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FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL

INFLUENCE OF THE STARTING TIME OF PRESLAUGHTER FEED WITHDRAWAL ON WELFARE OF PIGS AND PORK QUALITY

Filipe Antônio Dalla Costa
Médico Veterinário

2016

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Dissertação apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Campus de Jaboticabal, como parte das exigências para a obtenção do título de Mestre em Zootecnia.

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FORNECIDO PELA PÓS-GRADUAÇÃO

DADOS CURRICULARES DO AUTOR

Filipe Antônio Dalla Costa, nascido em 21 de novembro de 1990 na cidade de Concórdia-SC. Formado no ano de 2013 no curso de Medicina Veterinária pela Universidade do Estado de Santa Catarina, iniciou seus estudos em bem-estar animal e qualidade de carne durante estágios extracurriculares na graduação. Em 2014 ingressou no mestrado pelo Programa de Pós-Graduação em Zootecnia na Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista "Júlio de Mesquita Filho", em Jaboticabal/SP, como bolsista do Conselho Nacional Científico e Tecnológico - CNPq e integrante do Grupo de Estudos e Pesquisas em Etologia e Ecologia Animal (Grupo ETCO).

“Os loucos que acham que podem mudar o mundo, são os que efetivamente o fazem.”

Steve Jobs

Aos meus pais, que se sacrificaram dia e noite em prol da minha educação para que eu pudesse estar aqui alcançando meus objetivos e sempre entenderam minhas decisões. A minha família, que mesmo eu estando distante, sempre me apoiou.

A todos os colaboradores rurais e da indústria que batalham todos os dias para melhorar as condições de manejo pré-abate.

Dedico.

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Sherbrooke, 10 décembre 2015

Objet: Certification CIPA

Le comité institutionnel de protection des animaux (CIPA) du Centre de recherche et de développement de Sherbrooke (anciennement *Centre de recherche et de développement sur le bovin laitier et le porc*) certifie que le projet intitulé:

« Influence de la mise à jeun avant l'abattage appliquée à la ferme ou à l'abattoir sur la perte de poids, la réponse comportementale, les caractéristiques du tractus gastro-intestinale et la variation de la qualité de la carcasse et de la viande de porc »

a reçu l'approbation du comité institutionnel de protection des animaux lors de la réunion régulière du 12 février 2013 (projet #419).

Bien à vous,

Mme Pauline Bilodeau,
Présidente du CIPA

Sherbrooke, December 10th, 2015

Object: ACC Certification

This letter is to certify that the project entitled:

"Influence of preslaughter feed withdrawal as either applied at the farm or in lairage on body weight losses, behavioral response, gastro-intestinal tract characteristics and carcass and meat quality variation in pigs"

has received the approval of the Sherbrooke Research and Development Center (formerly *Dairy and Swine Research and Development Centre*) Animal Care Committee (ACC) at the meeting held February 12th, 2013 (project #419).

Sincerely,

Pauline Bilodeau

Mrs. Pauline Bilodeau,
President of the local Animal Care Committee

Canada

INFLUENCE OF THE STARTING TIME OF PRESLAUGHTER FEED WITHDRAWAL ON WELFARE OF PIGS AND PORK QUALITY

ABSTRACT – The objective of this study was to evaluate the effects of starting time of feed withdrawal interval preslaughter at the farm (FARM) or at the plant (PLANT) on animal losses, behaviour during loading and lairage, blood parameters (lactate and creatine kinase) and meat quality traits (pH, colour and drip loss) in pigs. A total of 700 pigs was distributed into two treatments groups. The FARM group was fasted for 18 h before the transport plus 6 h before slaughter, including 2 h transport and 4 h lairage, and the PLANT group was fasted for 24 h (considering 2 h transport plus 22 h at lairage), totalizing 24 h of fasting time before slaughter for both groups. The treatments neither influenced transport losses nor blood parameters. However, PLANT pigs had longer fights ($P < 0.05$) than FARM pigs at lairage and produced darker ($P = 0.02$) and drier ($P = 0.03$) loins. Based on these results, it is recommended apply feed withdrawal time before loading, which showed improvements to pigs' welfare, due to the lower number of fights during lairage at the slaughter plant, and produced better pork quality.

Keywords: animal welfare, animal losses, fasting, stress, preslaughter

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ACRONYMS AND ABBREVIATIONS LIST

ACTH: Adrenocorticotropic Hormone

ATP: Adenosine Triphosphate

CK: Creatine Kinase

CO_2: Carbon Dioxide

CRH: Corticotropin-releasing Hormone

DFD: Dark, Firm and Dry

DOA: Dead-on-arrival

JCS: Japanese Colour Scale

NAI: Non-ambulatory, Injured

NANI: Non-ambulatory, Non-injured

PC: Phosphocreatine

PFN: Pale, Firm, Non-exudative

pH_u: ultimate pH

PSE: Pale, Soft, Exudative

RFN: Red, Firm, Dry

RSE: Reddish-pink, Firm, exudative

CHAPTER 1 – General Considerations

1. Introduction

Feed withdrawal is a recommended practice for on-farm preparation before pigs' transport. This practice consists in denying pigs the access to feed, while keeping free access to water (DALLA COSTA; DALLA COSTA, 2014a). The appropriate application of feed withdrawal benefits both producers and slaughterhouse managers, as it helps to reduce transport losses (GUÀRDIA; GISPERT; DIESTRE, 1996; STEWART et al., 2008) and carcass contamination due to lower risk of gut contents spillage during carcass evisceration (SAUCIER et al., 2007), besides improving pork quality (FAUCITANO; CHEVILLON; ELLIS, 2010a). However, Aalhus (1992) and Viau and Champagne (1998) reported that this practice is not always consistently applied by the producers who prefer keeping pigs on feed until departure and starting fasting interval from farm departure to the slaughterhouse, as confirmed by a survey conducted at swine farms in Quebec, reporting that only 15% of pigs were fasted before transport (VIAU; CHAMPAGNE, 1998). This practice implies that to respect the fasting interval needed to obtain empty stomachs at slaughter a longer lairage time must be applied at the slaughterhouse.

The causes of the misapplication of feed withdrawal on-farm are associated to the split marketing production system which is very common at finishing farms in North America and in some European countries, such as France and Spain. It implies the removal of the heaviest 25 to 50% of pigs from a pen to market them 1-2 weeks earlier than the other penmates. The advantage of this practice is the reduction of production costs and the opportunity to ship batches with a more uniform market weight to the abattoir (SCROGGS et al., 2002). Whereas, the disadvantage is the risk of slower growth rate of pigs remaining in the pen that were also withdrawn of feed. A solution can be the use of shipping rooms where the heaviest pigs can be transferred ahead of time before loading, with local feed withdrawal drawing the feed there (VIAU; CHAMPAGNE, 1998).

The objectives of this study were to assess the effects of starting time of preslaughter feed withdrawal at the farm or at the plant on animal losses during transport, as well as behaviour during loading and lairage, besides evaluating blood parameters, carcass traits and pork quality.

2. Literature review

2.1. Animal welfare

According to Hughes (1976), animal welfare is a state of complete mental and physical health, where there is harmony between animal and environment. Similarly, Broom (1986) defined welfare of an individual as its state as regards its attempts to cope with its environment. While the Broom's definition refers to how well or how badly coping attempts succeed, the Hughes's definition refers only to situations when animals succeed in its attempts to cope with the environment. Therefore, when animal welfare can be measured from a state of very poor to very good (BROOM, 1991), the Broom's definition which is broader (Figure 1) is considered as more useful by the scientific community.

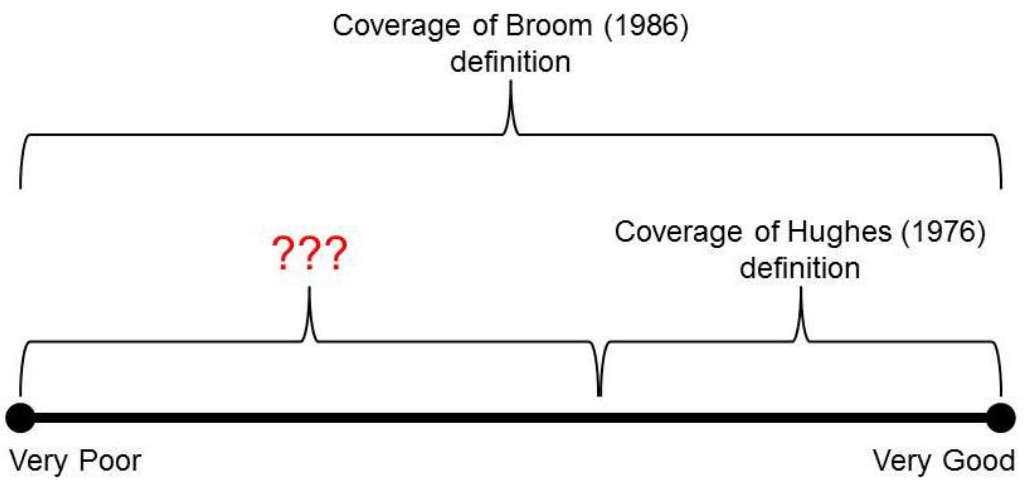

Figure 1. The coverage of animal welfare definitions (adapted from PARANHOS DA COSTA; OLIVEIRA and RUEDA, 2013).

The industry is giving more attention to animal welfare not only because of opportunities offered by new markets, but also due to financial losses related to the production of meat with quality defects, such as PSE (pale, soft, exudative) and DFD (dark, firm, dry) pork (FRASER; BROOM, 1997) .

2.2. General principles of stress

Stress is defined as an internal organism's state of perturbation on physiological homeostasis or psychological well-being (COMMITTEE ON ALLEVIATION OF DISTRESS IN LABORATORY ANIMALS, 2008). It occurs when the organism fails in coping with environmental changes, resulting in over taxation of the control systems and, consequently, compromised fitness (BROOM, 1988). In response to stress, the organism makes some significant biological changes as showed by the behavioural and physiological adaptive responses. When stress results in a "biological cost", it is considered as distress (MOBERG, 2000). Distress occurs when the adaptation processes fail and the organism cannot return to its physiological and/or psychological homeostasis (MOBERG, 1987; CARSTENS; MOBERG, 2000). Strong challenges may overload the pigs' capacity to cope with the stressor and result in death (HEMSWORTH et al., 2015).

Behaviour changes are the first and most efficient response to a stressor for the animal (VON HOLST, 1998; MOBERG, 2000). However, under given circumstances, behavioural changes alone may not be enough or appropriate to cope with a stressor. In this case, the stress response done by the autonomic nervous and neuroendocrine systems is intensified. The stress response is multifactorial and may be influenced by a combination of several factors, such as the type and duration of a stress event, animal age, genetics, coping patterns and social status of the animals (VON HOLST, 1998; SALAK-JOHNSON; McGLONE, 2007). The physiological reaction to a stressor is shown in Figure 2.

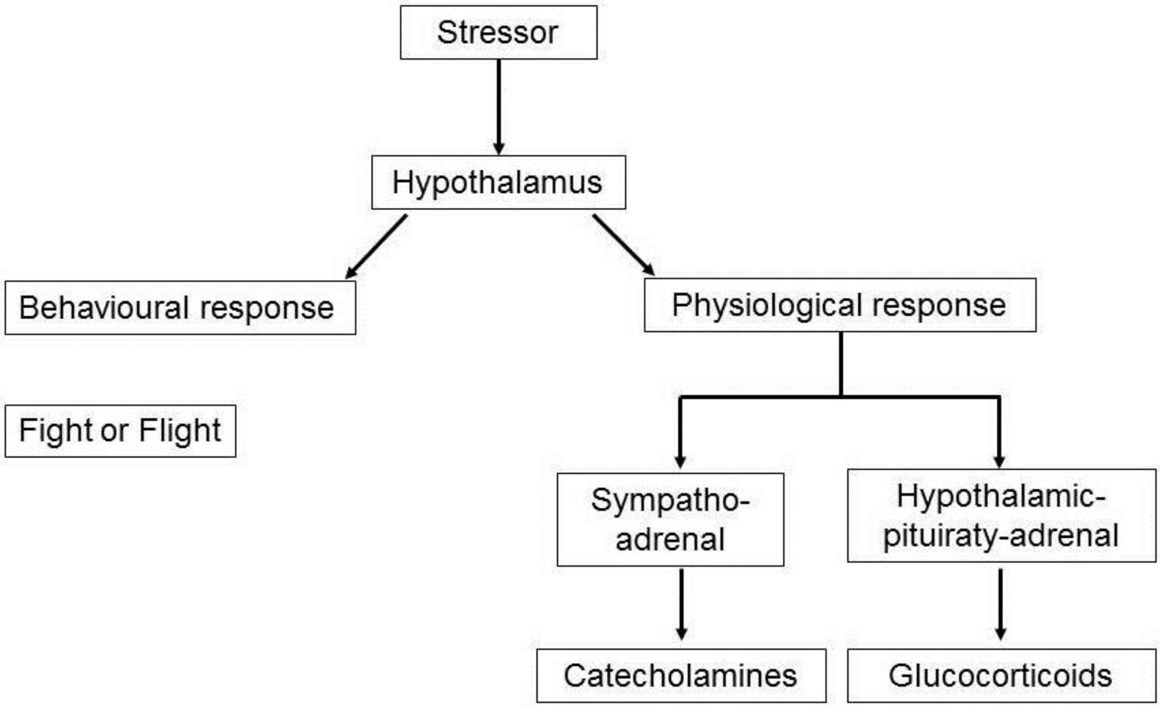

Figure 2. Animal physiological reaction to a stressor (adapted from CARRAGHER; MATTHEWS, 1996).

2.3. Behaviour

In response to a potential stressful stimulus, animals develop some adaptation mechanism to keep the homeostatic balance (GRANDIN, 1998; MOBERG, 2000). First, the animals show behaviour reactions to the environment (MOBERG, 2000). The behavioral reaction is quick, especially in acute stressful situations involving fear (DE PASSILLÉ et al., 1995). For example, increased vocalisation and greater number of slips and falls are behavioural signs of the pig response to stressful situations, e.g. rough handling during preslaughter procedures (WARRISS et al., 1994; GRANDIN, 1998a; CORREA et al., 2010; DOKMANOVIC et al., 2014).

2.3.1. Normal and abnormal behaviour

Since it measures animal own decision-making process using non-invasive methods, animal behaviour is an important tool for animal welfare assessments (DAWKINS, 2004).

Naturally, pigs can interact with their environment through their sensory abilities, such as hearing, vision, touch, olfaction and taste (HELD et al., 2009). This interaction results in behaviour patterns, such as sexual receptiveness and spatial intrusion (HELD et al., 2009). Normal behavior is defined as the collection of behaviours animals have developed during the evolutionary process and would express in a natural environment (LIDFORS; BERG; ALGERS, 2005). Basically, pigs perform three types of natural behaviours: 1) behaviour they want to express (e.g. nest building at the end of gestation); 2) behaviour performed only under specific conditions (e.g. panting or shivering under thermal stress); and, 3) behaviour they do not want to express (e.g. separation from a group; OLSSON; WÜRBEL; MENCH, 2011).

On the other hand, when normal behaviour is hindered, the abnormal behaviour and stress may be induced (LIDFORS et al., 2005). Abnormal behaviour is defined as an uncommon response to a specific combination between motivational elements and stimulus (JENSEN, 1986) and indicates a failure in the animal attempt to cope with the environment (JENSEN, 1986; LIDFORS et al., 2005). According to Jensen (1986) and Mormède (2008), abnormal behaviours in pigs may be classified as: 1) apathy or boredom, indicating a non-adaptation to a novel environment; 2) aggression, induced by competition for food and social hierarchy; 3) stereotypies, which is an unvaried sequence of movements repeated with no obvious purpose due to poor environmental stimulation and frustration; and 4) cannibalism (i.e. tail biting), induced by poor environmental stimulation.

2.3.2. Postural behaviour

Observation of postural behaviour is a method to determine stress levels imposed to animals during preslaughter handling (EWBANK; BRYANT, 1972; MOSS, 1978). Pigs can seat or lie down during lairage in order to relax or rest due to physical fatigue (BRANDT; AASLYNG, 2015). The shorter time to lie down at lairage may be an indicative of physical fatigue caused by the additive stress effect during preslaughter handling (BRANDT et al., 2013). Usually, pigs seat down within 1 hour of lairage (MOSS, 1978; BRANDT et al., 2013). There is an effect of feed withdrawal on behavior during lairage. Brown et al. (1999) observed a greater activity of fighting and drinking on arrival at the lairage pen in pigs fasted for longer time (18 vs. < 1 h). Fernandez et al. (1995) reported that fed pigs spent significantly more time lying down (10 min) than 24 h fasted ones (5 min). The increased proportion of pigs lying down at lairage is associated with the reduction in fighting behaviour, as shown by previous studies (MOSS, 1978; FERNANDEZ et al., 1995; BROWN et al., 1999). Independently of the reason, in order to relax or rest, pigs lying seem to be a welfare advantage, because it reduces the number of fights resulting in lower risk of skin damage (BRANDT et al., 2013).

Rabaste et al. (2007) found an effect of group size on lying down behaviour, where pigs kept in smaller groups (10 vs. 30 pigs) tended to spend more time lying down. The environmental temperature during the lairage period influences lying behaviour (FREAQUEZA et al., 1998; EKESBEO, 2011). Pigs lie close together during cold stress, whereas when it is warm, they lie without direct body contact (EKESBEO, 2011). Fraqueza et al. (1998) observed a greater percentage of pigs lying down at 35^{o}C for 0.5 or 3 hours than at 20^{o}C, which is correspondent to the thermoregulatory behaviour during attempts to increase body heat conduction to the floor. Weeding et al. (1993) also verified a larger number of pigs lying down for longer periods when kept in lairage above 25^{o}C and not cooled by showering.

2.3.3. Fighting behaviour

Fighting is a normal social behaviour in group of pigs, and is easily observed when unknown pigs are mixed into a group (RHIM et al., 2015). Usually, fighting behaviour occur in order to establish a clear dominance hierarchy (EWBANK, 1976). Situations such as mixing groups and competition by resources (i.e. food shortage, high density and restricted feed space) may increase fights (EWBANK; BRYANT, 1972; LØVENDAHL et al., 2005). Aggression can also occur due to fearful stress, and as a spontaneous response to sudden pain (GREGORY, 2004). During preslaughter handling, no mixing is a practical recommendation. However, under commercial condition, mixing is a common practice, which causes intensive aggression in commercial housing systems for pigs (RHIM et al., 2015). Generally, pigs can be mixed at the farm during fasting in the expedition room or loading into the truck, or even at the slaughter plant at the lairage. The fighting behaviour sometimes results in damages to health and induces physiological stress responses (MEESE; EWBANK, 1972; EWBANK; BRYANT, 1972; AREY; EDWARD, 1998; PITTS et al., 2000). Physical activity in lairage is the most potent factor stimulating muscle glycogenolysis in pigs. Brandt et al (2013) found a significant correlation between creatine kinase at exsanguination and skin damage score. Creatine kinase measurement can be an indicator of skin or muscle damage and it can be obtained from just one of the measurements (BRANDT et al., 2013). Problems associated to fighting include high bruises scores on carcass what affects meat quality (STOOKEY; GONYOU, 1994; WARRISS, 1996; GISPERT et al. 2000), and represent a significant economic cost to pork chain and serious animal welfare issue (AYO et al., 1998; O'CONNELL; BEATTIE, 1999).

Most of the fights occur during the first 60 min of lairage (MOSS, 1978; GEVERINK et al., 1996; FRAQUEZA et al., 1998). In mixed groups, Meese and Ewbank (1973) suggested that fights are practically eliminated after 24 hours and the social order is defined within 48 hours. The fasting condition affects fighting behaviour in pigs. Brown et al. (1999) found a greater number of fights on arrival at the lairage pen in pigs fasted for longer time (18 vs. < 1 h). Fernandez et al. (1995) reported a greater activity in fasted pigs (24 vs. 0 h) than fed ones. However, the authors did not found difference between treatments for the latency before the first aggressive interaction. Pigs fight more when kept in lairage for longer periods (overnight to > 24 h vs. 3 h) as a result of mixing and frustration due feed withdrawal (MURRAY; JONES, 1994; WARRISS et al., 1998b; GUÀRDIA et al., 2009).

The skin bruises are direct associated to stress suffered by pigs, as demonstrated by higher concentrations of cortisol and creatine phosphokinase in blood after slaughter (WARRISS; BROWN, 1985; WARRISS, 1996). Fernandez et al. (1995) suggested that the activation of the hypothalamic-pituitary-adrenal axis in response to both novel environment and aggressive encounters is not influenced by food deprivation. However, literature is scarce on the effect of feed withdrawal on sympatho-adrenal reactivity to stress.

Fighting behaviour can be direct or indirect measured by behaviour observations or counting the lesions on the skin or carcass, respectively. However, because observing behaviour is time consuming and impractical, counting skin or carcass lesion is more efficient, quicker and easier to determine fighting in pigs.

2.4. Physiology of the stress response

The autonomic system (i.e. the sympatho-adrenal medullary) and the hypothalamo-pituitary adrenal axis are activated in order to protect the biological system and to cope with the stressor through an increased synthesis of catecholamines and glucocorticoids (ULRICH-LAI; HERMAN, 2009; TURNER et al. 2012). The first activation occurs through a rapid response to stress, so called "alarm", "emergency syndrome" or also "fight-or-flight" (CANNON, 1929; MOBERG, 2000).

The stressful stimulus, external or internal, is transmitted to the hypothalamus which secretes the corticotropin-releasing hormone (CRH). This hormone is carried by the hypothalamo-hypophyseal portal system to the anterior lobe of the pituitary gland which is responsible for the synthesis and secretion of adrenocorticotropic hormone (ACTH). Its principal effects are stimulation of the cortex of the adrenal glands, resulting in synthesis and release of glucocorticoids (cortisol) and mineralocorticoids (adrenaline and noradrenaline) into blood (MATTERI; CARROLL; DYER, 2000; ULRICH-LAI; HERMAN, 2009).

2.4.1. Physiological parameters of stress

2.4.1.1. Cortisol

Cortisol is the major glucocorticoid used for the assessment of pigs’ welfare. Cortisol levels can be a blood indicator of long-term psychological stress response (fear, alert) rising in less than 30 min in response to a stressor and returning to the basal level in 3 h (NYBERG et al., 1988; BRADSHAW et al., 1996; EKKEL et al., 1997; RUIS et al., 2001; MORMÈDE, 2008). Cortisol is secreted by the cortex of adrenal gland following the activation of the hypothalamic–pituitary–adrenal (HPA) axis in response to a stressor (WILLIAMS, 1983; NORMAN; LITWACK, 1997;

BOLANDER, 2004). However, a particular attention must be paid in the interpretation of blood cortisol variation, as its blood levels follow a circadian rhythm, reaching a peak in the early morning and declining steadily during daylight hours and reaching the lowest level in the evening (RUIS et al., 1997; HAY et al., 2000; MORMÈDE, 2008).

2.4.1.2. Lactate and creatine kinase

Lactate concentration in blood is a very short-term indicator of physical stress which reaches the peak within 4 min and returns to basal level in 2 h (ANDERSON, 2010). Lactate is produced from pyruvate during normal aerobic metabolism of body muscle tissues. However, in anaerobic conditions glucose is broken down and oxidized to pyruvate, which in turn is converted into lactate in the striated muscle and liver (VOET; VOET, 1995; HARRIS, 2004).

The creatine kinase (CK) enzyme is also an indicator of physical stress and muscle damage, but in the long-term as it takes up to 6 h to achieve the peak and returns to basal levels 48 h later (ANDERSON, 2010). The tissue of skeletal muscle depends on the availability of adenosine triphosphate (ATP) for a proper functioning (DAROIT; BRANDELLI, 2008). Generally, the muscle stores energy in a phosphocreatine (PC) form which can be rapidly converted into ATP through a reaction catalysed by CK (VOET; VOET, 1995). The ATP is consumed during high metabolic activity and is regenerated thanks to the CK catalysing the transphosphorylation reaction between PC and adenosine diphosphate (WALLIMANN et al., 1992; WYSS et al., 1992).

2.4.1.3. Heart and respiration rate

Heart rate variability has been largely used as a welfare indicator in animal production that increases in stressful situations (TERLOUW et al., 2004; BROOM; FRASER, 2007; MILLS et al., 2010) and during physical activity (PÖSÖ; PUOLANNE, 2005). Independently the stress type, either psychological (i.e. presence of a predator or human contact) or physical (i.e. during loading and unloading), the heart rate measurement is a very rapid stress indicator (WARRISS, 2010) that provides the number of heart beats per unit time (MILLS et al., 2010). This measurement is defined as tachycardia to an 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 or relaxing. During preslaughter handling, heart rate is increased during the use of electrical goads (KUCHENMEISTER et al., 2005; CORREA et al., 2010), at loading (van PUTTEN; ELSHOF, 1978), transport (GEVERINK et al., 1998; WARRISS, 1998) and according to social stress (de JONG et al., 2000).

The respiration rate is a stress indicator influenced by factor such as emotional disturbance without body activity (MELLOR; MURRAY, 1989; LORSCHY, 1997), physical exercise and fatigue (ZHANG et al., 1992; RITTER et al., 2007) and heat stress (FORREST et al., 1968). The heat stress increases breathing rate in order to control body temperature (MOUNT, 1972; ZHANG et al., 1992; BROWN-BRANDL et al., 2001) through the evaporative heat loss. Sweting is not an efficient tool to cool pigs; therefore, in situations they do not have an external source of water for evaporation from body surfaces, they use the panting for evaporative cooling (INGRAM, 1965; HALEY et al., 2008b). So, the breathing rate and panting behaviour can be used as a stress indicaticator.

2.4.1.4. Body temperature

Body temperature is an indirect indicator of the metabolic rate (WEBB, 1995) that provides information about welfare status due to its impacts on pigs' physiology. Thermoregulation is controlled by the hypothalamus that acts as a thermostat (CHARKOUDIAN, 2003; CAMPBELL, 2011) and is fundamental to maintenance of homeostasis (TERRIEN et al., 2011). The stress response increases body temperature due to the stimulation of the autonomic nervous system by the hypothalamus, that induces changes in the peripheral vascular tone and blood flow on the animals' body (BLESSING, 2003; MITCHELL, 2013). During a stressful situation, there is an activation of HPA axis that results in alterations in heat production and dissipation by increasing the blood catecholamines and cortisol levels, and blood flow (SCHAEFER et al., 2002).

In heat stress, the blood flow to skin increases to 6 to 8 L/min due to a peripheral vasodilatation caused by the sympathetic vasodilator system (JOHNSON; PROPPE, 1996; as cited by CHARKOUDIAN, 2003). While in cold stress, the sympathetic vasoconstrictor system reduces blood flow to skin by shifting blood flow from the periphery to deep veins (ROWELL, 1983), which decreases the heat dissipation (CHARKOUDIAN, 2003).

The thermal equilibrium of pigs result comes from the balance between heat production and heat loss. Basically, in animals, this control is done by sweating (heat stress) and shivering (cold stress; CHARKOUDIAN, 2003). However, as pigs do not have functional sweat glands, the thermal regulation of pigs under heat stress mostly depends on wallowing, thermal panting (evaporative cooling) and heat dissipation (peripheral blood flow; INGRAM, 1965). Thermal stress is associated with other variables, such as: heart rate, rectal temperature, blood pressure and urinary excretion (PATIENCE et al., 2003).

2.5. Animal losses

Losses of pigs during or after transport are not only indicative of a welfare problem, but also represent an economical loss for the pork production chain (SUTHERLAND et al., 2008; RITTER et al., 2009; DALLA COSTA; DALLA COSTA, 2014b). Transport losses are defined on arrival at the plant as dead-on-arrival (DOA), as non-ambulatory injured pigs (NAI), and non-ambulatory non-injured pigs (NANI; SUTHERLAND et al., 2008; RITTER et al., 2009). While NAI pigs are easily identified by a body injury (e.g. lameness due to broken leg or claw problem), NANI pigs present signs of acute stress, such as difficulty in moving, open-mouth breathing, skin discoloration, muscle tremors and abnormal vocalizations (ANDERSON et al., 2002; ELLIS et al., 2003).

The incidence of transport losses can be affected by several factors, such as poor preparation of pigs on farm (no or too short fasting interval prior to transport), handling at loading and unloading at the slaughterhouse (i.e. use of electric prods;

PEETERS et al., 2004; CORREA et al., 2010), environmental conditions (CLARK, 1979; TARRANT, 1989), health problems (TARRANT, 1989), and transport conditions (TARRANT, 1989; WERNER; REINERS; WICKE, 2005; RITTER et al., 2008). The proportion of fatigued pigs is greater during the winter, while dead pigs represent the greatest portion of total losses during summer (FITZERLAND et al. 2009). The author also found an increase of 7.55-fold in total losses when transport density was changed from the minimum (212.38 kg/m^2) to the maximum (338.64 kg/m^2) density. Similarly, Ritter et al. (2006) found that total of injured pigs, fatigued pigs, and total losses were reduced by 0.35, 0.37, and 0.52%, respectively, when transport density was reduced from 0.48 to 0.39 m^2/pig.

Some potential causes of pigs dead-on-arrival are animal genetics, slaughter weight and increased production size (TARRANT, 1989; MURRAY; JOHNSON, 1998; WARRISS, 1998; ELLIS et al., 2003; WERNER; REINERS; WICKE, 2005; VECEREK et al., 2006). Fatigued pigs display signals such as open-mouth breathing, skin discoloration, muscle tremors, and abnormal vocalizations (ANDERSON et al., 2002), which are characteristic of acute-stress. A positive association (r = 0.81) between dead and non-ambulatory pigs (HAMILTON et al., 2003) suggests a common cause for dead and non-ambulatory pigs (RITTER et al., 2009).

Fasting the pigs before loading reduce losses during transport (SMITH, 1937; GISPERT et al., 2000; RITTER, 2007; AVERÓS et al., 2008). Pigs transported with full stomach can suffer motion sickness and vomiting (BRADSHAW et al., 1996; GRANDIN, 2014). In this case, their cortisol levels are raised resulting from travel sickness (GISPERT et al., 2000). Áverós et al. (2008) reported a double risk of mortality (0.3 vs. 0.54%) for not fasted pigs before loading (0 vs. <12; 12-18; >18 h). A feed withdrawal of 12-18 h before loading is considered suitable to prevent transport losses and meat quality defect (WARRISS, 1985, 1992; CHEVILLON, 1994; GUÀRDIA et al. 2004, 2005).

2.6. Carcass quality

2.6.1. Carcass yield

Some parameters such as carcass weight, fat depth, muscle depth, length, shape, distribution of joints, sex, and presence of skin lesions have to be considered to estimate carcass quality. The carcass yield is calculated according to the proportion between the weight of the carcass life weight (WARRISS, 2010a), such as the equation:

$$\text{Carcass Yield} = (\text{Carcass weight/live weight}) \times 100.$$

Carcass yield is a useful measurement for pork carcass grade, which is estimated to be around 75% (FORTIN et al., 2003; POMAR et al., 2008; WARRISS, 2010a).

The carcass yield can be affected by fasting time (ELLIS, 1998; BEATTIE, et al., 2002; KEPHART; MILLS, 2005; FAUCITANO et al., 2006; Rodriguez et al. 2008; Faucitano et al. 2010a; PANELLA-RIERA et al., 2012), feed texture and meal frequency (FAUCITANO et al., 2006). The fasting time increase cold killing-out mainly due to a reduction in gut fill and offal weight (ELLIS, 1998; BEATTIE et al., 2002).

In the carcass, the proportion of tissues of interest is determined by the lean yield, which is calculated by dividing the weight of the tissues of interest by the overall or partial carcass weight (POMAR et al., 2008). In commercial conditions, the lean yield trait is estimated according to subjective (visual) or objective (caliper and optical probes) carcass measurements (KAUFFMAN; WARNER, 1993). In Canada, it is estimated by measuring fat thickness and muscle depth at the ¾ last ribs using an optical probe (FORTIN et al., 2003). Lean yield is mostly affected by genetics (SELLIER, 1998; GISPERT et al., 2007), nutrition (ANDERSEN, 2000) and sex (LATORRE et al., 2003; 2004).

The live weight loss of pigs withdrawn of feed preslaughter may reach up to 5% in the first 24 h corresponding to around 5 kg body weight loss at an approximately rate of 0.2% per hour, or 0.25 kg/h (PELOSO, 2001). Carcass weight does not appear to be affected during the first 24 h as live weight losses are more associated to the excretion of urine and feces than to utilisation of body tissues (BEATTIE et al., 2002). Chevillon, Dubois and Vautier (2006) only found a significant effect of feed withdrawal on carcass weight loss from 24 h fasting on with losses being estimated at approximately 100 g/h starting (CHEVILLON, 2001) corresponding to 1% decrease in carcass yield (FAUCITANO et al., 2006).

The small reduction in carcass weight (360 g/pig) and yield (0.33%) observed after 24 h fasting (CHEVILLON et al., 2006) corresponds to the removal of one meal (1.3-2 kg of feed) per day (KEPHART; MILLS, 2005; FAUCITANO et al., 2010). This feed cost can be thus saved by the producer as feed ingested by pigs in the last 10 h won't be converted into muscle tissue and carcass weight. Indeed, after its intake feed takes 4 h to 8 h to be absorbed in the small intestine and most of its nutrients take 9 h before being released into the blood circulation (WARRISS, 1985).

However, other studies failed to find effects on carcass weight losses even after 60 h feed withdrawal (ELLIS, 1998; TURGEON, 2003). The reason for this discrepancy in the results may be the differences in preslaughter stress levels, genotype or slaughter weight between studies (FAUCITANO et al., 2010a). According to Warriss (1985), transport to slaughter may contribute more to the live weight weight loss rate than fasting alone. Furthermore, researches showed that the recommended 24 h maximum fasting interval does not truly apply to pigs with high body energy stores (muscle glycolytic potential), such as pigs carrying the Hal^n or RN^- gene (MURRAY et al., 1989; BIDNER et al., 1999, 2004), and heavy pigs (> 170 kg; NANNI COSTA et al., 2002), for which a feed withdrawal time between 30 and 48 h appears more appropriate to reduce the risk of PSE pork production at no detriment for carcass weight or yield (MURRAY et al., 1989; LO FIEGO et al., 1997; BIDNER et al., 1999, 2004).

The nutritional condition of pigs can also influence weight losses during fasting time. Studying the effects of feed withdrawal time (4, 14 or 24 h), meal frequency (2 vs. 5 meals per day) and feed type (mash vs. pellet), Faucitano et al. (2006) reported a higher carcass yield in pigs fed a pelleted diet compared with a mash one (81.2 vs 79.3%), and in pigs fed 5 meals/day (80.7 vs 79.8%), regardless of the pre-slaughter feed withdrawal time applied. These findings were attributed to the differences in stomachs weight removed on the dressing line.

2.6.2. Skin lesions

Skin lesions can be classified as superficial (scratches) or deep (bruises). Bruises are formed by the blood leaked from capillaries and trapped under the skin (ROBIN et al., 2015).

The presence of skin lesions is an important carcass quality trait for the market resulting in up to 6% carcass downgrading (MLC, 1985). According to North American pork industry estimates (VANSICKLE, 2002; SCHULTZ-KASTER; HILL, 2006; RIENDEAU, 2011), lesions alone contribute from $0.08 to $0.44 value loss per carcass. Their assessment also provides valuable information about animal welfare during preslaughter period (DALMAU et al., 2009; EUROPEAN FOOD SAFETY AUTHORITY, 2012) in which the most common sources of skin lesions are fighting (MOSS, 1978; GUISE; PENNY, 1989), rough handling (GEVERINK et al., 1996; RABASTE et al., 2007; FAUCITANO et al., 2008) and high density (GUISE; PENNY, 1989; BARTON-GADE; CHRISTENSEN, 1998).

The degree of carcass skin lesions is measured subjectively using a pictorial chart, either providing a general score based on the carcass appearance (1 = none to 5 = severe; MLC, 1985; Figure 4) or counting the bruises by anatomical location (ITP, 1996; BARTON-GADE et al., 1996a; Welfare Quality, 2009), and defining their shape to identify the infliction source (e.g., fighting, handling and overcrowding; ITP, 1996). Information about the bruise infliction can be taken by bruises' colour, with red bruises being the most recent ones (FAUCITANO, 2001).

2.7. Conversion of muscle into meat

The muscles still metabolise energy, contract and produce heat after death. However, oxygen is not delivered to the cells and the metabolic end products are not removed, because there is no blood circulation (GREGORY, 1998). Under anaerobic conditions, lactic acid is produced from glucose either naturally present or derived from muscle glycogen to produce adenosine triphosphate (ATP; GREGORY, 1998; HUFF-LONERGAN; LONERGAN, 2005). The glycolysis process stops with the complete depletion of glycogen or, the accumulation of lactic acid when a very low pH is reached (BOWKER et al., 2000; POSO; PUOLANNE, 2005). During this stage, the lactic acid accumulated in muscle decreases pH from a near neutral pH of 6.8-7.2 to about 5.6-5.8 (Figure 4).

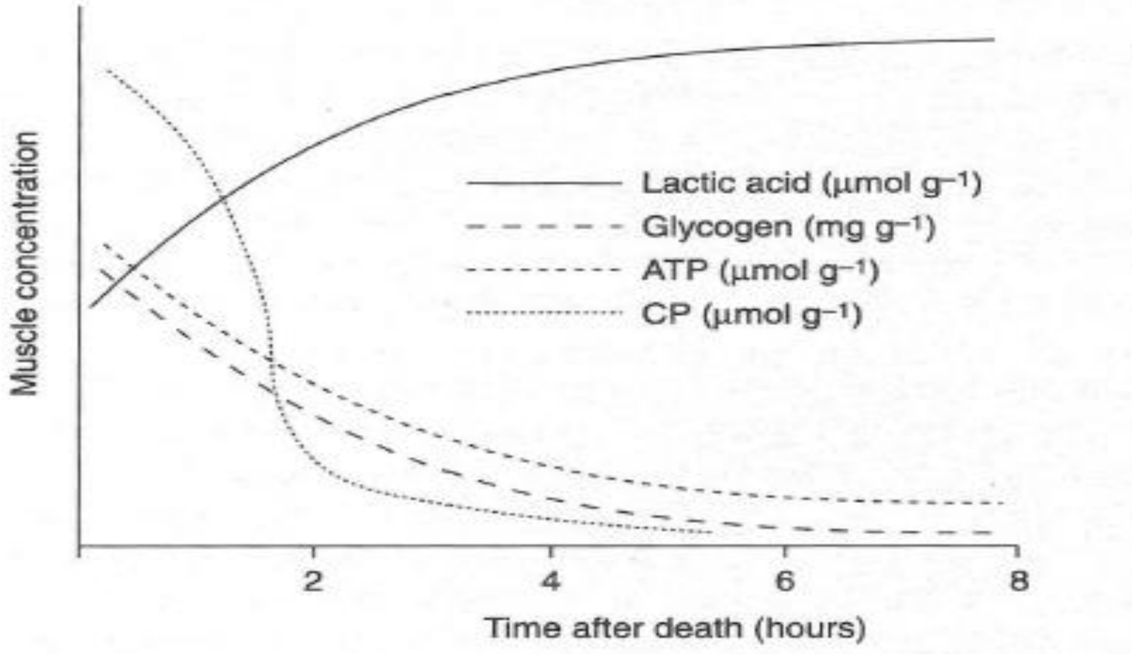

Figure 3. Changes in glycogen, phosphocreatine (PC), ATP, and lactic acid concentrations during *post-mortem* metabolism (TARRANT; McLOUGHLIN; HARRINGTON, 1972).

The increased acidity causes a reduction in water binding ability and calcium release triggering muscle contraction. The cells do not have energy left and the metabolism stops at this stage. The intracellular pH arrives at its final level (GREGORY, 1998; BOWKER et. al., 2000; RYU; CHOI; KIM, 2005). Rigor mortis is a muscle state achieved when ATP is depleted being impossible to expulse Ca^{2+} from the sarcoplasm and depicts the beginning of permanent acto-myosin crossbridges (GREGORY, 1998; BOWKER et al., 2000).

2.8. Meat quality

Meat is defined as the final result of a sequence of biochemical and physical events during the conversion process from muscle into meat in the *post-mortem* period, which takes from 1 day to 2 weeks (HONIKEL, 2004). Furthermore, 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.

The following four factors can be associated with the abnormal *post-mortem* metabolism and meat quality variation: 1) the Ca^{2+} regulation; 2) the muscle ATPase activity; 3) glycogenolytic enzymes activity; and 4) muscle glycogen levels regulation. These factors in combination with muscle temperature changes influence the *post-mortem* glycolysis affecting pH fall rate and other meat quality traits, such as colour and water-holding capacity.

2.8.1. Meat pH

In normal conditions, during the conversion of muscle into meat muscle pH declines gradually from approximately a value of 7.0 to around 6.1 within 1 h after slaughter due to lactic acid accumulation (LAWRIE, 1966; BENDALL, 1973). It finally achieves the ultimate value (pH_u) of 5.6-5.8 at 24 h *post-mortem* (SELLIER; MONIN, 1994).

However, *post-mortem* pH can vary depending on the glycogen content in the muscle at the time of slaughter. High levels of glycogen just prior to slaughter lead to higher lactic acid concentration in the muscle producing an approximately three-fold more rapid than normal pH decline (lower than 6 at 45 min *post-mortem*; WARRISS; BROWN, 1987; KŌHLER, 2001). This rapid pH fall combined with high muscle temperature (35-38^{o}C), denatures myofibrillar proteins leading to a loss of their water binding capacity and PSE pork (GREGORY, 1998; NICOLAIEWSKY, 1998, TERRA, 1998).

When pigs are submitted to prolonged stress just before slaughter, because their glycogen reserves will be low at the time of slaughter, the muscle will not have enough substrate to produce lactic acid during the conversion of muscle into meat, resulting in higher ultimate pH (pH_u) than 6, which is indicative of the DFD meat quality defect (TARRANT, 1989; CARRAGHER; MATTHEWS, 1996; GREGORY, 1998).

Feed withdrawal before slaughter is instrumental for raising muscle ultimate pH through the consumption of glycogen stores in the muscle (WARRISS, 1982;

JONES; ROMPALA; HAWORTH, 1985; EIKELENBOOM et al., 1991; MURRAY; JONES, 1994; TURGEON, 2003; GUÀRDIA et al., 2004, 2005; FAUCITANO et al., 2006; DALLA COSTA et al., 2010) resulting in improved water-holding capacity and colour (EIKELENBOOM et al., 1991; MURRAY; JONES, 1994; GISPERT et al., 2000; GUÀRDIA et al., 2005).

Faucitano et al. (2006) showed that 24 h feed withdrawal may increase the ultimate pH of 0.07 units in the *longissimus* muscle compared with 14 h. However, long fasting periods (> 22 h) may also increase the prevalence of DFD pork due to muscle glycogen exhaustion (EIKELENBOOM et al., 1991; GISPERT et al., 2000; GUÀRDIA et al., 2005). Whereas, short fasting periods may increase the risk of PSE pork production at a fasting time below 18 h (GUÀRDIA et al., 2004). However, other studies report little or no effects of preslaughter fasting on the ultimate pH value (DE SMET et al., 1996; MURRAY et al., 2001; BEATTIE et al., 2002; MORROW et al., 2002). The discrepancy in these findings indicates that the effects of feed withdrawal on pork ultimate pH are dependent on the pre-slaughter handling practices (i.e. mixing, long transport or lairage time) which are applied differently between studies (FERNANDEZ; TORNBERG, 1991; LEHESKA et al., 2003; BERTOL et al., 2005; FAUCITANO et al., 2006, 2010a) and the genotype (DE SMET et al., 1996). These effects are also muscle type-dependent, with higher ultimate pH value being more easily observable in locomotors muscles (i.e. shoulder and ham muscles) having a lower glycolytic potential (lower glycogen stores) and getting fatigued more rapidly (HAMBRECHT et al., 2005a; ROCHA et al., 2015).

2.8.2. Meat colour

Meat colour is one of the most valuable meat quality properties for the pork industry (WARNER; KAUFFMAN; RUSSEL, 1993) as it may influence decision making of consumers at the retail level (BREWER; McKEITH, 1999; ANDERSEN; OKSBJERG; THERKILDSEN, 2005; GRUNERT, 2005).

Colour in meat is a perception of its optical properties. Meat is a translucent material (allowing light to pass through it). Therefore, light cast interact with the meat and part of it is absorbed and part scattered (WARRISS, 2010a). Absorption is mainly influenced by the concentration and state of pigments, mainly myoglobin (WARNER, 1994; MANCINI; HUNT, 2005). A higher concentration of myoglobin produces a brighter red pork colour (YOUNG; WEST, 2001). The myoglobin forms are dependent from the availability of oxygen and the oxidation process. Myoglobin exists in three-forms: purple-red deoxymyoglobin in the absence of oxygen, bright red oxymyoglobin formed in the presence of air and brown metmyoglobin, resulting from myoglobin oxidation (TERRA, 1998). When freshly cut meat is exposed to air, the oxygenation or “blooming” takes 10-30 min resulting from the oxygen binding to myoglobin (CORNFORTH; JAYASINGH, 2004).

The structural properties in the superficial layers of the muscle influence the light scattering characteristics of meat. In meat, light scattering originates at the boundary between myofibrils and the sarcoplasm, and within the myofilament lattice of the myofibril, which happens due to instability of the sarcoplasmic and myofibrillar proteins (myosin). The aforementioned *post-mortem* muscle protein denaturation, in fact, leads to a “white precipitate” of denatured sarcoplasmic proteins accumulated on the myofibrils which disguise the red colour and make a semi-opaque background (JOO et al., 1999) reducing light diffusion into the material (MANCINI; HUNT, 2005). Due to that, there is a lower light absorption and a higher light reflection resulting in a lighter appearance producing PSE meat. Consequently, the light penetrates to a shallower depth and is adsorbed to a smaller extent by myoglobin. In PSE muscles, the increased scattered light occurs because of the reduced gaps between myofibrils. On the other hand, the dark red-pink colour of DFD pork is because the tissues are more translucent and scatter or reflect less light (BARTON-GADE; OLSSEN, 1987; BREWER, McKEITH, 1999). The higher pH of DFD pork enhances the darkness because of less free water available in the muscle due to more water still bound to proteins and, as a consequence, light scattering is reduced (BREWER et al. 2001).

Sources of variation of pork colour include genetic selection, nutrition, pre-slaughter handling, *post-mortem* carcass handling (i.e. cooling rate and technique), processing and holding times and temperature before packaging or further use, packaging, distribution and marketing, including lighting and other display conditions (MANCINI; HUNT, 2005).

The L*, a*, b* are coordinates of the tristimulus on the CIELAB colour space and can specify various colour space by determining the components of lightness and chromaticity. When colour is expressed in CIELAB, L* defines lightness, a* denotes red-greenness and b* yellow-blueness; CIELAB, 1976). Generally, these colour coordinates are measured by the Minolta Chromameter which evaluates the reflectance of the myoglobin and haemoglobin after exposure to oxygenation (BREWER et al., 2001). For instance, the greater the myoglobin concentration, the lower the lightness or L* value. Nevertheless, the myoglobin state affects the lightness ratios being higher for oxidised and lower for oxygenated state (FERNANDEZ-LOPEZ; PEREZ-ALVAREZ; SAYAS-BARBERA, 2000).

The consumer's colour perception at the retail level can be simulated by the subjective colour assessment using the Japanese Colour Scales (JCS; NAKAI et al., 1975). The JCS are six plastic disks (from 1 = very pale to 6 = very dark) with a meat-like appearance developed by using objective colorimetry.

2.8.3. Drip loss

Of all main muscle characteristics that affect appearance, shrinkage, processing properties, and palatability, drip loss is perhaps the most important for the meat industry economics and consumer perception (KAUFFMAN et al., 1986) due to loss of saleable weight and of a significant amount of proteins, water soluble vitamins and amino acids (112 mg of protein/mL of fluid; SAVAGE; WARRISS; JOLLEY, 1990).

Drip is defined as an aqueous solution purged from meat containing sarcoplasmic proteins, such as myoglobin which gives the characteristic red colour to it (SAVAGE et al., 1990).

Most animal muscles contain approximately 75% water held by 20% proteins. Most water (85%) is inside muscle cells, while the remaining 15% is located around them (HUFF-LONERGAN; LONERGAN, 2005). While half of the water contained in muscle cells is linked to muscle fiber proteins, the other half is linked to sarcoplasmic proteins. Water in the muscle presents a variable level of binding to the protein, with only 5% fully bound (HUFF-LONERGAN; LONERGAN, 2005). The mobility of water is restricted by capillary forces and osmotic pressure in the live animal at normal pH. However, as mentioned before, when the pH falls close to the isoelectric point (pH 5.0 - 5.1) of meat proteins there is a considerable reduction in their hydration and in the ability to hold water tightly (TOLDRA, 2003).

The lateral shrinkage of muscle fibres is the single most important factor for drip loss formation (OFFER; COUSINS, 1992). The shrinkage of myofibrils and denaturation of proteins causes a dislocation of water within or out of the fiber. During rigor development there will be less space available among the muscle fibers for the retention of immobilised water which becomes free and flows out the fibrils space around fibres and fibres bundles (OFFER et al., 1992).

As it depends on the rate of pH drop in early *post-mortem* muscle, drip loss rate is influenced by several factors such as genetics (FUJII et al., 1991; HUFF-LONERGAN; LONERGAN, 2005), animal and carcass handling (JENNEN et al., 2007), temperature management *post-mortem* (JENNEN et al., 2007), nutrition (LYNCH et al., 1998; D'SOUZA et al., 2005; JENNEN et al., 2007) and processing (JENNEN et al., 2007).

Drip loss can be assessed by methodologies based on fluid absorption, such as the filter paper (KAUFFMAN et al., 1986), gravity, such as the bag and EZ-drip loss methods (HONIKEL, 1998; CHRISTENSEN, 2003; CORREA et al., 2007), and pressure, such as the filter paper press (GRAU; HAMM, 1953).

2.9. Meat quality categories

Pork meat was initially divided into three quality categories based on pH, colour and drip loss variation, such as: normal (or RFN: red, firm, non-exudative), PSE and DFD pork. Pork classified as PSE has distinctly reduced processing and cooking yields (BOLES et al., 1991; PERSON et al., 2005), as well as decreased tenderness and juiciness (BENNETT et al., 1973). Pork classified as DFD appears very dark and sticky due to the self-cohesiveness of the myofibrils and their high water-binding capacity (FISHER, 2005). Furthermore, the high pH value, which is conducive to bacterial growth, results in shorter shelf-life for this meat type (FISCHER, 2005; HOLMER et al., 2009; FAUCITANO et al., 2010b).

PSE meat has been described as having an initial pH lower than 5.8 (SELLIER et al., 1994), pHu value between 5.5 and 5.7 (SELLIER et al., 1994), drip loss higher than 5% (WARNER; KAUFFMAN; GREASER, 1997; JOO et al., 1999) and Minolta colour L* value higher than 50. Whereas, DFD pork is described as having a high pHu (> 6.0), very low drip loss (< 2%) and colour L* values lower than 42 (WARNER, 1994; CORREA et al., 2007).

PSE and DFD conditions are commonly considered as a stress-related muscle disorders. The occurrence of PSE pork has been associated to animal exposure to acute stress just before slaughter, such as the use of electric prods, no or too short fasting duration or animal genetic background (Hal gene). Whereas, exposing animals to a long period of stress, such as long fasting periods combined with long transportation and lairage time and mixing cause the depletion of muscle glycogen reserves and may increase the incidence of DFD pork (VAN DE PERRE et al., 2010; WARRISS, 2010b).

However, two new quality categories have been added for a more reliable quality assessment considering the variation in either colour or drip loss, namely RSE (reddish-pink, firm, exudative) and PFN (pale, firm, non-exudative) pork (WARNER et al., 1993, 1997; VAN LAACK et al., 1994; Table 1).

Table 1. Pork quality based on parameters of ultimate pH (pHu), drip loss (DL), subjective colour (JCS), and objective colour (L*) (CORREA et al., 2007)

Quality classes[1]	**pHu**	**DL**	**JCS**[2]	**L***
PSE	<5,5	> 5%	1 – 1,5	>50
Moderate PSE	5,5 – 5,6	> 5%	2 – 3	≥ 50
PFN	5,5 - 5,8	< 5%	<3	> 50
RSE	5,6 - 5,8	>5%	3	42 – 50
RFN	5,6 - 5,8	2 – 5%	3	42 – 50
Moderate DFD	5,8 – 6,1	< 5%	3 – 4	42 – 45
DFD	> 6,1	<2%	≥ 4	≤ 42

[1] PSE (pale, soft, exudative), PFN (pale, firm, non-exudative), RSE (red, soft, exudative), RFN (red, firm, non-exudative), DFD (dark, firm, dry)
[2] Japanese Colour Standards from 1= pale to 6 = dark (NAKAI et al., 1975)

PFN pork has normal structure (firmness) and pH_u (5.5 – 5.8), but shows a paler colour, with an L* value as low as the PSE meat (> 50). The RSE meat is characterised by a normal pH_u value (between 5.6 and 5.8) and a reddish-pink colour (L* between 42 and 50), but a drip loss as high as in the PSE meat class.

The PFN and RSE meat quality classes have been recently reported as major quality defects in Canada, representing more than 13% of all defects comparing to PSE (13%) and DFD pork (10%; MURRAY et al., 2001). Additionally, Faucitano et al. (2010b) reported that 47% of meat quality defects were classified as RSE meat, whereas PSE and DFD represented only 21 and 2%, respectively. However, despite the economic importance, the causes of RSE meat occurrence remain unknown (VAN LAACK et al., 1994).

2.10. The effects of preslaughter handling practices on animal welfare and meat quality

2.10.1. Preslaughter feed withdrawal

Feed withdrawal is a common practice for on-farm preparation before transport that is regulated by codes of practice (AAFC, 1993). It consists in removing the access to feed, while keeping free access to water, during a defined interval prior to slaughter. Communication between producer and slaughter plant is necessary to inform the loading time one day before loading and to prepare the animals to loading. Total feed withdrawal interval before slaughter has to be planned considering the on-farm feed withdrawal interval, time spent during loading, transport and unloading, and lairage period (DALLA COSTA; DALLA COSTA, 2014a).

Starting feed withdrawal time at the farm may result in economic benefits for the pork chain, such as feed saving (KEPHART; MILLS, 2005), better pigs' welfare during transport (BRADSHAW et al., 1996; GUÀRDIA et al., 2005; CORREA, 2011) resulting in decreased animal losses (GISPERT et al., 2000; AVERÓS et al., 2008), reduced carcass contamination due to a lower risk of gut contents spillage during carcass evisceration (FAUCITANO et al., 2010a) and improved pork quality due to greater easiness of handling (EIKELENBOOM; HOVING-BOLINK; SYBESMA, 1991; GUÀRDIA et al., 2004, 2005). It also helps reduce wastes to be disposed of at the slaughter plant (MURRAY et al., 2001).

Overall, recommendations about the ideal duration of fasting preslaughter from 5 h in Canada (AAFC, 1993) to 48 h in USA (MILLER et al*.,* 1997). Table 2 shows some feed withdrawal intervals recommended in different countries. Generally, a feed withdrawal of 12 hours is applied on-farm in Brazil. The Brazilian legislation recommends a feed withdrawal between 8-24 hours at the slaughter plan (INSTRUÇÃO NORMATIVA nº 3/2000; PORTARIA Nº 711). However, based on a review of literature reports, Faucitano et al. (2010a) suggest a period of 24 h between the last meal and slaughter as an acceptable compromise to obtain optimal carcass yield and pork quality.

Table 2. Feed withdrawal interval applied in different countries (FAUCITANO, 2007; DALLA COSTA; DALLA COSTA, 2014a)

Country	On-farm (hours)	Total (hours)
United Kingdom	4-8	18
Brazil	-	24
European Community	10	-
Canada	5	24
France	12-18	24-28

2.10.2. Physiological parameters

During preslaughter handling, Dalla Costa et al. (2008) reported an increase of 2.67 times in salivary cortisol level, rising from 0.267 on-farm to 0.714 after transport and unloading (Figure 3).

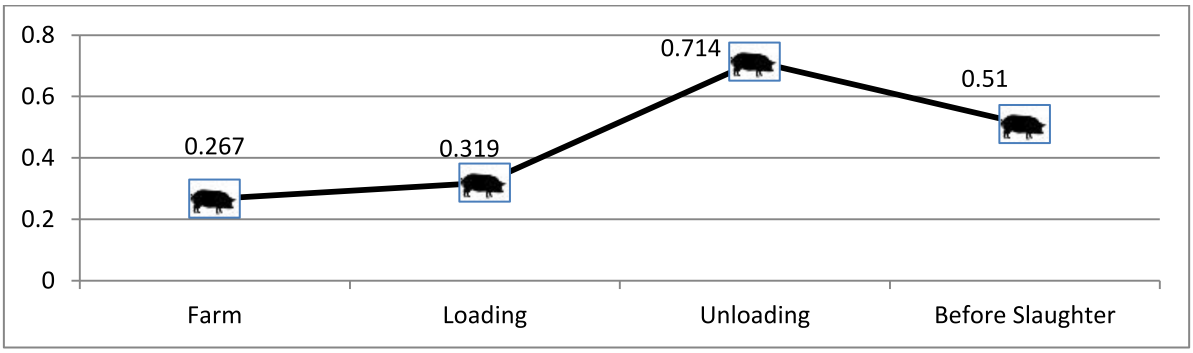

Figure 4. Variation in salivary cortisol levels (µg/dL) of pigs during preslaughter handling (adapted from DALLA COSTA et al., 2008).

Feed withdrawal period may influence cortisol levels in blood (PARROTT; MISSON, 1989; GISPERT et al., 2000; FAUCITANO et al., 2006). However, the literature results are contradictory. Gispert et al. (2000) reported lower level of cortisol in blood of pigs fasted on-farm from 12 to 18 h than those fasted for <12 h or >18 h. Whereas, other studies found increased blood cortisol levels starting from 15 h of fasting (HOUPT et al., 1983; PARROT; MISSON, 1989). Faucitano et al. (2006) also found higher cortisol level in the urine of pigs fasted for 14 h compared with pigs fasted for 4 and 24 h. It is hard to explain how feed withdrawal affects the elevation of cortisol levels, as the effects are multifactorial (VON MICKWITZ, 1982), with cortisol levels being also affected by travel sickness during transport (BRADSHAW et al., 1996) and fighting during lairage (GISPERT et al., 2000), both responses due to increased demand for energy supply (Von MICKWITZ, 1982).

The concentrations of blood lactate and CK do not seem to affect by fasting time (WARRISS et al., 1992; GISPERT et al., 2000). Leheska, Wulf and Maddock (2003) found no effect of fasting (48-h prior to harvest) or no fasting of pigs on lactate levels. Gispert et al. (2000) and Dalla Costa et al. (2008) reported no effects of fasting during on-farm feed withdrawal (< 12, 12-18 and > 18 h; 9, 12, 15 and 18 h, respectively) on lactate and CK levels. Edwards et al. (2010) reported an increase in lactate level prior to loading and transport from 4.3±0.3 mM (basal level) to 7.4±0.4 mM after pre-slaughter handling, movement, and stunning.

2.11. Loading

Loading pigs onto the truck at the farm represents the greatest challenge pigs have to cope with preslaughter and also plays an important role in the pork industry economics because of its impact on animal losses, carcass condemnation and meat quality variation (WARRISS, 1998; GARCIA; McGLONE, 2014).

The stressfulness of the loading procedure results from the combination of different factors, such as group splitting in the finishing pen, distance moved from the pen to the load point, group size, handling system and eventually the design of the loading device (either ramp or quay).

The effects of loading procedures are included in those of the farm of origin, which has been showed to be a major contributor for animal losses during transportation, general stress response and carcass and meat quality variation (BROWN et al., 1999; DALLA COSTA et al., 2007; DEWEY et al., 2009; FITZGERALD et al., 2009).

2.11.1. Exiting the farm gate

Based on heart rate and blood cortisol variation, sorting pigs in the fattening pen and moving them to the loading area are the first intense stress and physical efforts which animals are exposed to during the loading process (BRADSHAW et al., 1996; CORREA et al., 2010; 2013). The major factors triggering this physiological response are group splitting in the finishing pen, walking a distance through the farm alley and interaction with the handler through narrow and enclosed spaces (handling).

Sorting pigs out of a pen can be very stressful to the group of pigs in the pen as it involves a close human-animal interaction and animal separation from the group (WAIBLINGER et al., 2006; JOHNSON et al., 2013). Group splitting at the time of loading is necessary to handle small groups of pigs through the alleys or to ship groups of uniform weight (split-marketing). Split marketing is very common at finishing farms in North America and in some European countries, such as France and Spain. It implies the removal of the heaviest 25 to 50% of pigs from a pen to market them 1-2 weeks earlier than the other pen-mates. The advantage of this practice is the reduction of production costs and the opportunity to ship batches with a more uniform market weight to the abattoir (SCROGGS et al., 2002).

In large swine units, animals are often imposed to walk long distances to get to the loading area. According to Ritter et al. (2008), the average distance over which pigs are moved to reach the loading area may be greater than 100 m. This situation may result in muscle fatigue during loading and transportation (RITTER et al., 2008). An increased frequency of open-mouth breathing and skin discoloration (signs of acute stress) at loading at the farm and at unloading at the slaughter plant have been reported in pigs moved over a long distance (up to 91 m) compared with those moved over a short one (< 24 m) to reach the loading area (RITTER et al., 2007, 2008). Therefore, the distance to the loading area should be minimized as much as possible (FAUCITANO, 1998) by using, for example, shipping rooms. Besides being a recommended practice for biosecurity and feed withdrawal control (CHEVILLON, 2005; CARR, 2006; FAUCITANO et al., 2010a), moving pigs to shipping pens improves animal welfare pre- and during transport. A 2 to 18 h wait in the shipping pen prior to loading was reported to reduce loading time, and pigs' heart rate and fatigue at loading, all resulting in 25% lower mortality rate during transport (CHEVILLON, 2005; GEESING et al., 2010).

At loading, pigs are usually moved using plastic sorting paddles and board, electric prods or flags. However, these tools do not have the same efficiency and the same effects on the behaviour and physiology of pigs during handling. Some authors describe the use of electric prods as being as stressful as the loading procedure (BECKER et al., 1985) and more aversive than 90% CO_2 gas inhalation (JONGMAN et al., 2000). Regulations and codes of practice recommend to limit, if not to avoid, the use of electric prods for pig handling at any stage of the preslaughter period (EC, 1993, 2002; CARC, 2001) because they increase moving time and animal losses (BENJAMIN et al., 2001; CORREA et al., 2010) besides making handling more difficult (BRUNDIGE et al., 1998; MCGLONE et al., 2004). They also produce a negative physiological (higher heart rate and blood lactate level) and behavioural (falls, slips, vocalization, overlaps, etc.) response (BRUNDIGE et al., 1998; BENJAMIN et al., 2001; BERTOL et al., 2002, 2005; HAMILTON et al., 2004; CORREA et al., 2010, EDWARDS et al., 2010), eventually resulting in poor pork quality (JONGMAN et al., 2000; CORREA et al., 2010). As Grandin (2001) points out, electric prods should be used only as last choice, and only when a fit animal refuses to move forward. Its abusive use may reflect the handler's lack of experience or training (FAUCITANO, 2001; CORREA et al., 2010; ROCHA et al., 2015) or the poor facilities design (DRIESSEN; GEERS, 2000; TORREY et al., 2013b).

Flags, paddles and plastic boards can be considered good alternatives to the use of electric prods. McGlone et al. (2004) compared the efficiency and effects of these tools, and concluded that the sorting board and the flag were the most efficient devices for moving pigs because they appear as solid and blocking walls. Whereas, when comparing the use of plastic boards combined with paddle and compressed air prod along the alley and the loading ramp, Correa et al. (2010) reported a similar efficiency between these two handling tools, although the use of compressed air prod increased loading time due to increased behaviour of 180^{o} turns, likely caused by the noise of the air compressor.

The correct choice of group size of pigs to move forward guarantees the smooth flow of pigs through the alleys and ramps, without stressing the animal and reducing the workload for handlers. Lewis and McGlone (2007) and Berry et al. (2009) reported a shorter loading time, a lower increase in heart rate and reduced animal losses on arrival at the plant when pigs were handled in groups of 5 to 6 compared to smaller or larger groups (< 5 or > 6 pigs).

2.11.2. Loading onto the truck

Although widely used, loading ramps may be considered detrimental to the welfare of pigs if they do not meet some specific criteria (BROWN et al., 2005; KEPHART et al., 2010). Pigs showed to have difficulties, as showed by increased heart rate and slower pace, when negotiating steep ramps > 20^{o} (van PUTTEN; ELSHOF, 1978; WARRISS et al., 1991).

The use of a staircase, including 8 cm high and 30 cm long steps, may be an alternative to reduce the slope of the ramp as it reduces slipping when the ramp is wet (i.e. presence of feces and urine; Grandin, 2000, 2008). Little spaced (15 cm) cleats also reduce slipperiness, especially on steep ramps, allowing an easier and safer movement of animals (WARRISS et al., 1991).

Given the difficult handling through ramps, alternative systems, such as a truck tailgate lift, hydraulic deck, and gantry have been developed and tested for their efficiency and effects on pig welfare at loading (CHRISTENSEN et al., 1994; BROWN et al., 2005; BERRY et al., 2012; CORREA et al., 2013; WESCHENFELDER et al., 2012, 2013). When compared to the ramp, the tail gate lift system showed to reduce the need of electric prods during handling (GRANDIN, 1991). However, other studies failed to find differences in heart rate, body temperature, blood cortisol concentration and meat quality in pigs loaded with a tailgate lift compared to pigs loaded with ramps (NANNI COSTA et al., 1999, 2002;

BROWN et al., 2005).

The loading gantry has also been tested as a promising alternative. It consists of an aluminum covered chute (7°slope) with two dual pivoting extension systems that allow proper positioning to both the barn and trailer. Berry et al. (2012) reported that when compared to a regular (19° slope) loading ramp, the use of the loading gantry reduced the electric prod use and produced fewer overlaps, slips, falls and balking incidences, pig vocalizations and transport losses (0.6 vs. 1%; $P = 0.03$).

2.12. Transporting pigs

Transportation is considered one of the most stressful event for pigs prior to slaughter (GEVERINK et al., 1998b) which may compromise pigs' welfare and pork quality (WARRISS; 1998, VON BORELL; SCHAFFER 2005; SCHWARTZKOPF-GENSEWIN et al., 2012). Major sources of stress during transport are ambient conditions (season of the year), vehicle design and loading density.

Season can affect the welfare of pigs during transport. Transport mortality rate and the percentage of panting pigs on arrival at the plant are reported to increase at temperatures above 20 and 17°C, respectively (SUTHERLAND et al., 2009; KEPHART et al., 2010). However, seasonal effects on total transport losses do not always correspond with increases in environmental temperature. Clark (1979) reported greater mortality losses in winter and Sutherland et al. (2009) found increased percentage of downers on arrival at the plant at ambient temperatures of 5°C and below.

2.12.1. Vehicle design

Vehicle design plays a major role in the incidence of animal losses during transport as reported in a recent Canadian survey where vehicle design was included in the 16% transporter contribution to the incidence of transport mortality rate (DEWEY et al., 2009).

The design of the truck used to transport pigs differs around the world, ranging from small and single-decked trucks to large and triple-decked punch-hole trailers, which can be equipped with fan-assisted ventilation depending on the geographic location and climate conditions. The results from comparative studies including trucks featuring ramps or hydraulic decks showed longer loading and unloading time, increased use of electric prods and higher proportion of animal losses in trailers equipped with internal ramps, such as the Canadian pot-belly (PB) trailer than in other vehicle types, equipped with hydraulic decks, such as a double-decked truck or a flat-deck trailer (ELLIS; RITTER, 2006; BARTON-GADE et al., 2007; RITTER et al., 2008; SUTHERLAND et al., 2009; KEPHART et al., 2010; CORREA et al., 2013).

The effects of vehicle design on animal welfare is also related to the variation in internal climate (temperature and humidity) resulting from differences in radiant heat load, the amount of water and heat produced by pigs, and the air flow pattern (LAMBOOIJ; SYBESMA, 1988). Heat stress is more important than cold stress, in terms of transport losses (SCHRAMA et al., 1996). Some trials conducted in Canada showed that the PB trailer was warmer than the flat-deck trailer either while stationary or moving due to the different pattern of the side openings and internal gates design (RITTER et al., 2008). Within the PB trailer, higher temperatures have been recorded in the front compartments of the middle and bottom deck (BROWN et al., 2011). The higher temperatures have been explained by the reduced ventilation (BROWN et al., 2011). Animal losses particularly increase when the vehicle is stationary under warm ambient conditions (RITTER et al., 2006) as in the absence of ventilation internal temperatures can be up to 9°C warmer than the external temperature (WESCHENFELDER et al., 2012; FOX et al., 2014). To improve the climate conditions in a stationary vehicle pigs should be cooled off by water sprinkling and/or fan-assisted ventilation (BROWN et al., 2011). Active ventilation contributes to reduce deaths during transport (NIELSEN, 1982). Whereas, when ambient temperature is above 20°C water sprinkling pigs for 5 min in a stationary truck before leaving the farm and during wait time at the plant proved to reduce drinking behaviour in lairage, physical fatigue at slaughter (lower blood lactate levels) and drip loss in pork meat (FOX et al., 2014; NANNONI et al., 2014). Christensen and Barton-Gade (1999) reported that when active ventilation and water misting/sprinkling are combined transport mortality rate can be significantly decreased as ventilation, besides removing the excessive humidity from the interior of the sprinkled truck, increases evaporative cooling in pigs.

The effects of vehicle design on pork quality are inconclusive. A higher incidence of PSE (pale, soft, exudative) pork was reported in pigs transported in European featuring fixed decks and ramps (GUÀRDIA et al., 2004; LAMMENS et al., 2007). Whereas, Correa et al. (2013) reported no effect on pork quality when comparing the PB trailer with a double-decked truck equipped with an upper moving deck during 2 h travel time. Weschenfelder et al. (2013) studied the pork quality variation in pigs either transported with the PB trailer or the flat-deck trailer featuring a middle and upper moving decks and found higher pH_u values in the hams of pigs being transported with the PB trailer but only after short distances (45 min).

2.12.2. Transport density

Transport density is the amount of space that pigs are provided on the truck during transportation. Even when loading densities are regulated by legislation, like in the case of the EU directive 95/29/EC, they are hardly met in practice as the chosen densities are frequently adjusted to transport conditions (weather and distance) and slaughter weight. In most EU countries and North America loading densities vary from 0.35 to 0.50 m^2/100 kg (WARRISS, 1998). Based on the measurements of the space needed for sternal recumbency, the minimum space required for slaughter pigs of 90-100 kg live-weight is equivalent to about 250 kg/m^2 (or 0.40 m^2; WARRISS, 1998; SCHWARTZKOPF-GENSWEIN et al., 2012).

High densities (< 0.40 m^2/100Kg) are described to increase temperature and decrease air circulation inside the truck, negatively affecting its microenvironment (WARRISS et al., 1998a). They also prevent pigs from lying down resulting in increased blood creatine kinase levels (GUISE; PENNY, 1989; LEE et al., 2000; RITTER et al., 2009; CHAI et al., 2010), rectal prolapses (GUISE; WARRISS, 1989), lameness (KEPHART et al., 2010), carcass bruises (NANNI COSTA et al., 1999) and meat quality defects (BARTON-GADE; CHRISTENSEN, 1998; CHAI et al., 2010). Whereas, lower densities (> 0.50 m^2/100 kg) result in fatigued pigs due the attempts to keep balance.

Transport floor space also has a major impact on dead and non-ambulatory pigs at the packing plant. Ritter et al. (2006, 2007) found increased mortality rate (0.08 to 0.27 %) at densities of 0.5 m^2/100 kg and 0.4 m^2/100 kg, respectively, and concluded that providing pigs (120 kg average weight) with 0.48 m^2/pig was the most effective practice to reduce transport losses.

However, some studies (KEPHART et al., 2010; PILCHER et al., 2011) reported that floor space (< 260 kg/m^2 and 0.415 m^2/pig, respectively) effects on the incidence of downer pigs can be exacerbated by short transportation time (< 2.5 h and < 40 min, respectively). Similar effects have been also reported on meat quality variation, with higher risk of PSE (pale, soft, exudative) pork occurring in short journeys (1 h) carried out at lower stocking densities (0.5 *vs* 0.25 m^2/100 kg; GUARDIA et al., 2004).

2.13. Unloading

On arrival at the plant, the primary recommendation is to begin unloading within 30 min and unloading to be completed within one hour to avoid heat and humidity rise inside the stationary truck and its negative consequences on animal welfare and meat quality of pigs, as previously described (AMI, 2012; DALLA COSTA; DALLA COSTA, 2014a). However, in commercial conditions, the waiting time to unload a truck after its arrival at the abattoir is very variable ranging from 5 min to 4 h (AALHUS et al., 1992; JONES, 1999). Research showed that as the waiting time increased by 30 min, the risk of pigs dying can increase by 2.2 times (HALEY et al., 2008a). Driessen and Geers (2001) also reported the greatest incidence of PSE pork after 30 min wait at 23ºC ambient temperature or after 53 min wait at 11ºC (70 and 45%, respectively). The presence of heat-stressed (or panting) pigs at unloading is a major risk factor for PSE pork production (VAN DER PERRE et al., 2010).

To meet this recommendation, a strict coordination of truck arrivals with the predicted number of pigs in lairage, lairage capacity and operation speed or the availability of a number of unloading docks allowing more than one truck to unload at the same time may shorten waiting times (WEYMAN, 1987).

If delay to unload is unavoidable, when pigs are waiting the truck, they must keep on moving, or sit in the shade or be cooled off using active ventilation and/or water sprinkling/misting in the truck (BENCH; SCHAEFER; FAUCITANO, 2008).

Although unloading can be considered less stressful than loading (based on heart rate and blood lactate level; CORREA et al., 2010; EDWARDS et al., 2010), poor handling resulting in carcass bruising and poor meat quality is inevitable at this stage, unless well-designed unloading facilities and trained personnel are used.

In practice the most common unloading device at the slaughter plant dock is the ramp, either combined to the truck ramp or lift. Keeping in mind that pigs have difficulties in descending a slope (BROWN et al., 2005), ramps steeper than 20º are not recommended as they result in greater pigs' heart rate, vocalization and backing up behaviour, increased handler interventions (voice and electric prods) and longer unloading time (WARRISS et al., 1991; RITTER et al., 2008; GOUMON et al., 2013; TORREY et al., 2013a, b). Higher pitched vocalization of pigs at unloading has been associated with greater risk of PSE pork production (van DER PERRE et al., 2010). The greater need for electric prod use observed on ramps may result in reduced easiness of handling (RABASTE et al., 2007) resulting in an higher incidence of fatigued pigs at unloading and lower pork quality (CORREA et al., 2013). When compared to ramps, the use of hydraulic lifts or decks reduces handling stress (lower heart rate), increases the easiness of handling and shortens off-load time (BROWN et al., 2005).

To ease handling of pigs, there should not be closed corner to negotiate, so they can move either when exiting the truck or unloading the dock. A greater heart rate and balking behaviour during unloading time have been reported in pigs facing angles or bends of 45° to 90° compared with 0° and 30° (WARRISS et al., 1992; GOUMON et al., 2013).

Handling problems due to hesitation and refusals of pigs to go forward resulting in greatest heart rate in pigs and handlers and greater number of pigs falling and slipping can also be caused by poor lighting, lack of shelter at the dock, the presence of a step (> 15 cm) or a gap between the truck deck floor and the unloading dock (SCAHAW, 2002; AMI, 2012; GOUMON et al., 2013) and the slippery ramp floor (TORREY et al., 2013b). Reducing the frequency of slips on the unloading ramp not only prevents injuries, but also improves pork quality (ROCHA et al., 2015).

2.14. Lairage

Other than feeding the slaughter line, lairage is important for welfare and meat quality reasons as it provides an opportunity for animals to recover from stress and fatigue with clear benefits for meat quality (WARRISS, 1987). Lairage time, handling, facility design (pens and alleys), environment, whether pigs are mixed or not are all related to the recovery rate of pigs and carcass and meat quality variation (WARRISS, 2003; FAUCITANO, 2010).

Under commercial conditions, the lairage time applied is very variable (from < 1 to 15 h), depending on the lairage area size and livestock delivery schedule (GEVERINK et al., 1996; GISPERT et al., 2000). In Brazil, the minimum recommended lairage time is 3 h (DALLA COSTA; DALLA COSTA, 2014b).

There is evidence that a lairage time of 2 h is a sufficient time for recovery as showed by the blood cortisol levels (WARRISS et al., 1992; PÈREZ et al., 2002) and results in pigs easier to move based on the lower frequency of electric prod use (MILLIGAN et al., 1998). No or short lairage (15 - 60 min) is not recommended as it results in higher 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; SHEN et al., 2006). Whereas, longer lairage time proved to increase blood lactate and creatine kinase CK levels, reduce the risk to produce PSE pork (2% at 10 h; GUÀRDIA et al., 2005; DALLA COSTA et al., 2009), but to decrease carcass weight and increase the percentage of DFD (dark, firm, dry) pork (WARRISS et al., 1998b; GISPERT et al., 2000). Guàrdia et al. (2005) reported a 12% risk to produce DFD pork after 3 h lairage reaching a 25% risk after overnight lairage. The increase in DFD pork proportion with lairage time is the result of reduced muscle glycogen content at slaughter caused by the combined effect of fasting and fighting (MOSS; ROBB, 1978; NIELSEN, 1982; DE SMET et al., 1996; WARRISS et al., 1998b; NANNI COSTA et al., 2002; GUÀRDIA et al., 2009).

Mixing unfamiliar pigs, which is a common practice at commercial slaughter plants, inevitably causes some fighting to establish a new hierarchy within the group

(BARTON-GADE, 2008; DEEN, 2010) which results in a significant rise in body temperature (lasting up to 8 h after mixing) and greater risk of DFD pork (WARRISS; BROWN, 1985). Several studies found a more extended fighting rate in pigs kept in lairage for longer periods (overnight to > 24 h vs. 3 h) as a result of mixing and feed withdrawal (MURRAY; JONES, 1994; WARRISS, et al., 1998a; DE JONG et al. 1999; GUÀRDIA et al., 2009). Brown et al. (1999) reported a greater activity (fighting and drinking) in lairage in pigs fasted for longer time (18 vs. < 1 h). Fernandez et al. (1995) also found a greater activity in fasted pigs (24 vs. 0 h) than fed ones. To limit fighting in lairage it is either recommended to keep pigs in smaller groups (15 - 40 pigs; GEVERINK et al., 1996; RABASTE et al., 2007) or to mix very large groups (up to 200 pigs; GRANDIN 1990; CHRISTENSEN; BARTON-GADE 1997) in the pen. However, several studies reported a greater activity and number of fights during lairage in larger groups (BRYANT; EWBANK, 1972; PETHERICK, 1983; GEVERINK et al., 1996). Basically, the behaviour of pigs in both small and large groups is influenced by the perception of pigs regarding group size and on the availability of resources (food, water, etc.) in the pen (TURNER et al., 1999). Moss (1978) reported a greater incidence of fights in groups of 10 pigs kept at a low density (0.85 m^2/pig) than in groups of 20 pigs stocked at 0.26 m^2/pig in the pen, which highlights the greater impact of space allowance on social behaviour of pigs in the lairage pen than group size (MOSS, 1978). Based on these results and on the evidence that most fighting occurs within the first 30-60 min of lairage (MOSS, 1978; GEVERINK et al., 1996), to restrict fighting between mixed pigs stocking densities of 0.42 m^2/pig for short lairage (< 3 h) and 0.66 m^2/pig for long lairage (> 3 h) are recommended (WEEKS, 2008). Pigs may fight more at a low density, because larger available space allows greater opportunities to interact and fight with more individuals, while at high density pigs mostly interact with the few pigs in proximity rather than moving around the pen preventing fighting from spreading all over the pen (WEEKS, 2008). However, the densities applied in commercial conditions vary from 0.3 to 2.7 m^2/pig (GISPERT et al., 2000; WEEKS, 2008).

Air temperature in lairage has an impact on pork quality variation (LAMMENS et al., 2007). Santos et al. (1997) reported that pigs have great difficulty in losing heat and showed signs of stress, such as increased respiration rate under extreme climatic conditions (> 30ºC and RH > 80%).

Showering pigs in lairage is used to cool pigs (3ºC decrease in body temperature) and improve pork quality by reduce PSE incidence (LONG; TARRANT, 1990). It can also reduce aggressive behaviour during lairage and increase the ease of handling upon entrance into the stunning chute (WEEDING; GUISE; PENNY, 1993). Other benefit would be the improved electrical stunning efficiency as wetting the skin lowers its impedance, resulting in an easy and rapid achievement of unconsciousness prior to slaughter (WOTTON, 1996a). However, showering is not recommended at temperatures below 5ºC, because it can result in pigs shivering and lead to DFD pork due to muscle energy depletion to maintain a constant body temperature (KNOWLES et al., 1998).

2.15. Handling pigs to slaughter

Handling and facilities quality in the pre-stun area is of primary importance, given the need to handle pigs faster, so as to follow the speed of the slaughter-line, while ensuring the welfare of pigs at this stage. The combination of higher slaughter speed, poorly designed handling systems and large groups during the short period between the exit of the lairage pen and stunning may, in fact, result in greater proportion of slips and high-pitched vocalisation, and increased use of electric prods, all associated with lower pHu and higher drip loss in pork meat (van DER WAL et al., 1999; CHEVILLON, 2001; HAMBRECHT et al., 2005; RABASTE et al., 2007; DOKMANOVIĆ et al., 2014; ROCHA et al., 2015). To ease handling and keep a smooth and consistent flow of pigs to the stunner it is recommended to move pigs in groups of 6-8 to 18-20 in order to maintain slaughter throughput rates from 150 to 900 pigs/h, respectively (CHEVILLON, 2001). Moving large groups of pigs (45 pigs) through the lairage alleys may result in a greater incidence of PSE pork and blood splashing (BARTON-GADE et al., 1992).

Critical factors at this point are the entrance into the race and the "stop-start" forward motion of pigs towards the stunner, which are both observed in races feeding electrical and CO_2 stunners. The entrance of pigs into a CO_2 stunner has been significantly improved by the Danish group-wise stunning system (CHRISTENSEN; BARTON-GADE, 1997). Compared to the traditional double race system, the group-wise handling system resulted in lower proportion of PSE and blood splashed pork due to reduced prodding and muscle exercise (CHRISTENSEN; BARTON-GADE, 1997; FRANCK et al., 2003).

2.16. Stunning methods

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

2.16.1. Electrical stunning

The electrical system is the most used stunning method for pigs. In this method, electricity is passed through the brain to cause instantaneous insensibility and unconsciousness by inducing a tonic/clonic epileptic fit, before any pain stimulus associated with the application itself is detected and transmitted to the central nervous system (WOTTON, 1996a). Electrical stunning must be applied properly to deliver a minimum electric current of 1.3 A in less than one second and ensure that pigs are instantly rendered unconscious, (HOENDERKEN, 1978).

Electrical stun can be either performed in three different forms. It can be done by applying an electric current across the head only (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) to induce cardiac ventricular fibrillation. When compared with carbon dioxide stunning, head-only electrical stunning increases the rate of the *post-mortem* muscle glycolysis (PSE pork) in pigs due to increased post-stun convulsions and produces a 20-fold increase in the incidence of haemorrhages in the muscle due to increased blood pressure and muscle activity (LAMBOOIJ; SYBESMA, 1988; WOTTON et al., 1992).

Compared to the head-only stunning, the head-to-chest system reduces the negative effects of electrical stunning because the application of the chest electrode inhibits the spinal nerve function reducing the post-stun clonic convulsions (GILBERT et al., 1984). However, when compared with CO_2 stunning, head-to-chest stunning still increases the incidence of PSE and blood splashing on the loins and hams (VELARDE et al., 2000a, 2001).

2.16.2. Carbon-dioxide (CO_2) stunning

Inhalation of carbon dioxide reduces the pH of blood and cerebrospinal fluid, which causes respiratory and metabolic acidosis, and consequently inducing a state of analgesia and anaesthesia in the animal (RAJ, 2008). In this system, pigs are loaded into a cradle and lowered into a concrete well pre-filled with a high gas concentration (85-90%). To avoid the presence of still conscious animals at the exit of the stunner, the minimum CO_2 cycle time should be longer than 130 sec. (VELARDE et al., 2000b). Pork quality is affected by gas concentration. Nowak et al. (2007) reported improvements in meat quality (higher pH_u values) after stunning with 90% compared to 80% CO_2.

2.17. Slaughter methods

In order to limit post-electrical stun convulsions and limit the effects on meat quality variation pigs should be bled promptly (< 15 sec in case of head-only stun; WOTTON, 1996b), whilst still in the tonic phase, and in the lying position. Woltersdorf and Troeger (1987) and Faucitano et al. (1998) observed a significant decrease in the incidence of PSE pork when animals were bled out rapidly in a lying position. As head-to-chest/back stunning induces cardiac arrest and thus to animal death, the promptness of bleeding is not an issue as it is only a procedure to drain blood out of the carcass. However, to prevent blood clots in large vessels and obtain a satisfactory bleeding, a stun-to-stick interval of 3 min is recommended (GREGORY, 1998).

Vertical bleeding is usually observed in CO_2 stunned pigs as the extremities of pigs are relaxed (no convulsions) at the exit of the system (FAUCITANO, 2010). The stun-to-stick time for pigs exiting from a CO_2 system depends on the time of exposure to the gas and the gas concentration ranging from 25-35 to 40-50 sec for exposure to 80% and 90% CO_2, respectively (NOWAK et al., 2007).

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