 0.001$) between meat classes. For meat to be classified as RFN, pH of the meat had

to be lower than 6 by definition (Weschenfelder et al., 2012). The pH of the RFN meat exudate is higher than 6 suggesting that either during the EZ-drip loss procedures at 4°C for 48h or during storage at -80°C, meat exudates composition was altered. Apart from DFD, the exudates from RFN and RSE pork classes showed the higher pH values compared to all the other meat classes ($P < 0.05$). The pH from DFD meat could not be measured due to the low quantity of meat exudate available for the analysis.

No difference in protein concentration was found between the different pork exudates ($P > 0.10$). These results disagree with Bowker and Zhuang (2013) who reported greater values for the drip protein concentration between dark and pale breast fillets. The lack of difference in protein content between pork quality classes in this study may be explained by the exudate storage conditions (-80°C) pending analysis that may have caused certain physicochemical qualitative changes in the samples, such as amino acid decarboxylation, formation of ice crystals resulting in protein denaturation (Petrović et al., 1993). No correlation between constituents, or ratio of constituents, with meat quality classes could be established firmly. Only the pH of the exudate can explain variation observed in microbial growth. To our knowledge, this is the first study on meat exudate composition from the different pork quality classes including PFN and RSE meats.

CONCLUSION

The results of this study showed that meat exudates contain low molecular weight compounds, such as glucose, G6P, lactate and protein that can be readily available for microbial growth. However, either the small size or the quality of samples available for this study prevented from identifying the contribution of these components to the variation in shelf-life between pork quality classes. New analytical techniques, such as high throughput mass spectroscopy coupled with machine learning strategies are now being tested as a mean to better profile meat exudate constituents when limited quantity of material is available for analysis such as in the case of meat exudates where microbial growth actually takes place on the surface of fresh intact meat.

Chapter 7: General conclusions, implications and perspectives

7.1. General conclusions and implications

The objective of the present thesis was to respond to the current demands of consumers and the pork chain for accurate tools to monitor the quality of preslaughter handling and ensure a better welfare for pigs. Stress experienced by pigs preslaughter has a direct impact on the physiological status of pigs before slaughter, as showed by the significant changes in body temperature, blood lactate and muscle glycogen levels resulting in pork quality variation.

The main hypotheses of the experiments described in this thesis were that the hand-held Lactate Scout Analyzer (LSA), animal welfare audit protocols through the pork chain and the infrared thermography are effective tools allowing the control of meat quality variation through a precise and reliable assessment of the animal welfare conditions of pigs preslaughter.

Overall, the LSA detected the increase in blood lactate concentration through lairage (from unloading to slaughter) reflecting the progressively increase of muscle activity and stress as the animals pass from a group to in-line or individual situation in the restrainer. More specifically, the measurement of blood lactate level of pigs in the restrainer as measured by the LSA appears to be the best indicator of the physical fatigue condition of pigs at slaughter. However, as far as meat quality prediction is concerned, the low correlations obtained between blood lactate and meat quality traits do not allow to indicate this measure as a reliable predictor of pork quality variation. These results may be explained by the too controlled *peri-mortem* handling conditions resulting in a stress level too mild to produce the necessary variation in blood lactate levels and meat quality traits.

The novel on-farm animal welfare audit protocol used in this study was able to assign one of four possible animal welfare category (Not classified; acceptable; Enhanced or Excellent), based on reference profiles for the principle-scores it obtained, and differentiate the quality of the raising system by the welfare conditions of the pigs audited in this study. However, the audit scores obtained at the farm could not explain the variation of pig behaviour at the slaughter plant. The results can be explained by the bias represented by the difference in the trucker handling skills at

loading and unloading that may have influenced the behaviour of pigs at these stages. By the way, these results give evidence of the importance of handler training on animal welfare. The results of this study also showed that pigs raised in animal welfare improved raising systems are more likely to produce PSE and RSE pork compared with those raised in conventional systems. Based on previous evidence, it is proposed the greater residual glycogen content in the muscle of these pigs at slaughter to be the most likely explanation for this result. Slips and electric prod use as audited by the novel protocol in the stunning chute area at the plant showed good preditors of drip loss variation in pork meat.

Finally, the results on infrared thermography showed that IR thermography is a promising technology to assess the body temperature variation in response to handling stress in live pigs, especially when this measure is taken behind the ears, compared to eyes, neck and rump surface. However, the correlations found in this study were low, although significantly, likely due to the small number of pigs used, and due to the use of only two physiological stress indicator (*i.e.* rectal temperature and blood lactate). Another implication in this study was the short-time stress imposed to the pigs and the low feasibility in taken thermal images using the eye location in a free-pen situation.

7.2. Futures perspectives

7.2.1. Hand-held lactate analyzer

The LSA technique was tested in conditions that were not particularly stressful and not representing the normal preslaughter situation. Further studies under more variable level of preslaughter stressful conditions are then required to validate its potential as a monitoring tool. Furthermore, the validation of whole blood lactate as a stress indicator and meat quality predictor should be done using other physiological stress indicators, such as heart rate, salivary cortisol, body temperature (i-button or IR) and glycolytic potential (in locomotory muscles), that can be also measured using stress-free or non-invasive techniques. The repetition of this study under less controlled or stressful conditions may also allow the necessary variation in exsanguination blood lactate levels and meat quality to determine a blood lactate cut-off level at bleeding. A LSA blood lactate threshold is requested by the industry to identify the critical points in the lairage area and

make the necessary adjustements in the handling practices or facilities design to ensure the production of more uniform pork quality.

7.2.2. Application of animal welfare auditing through the pork chain

The previous study published in this thesis showed that the quality of the raising system at the farm of origin as assessed by the WQ® and CPC protocols was an important source of variation in the behavioural response of pigs to preslaughter handling as assessed by the WQ® and AMI protocols, and pork quality. However, the WQ® scores obtained on farm in this study may were biased by the inclusion of criteria, such as castration, which is a negative criterion in the WQ® protocol, but is an accepted practice in the Canadian pig production. Furthermore, other criteria, such as ammonia levels at the farm or presence of frostbites on the pig's body or carcass, are missing in the existing protocols, but should be considered when assessing animal welfare at Canadian farms and slaughter plants as they are specific to our production and marketing conditions. Therefore, a new study aiming to develop and transfer to the Canadian pork industry a standardized and scientifically proven "custom-made" animal welfare assessment protocol including criteria that are more applicable to the Canadian pork production conditions (from farm to slaughter) and that would help the Canadian pork chain to better monitor the welfare of pigs under the current marketing process conditions would be intresting.

7.2.3. IR thermography technology

The study aiming at assessing the efficiency of the IR technology at different body locations should be repeated by including more objective animal welfare measures, such as behaviour and a larger number of physiological indicators, such as heart rate, surface body temperature and blood stress indicators (lactate, salivary cortisol, etc.), the application of different degrees of physical stress (from gentle to very rough and short- to long-term) and larger population are necessary to validate the efficiency of the IR technology as a measure of the physiological conditions of pigs in response to stress.

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