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Risk Factors affecting carcass and pork quality in pre-slaughter period

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Summary

Skin damage and meat quality are today very important issues for the whole pig industry as well as animal welfare too. These contents have infact a ethical and, above all, important economical implications.

Several studies have just shown that pre-slaughter phases are very critical for the animal welfare and consequently for final meat quality traits.

In a very specilized country as Italy in heavy pig rearing for DPO productions, which are the most famous and eaten for their high quality, is foundamental to limit carcass and meat defects.

To better investigate the real impact of pre-slaughter period and its entity on carcass apperance and meat quality, a Risk Assessment approach was applied in this phase to have a widely idea of which are the potential causes of damage and worsening of carcass and meat and in which moment they occour.

Risk Assessment (RA) consisted in the observation of the potential causes of carcass lesions occurred during unloading at the slaughterhouse and during driving of animals to the stunning point. A total of 1680 Italian heavy pigs were examinated and frequency of pigs'behaviour was recorded and related to carcass damage on, ham, loin and shoulder/head. The operators'handling was recorded too. It was found that, the main potential cause of damage was the use of driving devices by operators.

The effect of pre-slaughter stress was also evaluated on some quality traits of pork. Twenty-eight pigs of three different breed (Italian large White, Italian Duroc and Pietrain) were subjected to two different handling manner (rough and genlty) before stunning and the level of lactate, pro-, macro- and total glycogen were evaluated in *longissimus dorsi* and *semimembranosus muscles.*

Pigs rough handled before death showed the tendency to presents muscles with lower level of pro-, macro- and total glycogen and higher level of lactate, with little differences among breeds within the parameters. These results indicate that the type of pre-slaughter handling and stress can potentially adversely affects meat quality and animal welfare.

Before the arrival at the abattoir, transport is well known to be another very stressful event for pigs which influence skin damage score and meat quality. In this work data from 3.650 heavy pig batches were collected to identify the relationship between the transport and ham aesthetical and technological defects that make them being rejected from DPO Parma Consortium. The effect of journey duration and season were related to the incidence of haematomas, lacerations, microhaemorrhages and veinig defects.

The results shown that short (<37 km) or long (>170 km) travel distances may have adeverse effects on the incidence of defects on fresh hams together the season of transport, where Autumn and Spingtime were found to be the seasons with highest incidence of ham defects.

This thesis confirms how ante-mortem handling is a very important factor not only for the welfare of pigs but also for the quality of carcass, and raw hams and for the final meat quality. Risk Assessment could represent a valid tool to monitor pre-slaughter handling under commercial conditions.

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General Introduction

The Italian pig commerce takes a key role in the national livestock and agriculture production. In the food industry, production of fresh pork and sausages accounts alone for 45% of meat industry sales (ISMEA, 2008). The national production is highly focused and specialized on heavy pigs for processing (95% of slaughtering's)

In particular, this type of rearing is aimed at DPO and GPI products representing more than 50% of the total value generated by the farms and about 25% of sales value generated by the meat industry (ISMEA, 2014). Denomination of Protected Origin (DPO) and Geographical Protected Indication (GPI) are brands which protects and enhances the quality of products offering to consumers a guaranteed purchases. This guaranty is assured by the stricted rules included in the respective production disciplinaries, one for each product. DPO and GPI indicates foods whose peculiar characteristics depend essentially from the territorial area in which they are exclusively produced and processed.

Parma and San Daniele hams, the symbol of the Made in Italy food worldwide, derive from this unique and of great quality and cultural value supply chain. The breeding of pigs for DPO supply chains is a large part of the Italian pig production and is concentrated in a fairly small area, where Piemonte, Lombardia, Veneto and Emilia Romagna regions represent the core, with almost 4000 farms corresponding to 89, 25% of the total (Regione Lombardia, 2014).The Italian province with the highest concentration of pigs is Mantova, where for each inhabitant there are on average four pigs.

Heavy pig

The type of heavy pigs is an Italian characteristic aimed to dry cured ham production, whose preparation requires thighs coming from pigs reared and slaughtered in specific areas and with quality characteristics indicated by the production regulations of the different Consortia. Heavy pigs intensively reared accounts for 30-35% of all herds and is concentrated in the Po Valley.

Pig breeds allowed to this production are principally Italian Large White, Italian Landrace and Duroc, as indicated in the Disciplinary of ham Production and other DPO pig products and reported by the Italian Herd Book for heavy pigs.

Heavy pigs are slaughtered at a minimum age of 9 months and an average live weight of 160 kg and more (Parma Ham Disciplinary,1992) in order to achieve carcasses with a good degree of marbling, large thighs with characteristics suitable for the processing and transformation into dry-cured ham or other meat products.

The processing industry requires fresh cuts characterized by a narrow range of weight and subcutaneous fat contents and with a high level of intramuscular fat to improve uniformity and quality of the end products (Piao et al., 2004).

Ham, especially DPO, is the product with highest commercial value of the Italian food industry and for this reason the thighs represent 55% of the commercial value of the heavy pig carcass, even though they represent only 18-20% of carcass total weight (Pastrello, 2011, Russo, 1990, Chizzolini et al., 1995).

DPO productions and meat quality

Parma ham is the Italian DPO product best known in the world and represents, alone, 90% of Italian DPO market with a production of almost 9 million hams branded in 2014.

The raw material, i.e. fresh legs, comes from heavy pigs reared in a limited area, in accordance with the Regulations of Parma Ham Consortium.

Factors as genetic type, sex, breeding, feeding and slaughter live weight allowed for such production are clearly reported in the Regulations to get a product with specific properties and a meat suitable for processing and transformation (Regione Lombardia, 2014).

All these aspects together with farming technologies and pre-slaughter treatment strongly influence the quality of the meat and the characteristics of the thighs (Rosenvold and Andersen, 2003).
The genetic type influences the chemical, technological and sensory properties of meat and derived products (Moretti et al., 2009). Over the years, the selection of breeds and hybrids destined to processing industries lead to a type of heavy pig which presents evident differences with the pigs slaughtered at 90-110 Kg live weight. Italian genetic breeding for the heavy pig is focused on high processing suitability and a fat covered thighs for seasoning, while around the world pigs are selected and characterized for the high percentage of lean cuts.

Sex and live weight affect both the characteristics of the carcass and meat: castrated females and males show heavier slaughter weight than intere females (Latorre et al., 2003, 2004) with an increase in carcass yield. Moreover in castrated females increases the fatness of primal cuts (loin+shoulder+ham) facilitating the drying and ripening process of the meat (Candek-Potokar et al., 2002) and improving the quality of dry-cured products for aroma and flavor (Ruiz-Carrascal et al., 2000; Banòn et al., 2003). Sex could influence other meat characteristics as reported by Candek-Potokar et al., 2002 who observed that hams from females had firmer texture than hams for castrated males and by Nold et al.(1999) and Latorre et al. (2003) who highlighted that barrows have lighter and more red colour compared with gilts and boars are leaner than barrows and gilts for the lower rate of intramuscular fat (Latorre et al., 2003). Differences in marbling between gilts and barrows were found by other authors (Furman et al., 2007).

The effects of sex and market weight on meat quality was demonstrated including also pH, drip loss,cooking loss, share force, juiceness and overall taste as underlined by Piao et al. (2004).

A recent study shows how the different rearing technologies are able to influence the chemical and sensory characteristics of pork (Bonneau and Lebret 2010). The space available to animals and the presence of straw bedding can lead to an increase in intramuscular fat with a positive effect on the flavour and juiciness (Lebret, 2008) and a reduction of cooking loss (Mandell et al., 2006, Matthews at al., 2001). In addition, the outdoor rearing system seems to influence the characteristics of carcass and intramuscular fat in meat, in relation to the climatic conditions (Edwards et al., 2005).

Feeding composition in protein, fatty acids and minerals content including integration in vitamins and antioxidants influence greatly the quality of the meat and derived products (Corino et al., 1999; Dugan et al., 2004; Rossi et al., 2010). Fatty acid composition influences several aspects of meat quality, including tissue firmness, shelf life and eating quality, particularly flavour (Teye et al. 2006, Isabel et al., 2003, Wood et al., 2003). Intramuscular fat content can be increased by feeding pigs protein/lysine-deficient diets in the growing or finishing phases (Teye et al. 2006, Castell et al., 1994, Cisneros et al., 1996 and Wood et al., 2004).

Also the pre-slaughter treatments such as the duration of journey, the density inside the vehicle and the management of the animals from the farm to the slaughterhouse, can affect pig welfare and pork traits. Pre-slaughter handling includes a wide range of different physically and psychologically stressful procedures (Grandin,1997) that could result in alteration of muscles metabolism and consequently in a worsening of meat quality (Muchenje, 2011).

Pre-slaughter treatment

After a rearing period of several months under specific housing conditions, pigs are transported on road from the farm to the slaughterhouse. The handling of animals for slaughter includes the exposition of pigs to novel procedures such as loading, transport, unloading at the abattoir, lairage and driving to the point where they are stunned. The combination of these stressful situations concentrated in a very short period of time may have a large effect on the welfare of pigs. For example, fear reaction can make handling difficult and causes potentially dangerous situation for animals (Driessen et al., 2013). Physical exercise of animals during the loading of the vehicle, the moving from a familiar environment into a novel one, the closed contact with stock people and type of handling have an important impact on animal stress, welfare, and meat quality. The high level of stress reached by pigs before death could increases physiological levels of blood cortisol, lactate, CPK and adrenaline (Troeger 1989; Støier et al. 2001; Hambrecht et al. 2005), heart rate and body temperature (Schaefer et al. 1989; Griot et al. 2000) whose may lead to defects such as PSE (pale, soft, exudative) or DFD (dark, firm, dry) meat (Kauffman et al., 1978). Ante-mortem conditions affects also skin damage score (Faucitano, 2001).

All these factors could represent a commercial problem for swine industry for the possible financial losses and as well as an ethical concern because can seriously compromise pigs' welfare too.

In order to ensure the welfare of pigs in pre-slaughter period, reducing stress and consequently increasing meat quality, there is the need to invest in new handling techniques, appropriate facilities (Goettems, 2011) and in training of all operators.

Loading

The transfer from the familiar pen to the interior of the trailer, associated with the physical effort induced by the coercion to walk through sloped ramps, make pigs nervous and more difficult to handle (Driessen 2013, Faucitano 2001).

Moving animals in small group sizes (Lewis and McGlone, 2007) and use appropriate devices as solid board (Hemsworth, 2000; Correa et al., 2010) help to better a handling and reduce stress in pigs. Unfortunately, the wrong habit of handling too many animals to the loading ramp of the truck is widely diffused in the conviction to accelerate the loading times but getting the opposite result. Due to the coercion of novelty balk, pigs try to escape or overcrowd to find protection into the group. Animals hesitation induces also stock people to an excessive use of driving tools, especially at the entrance of the loading ramp, to move quickly on pigs.

Although a common practice, rough handling should be avoided for its detrimental effects on animal welfare: it amplified stress in pigs due to fear and agitation. Exceeding in using ectrical prods has been related to increased heart rate, higher levels of lactate, incidence of fatigued pigs and resulted in poorer meat quality too (Weschenfelder, 2013). Prods or sticks has been demonstrated contribute to increase skin damage score (Faucitano, 2001) and incidence of PSE pork (Driessen et al., 2013).

The design of loading facilities is another factor that influence animal welfare and meat quality. In order to drive pigs calmly without panic, slips and falls, there is a need that ramps and trailers have no slippery floor. Moreover, ramps' inclination shouldn't be above 30°, avoiding excessive physical effort. A hydraulic lift has been recommended than ramps because it makes the loading operation easier and quicklier, reducing the need to constraint pigs (Driessen et al., 2013).

To maintain the order during moving is also better not mixing unfamiliar pigs coming from different pens or batchs . In fact, mixing pigs is often made at loading to fill at maximum capacity the decks of lorry (Faucitano, 2001, Gispert et al., 2000). Mixing pig leads to animal fighting , especially during lairage time, with an increase of stress and skin damage (Aaslyng et al., 2013).

Transport

The conditions under which slaughter pigs are transported represent a critical point in pre-slaughter period because can seriously affect pigs' welfare as well as carcass damage score.

During transport, pigs are exposed to several stressful situations, such as unfamiliar and loud noises, new smells, vibrations, lower individual space besides sudden speed changes of the truck and a frequent contact with handlers (Grandin, 1997). Vehicle design, placement and microclimate inside the vehicle, season, stocking density, duration of the journey and driving conditions are the main factors reported to affect animal welfare and/or the quality meat.

The driving style of the trucker influences the behaviour of the pigs during transport. When driving is rough animals are subjected to rapid movements i.e. sharp accelerations and stopping which may cause slips and topples of pigs resulting in bruising, muscular fatigue, fear (Driessen et al., 2013, Randall et al., 1995). The physical effort to remain standing, in order to cope with the high level of vibrations result in higher skin damage score and DFD pork (Barton-Gade et al.,1996b) as standing pigs are more prone to falling or trampling (Dalla Costa et al., 2007, Barton-Gade et al., 1996b). A better driving allows pigs to adapt the high level of vibrations with the standing or lying position (Driessen et al., 2013).

Randall et al., 1996 found that also vehicle type and and the animal location inside (Barton Gade et al., 1996b, Guise & Penny 1989) are also important both animal welfare and carcass quality: a large body fixed truck assure a better comfort level and the front and rear compartment have a significant effect on meat quality and carcass blemishes (Dalla Costa et al., 2007). In addition, the deck of the trailer is important for bruising issue too because pigs transported in the lower deck show higher damage scores in the middle and shoulder than pigs transported on the upper deck (Faucitano, 2001, Barton-Gade et al. 1996a).

Loading density in the transport vehicle is an important factor in pre-slaughter stage contributing to variation in presence of carcass damage and pigs' welfare in relation to environmental temperatures.

EU directive 95/29/EC rules European densities of transport fixed at 250 kg/m2 or 0.44 m2/100 kg for normal slaughter pigs of 90-100 kg live-weight (Warriss, 1998) to ensure animals the space to lie down in their natural position. Regulation CE N.1/2005 also highlighted this loading density threshold. Out of Europe, this stocking density is rarely applied in commercial condition as space allowances are frequently adjusted according to different transport conditions (Faucitano, 2001) and economical pressures (Warriss, 1998) with real densities ranging from 0.35 to 0.50 m2/100 kg (Weschenfelder, 2013). Nevertheless, there are some evidences that at low stock densities pigs can change behaviour leading to greater skin damage incidences (Faucitano, 2001). The negative effects of high densities on meat quality can be observed alone or combined with other effects, as reported by Carr et al. (2008) who found that pigs being handled roughly at loading and transported at high density produced darker pork for lower L* values (Weschenfelder, 2013).

In general, the journey duration could exacerbate the stress of transport in pigs with a worsening of welfare condition. A longer travel time increases fatigue symptoms such as tremors, hyperventilation and erythema, as well as incidence and degree of skin bruising (Mota-Rojas et al., 2006). Gallo et al., 2001 found that after longer journey more animals show lesions. Long distance transportation results also in depletion of energy for prolonged stress of pigs leading to darker carcasses (DFD meat) and a reduced carcass yield (Mota-Rojas et al., 2006). Controversially, some authors have demonstrated that pigs transported for short distances failed in recovering from loading and transport stress producing PSE meat (Sheeren et al., 2014, Pèrez et al., 2002) more difficult to handle (Grandin, 1994).

Besides transport distances are associated with greater mortality. Barton-Gade et al. (2007) and Warris (1998) reported that deaths are more frequent on longer journeys, Werner et al. (2007) affirmed that travel distances longer than 8 h and shorter than 1 h, both, negatively affected animal welfare with increased mortality rates. In any case pigs transport carried out with a normal truck shall not exceed 8 h, but can be prolonged up to 24 h with a special vehicle provided with adequate ventilation system (Brown et al., 1999) and with continuous access to water during the journey (Bench et al., 2008; Reg. 1/2005 CE).

The duration of transport is however conditioned by the season because external temperature is an additional factor affecting pigs welfare during transportation. In hotter months was observed a reduction of meat quality as a consequence of heat-stress that involves fatigued pigs (Mota-Rojas et al. 2006). Correa et al. (2013) and other authors showed a higher risk of PSE pork in summer and DFD in winter. In this latter season there is evidence of greater frequency of skin damages (blemishes, haematomas and blood splashing), probably caused by slips and falls for slippery icy floor (Schereen et al, 2014, Gozlavez et al., 2006). Transport duration and temperature were found to affect body weight, with the highest weight losses apparent after 24 h of transport at 35°C (Lewis et al., 2005; Berry & Lewis, 2001a).

Unloading

At the slaughterhouse, pigs should be unloaded as soon as possible (AAFC, 1993) to avoid temperature stress and its negative consequences on meat quality and animal welfare: an increase in carcass bruises and in incidence of exudative pork for unloading times longer than 30 min was found by Weschenfelder (2013) and Driessen & Geers (2001). If delay may unavoidable, animals have to be provided with adequate ventilation (Szent István University, 2009).

Even if is considered less stressful than loading, unloading represents a source of stress due to novel environment and handling that may cause fear (Dalmau et al., 2009). The physical effort lead to an increase of heart rates and of plasmatic cortisol and creatine kinase concentrartion (Geverink et al., 1998, Kim et al., 2004, Brown et al., 2005). Problems can be caused by the lack of sheltered quays when animals are subjected to wind, rain and sunlight they balk and may refuse to exit the lorry.

The reluctance of pigs to go forward can be also caused by poor lighting and inappropriate design and location of the unloading area. Different colours and shadows may frighten the animals and they preferably walk form a dark to a lighter place (Driessen et al., 2013). The use of a hydraulic lift to offload allows a gradual emptying of the truck by compartment using paddles or boards only, increasing the easiness of handling and reducing the time of procedures (Weschenfelder, 2013, Jones, 1999). The unloading space must be structured in order to driving pigs straight to lairage without any obstacles as bottlenecks or corners, that could damage the skin during the passage. The condition of the surrounding environment play a key role to for pigs' responses (Faucitano, 2001) and meat quality (Van de Perre et al., 2010) as well as the quality of management by operators.

Several studies (Driessen et al., 2013, Rabaste et al., 2007) reported that pigs being handled gently at unloading were less stressed and adapted better to the lairage pen environment than pigs being handled with electric prods. Frequent use of electric prods results in fear and stress, making pig driving more difficult due to an increase of mounting, slipping and turning (Rabaste et al., 2007) and it is responsible for considerable increases in skin damage on the carcasses (Rabaste et al., 2007, Geverink et al., 1996). Damage to the surface of the carcass after dehairing is a serious commercial problem, since it downgrading the value of the carcass and adding costs to remove blemished tissue and reducing the speed chain (Faucitano, 2001).

Lairage

Among the pre-slaughter factors herein examined, lairage was the most important source of variation determining meat quality.

Resting time at abattoir provides an opportunity for animals to recover from stress and fatigue of transport and unloading (Warriss, 1987), limiting the presence of defects in carcass and meat (Faucitano and Geverinck, 2008, Warriss, 2003). Furthmore resting creates a reservoir of animals aimed at maintaining the constant speed of the slaughter line.

There is evidence that a lairage time of 1-3 h is optimal and recommended to recover from stress prior to arrival at the slaughter plant (Pannella-Riera et al. 2012, Lammens et al. 2007) and to have muscle temperature and ultimate pH in a good range for meat quality (Zhen et al., 2013). Several studies shown that short lairage durations resulted in pigs with higher blood level of cortisol, lactate and CK (Salajpal et al., 2005, Saco et al., 2003). No resting times lead to high incidence of blood glucose levels (Zhen et al., 2013) and PSE meat (Fortin, 1989; Eikelenboom et al., 1991). In contrast, longer lairage time provoked greater blood levels of acute phase proteins (Saco et al., 2003) and fighting (Nanni Costa et al., 2002) associated to increased skin damage scores (Guàrdia et al., 2009, Nanni Costa et al., 2002) and a higher risk of DFD pork (Guàrdia et al., 2005). Moreover, Warriss et al. (1998a) observed a significant reduction of carcass weight and backfat thickness after overnight lairage. Pigs held in lairage the night before slaughter exhibited skin damage in all carcass almost tripled compared with shorter lairage and a little tendency to DFD condition (Nanni Costa et al., 2002). Overnight lairage leads also to a depletion of glycogen and a decrease of pH at 1.5 h, colour score and drip loss as well as an increase of ultimate pH. Lairage time had a significant effect on the veining defect. Prolonged lairage time increased the presence of the visible vein network defect on the medial side of the ham (Zgur, 2014). In practice, the resting times applied are varying from 1 to 15 hours depending on the abattoir size, availability of pigs for slaughter, transport time, handling procedures and environmental conditions (Gispert et al., 2000).

Handling, facilities design, environment conditions and mixing are other aspects of lairage which influence both welfare and meat quality.

Adequate space allowance in resting pens reduce aggressive encounters as reported by Rabaste et al. (2007) who observed that larger groups pigs in the holding pen spent more time standing, fighting and were more involved in behaviours as bites and head knocks than pigs kept in smaller groups. Stress and aggressiveness are also linked to the time of withdrawal (Nanni Costa et al., 2002). Optimal feed withdrawal times are suggested to be in between 16 to 24 hours (Eikelenboom et al. 1991) or 12 to 18 hours. If feed deprivation is too long (e.g. with overnight lairage), energy reserves are empty and there is not enough glycogen to assure a sufficient pH decline increasing the risk of DFD (Gispert et al. 2000).

Even if the mixing of unfamiliar pigs in lairage is a very common practice, it induces high levels of aggression among pen mates (Faucitano, 2001, Guise and Penny 1989, Warriss 1996, Ekkel et al. 1997), which increases skin damage and promotes the development of PSE/DFD meat (Brown et al., 1999; Gispert et al., 2000). To reduce the negative impact of this practice on welfare and skin damage, large pens should be equipped with mobile dividers in order to keep pigs in small batches avoiding mixing (Faucitano, 2001, Barton-Gade et al. 1992).

Despite the role of lairage as well as the relevance of resting area that allows animals to recover from transport stress (Faucitanto, 2001), temperature at lairage would also represent an important risk factor for animal welfare and meat quality traits (Lammens et al., 2007). Under extreme temperature conditions (> 30ºC and RH > 80%), pigs have great difficulty in losing heat and show signs of stress, such as respiration rate and panting (Santos et al., 1997) with a consequent high lactate and CPK levels in the blood and a more than two-fold higher incidence of PSE meat (Warriss et al., 1994). The lack of environmental control may lead to further economic losses due to death.

A method to improve pigs'welfare in extreme heat conditions, especially during hot weather, is showering pigs for some minutes in order to reduce the body's surface temperature of 3-4 ºC. However, intermittent and frequent showers are detrimental, as they prevent pigs from resting and lying down (Stzen Istavàn University). A quick shower during overnight lairage may be needed to avoid skin reddening (Stzen Istavàn University). At temperatures below 5ºC, showering could cause animal shivering and may lead to darker pork (DFD) due to muscle energy depletion to maintain constant the body temperature (Knowles et al., 1998).

Showering provided some minutes before slaughter decreases muscle temperature and may lead to a better meat quality. Showering pigs in lairage also reduces aggressive behaviour and facilitates pigs handling upon the entrance into the stunning chute (Weeding et al., 1993), besides increases electrical stunning efficiency resulting in an easy and rapid loss of consciousness prior to slaughter (Wotton, 1996).

Stunning

During conduction to stunning, animals could be very stressed, due to the fast handling in small group combined with poorly designed handling systems and rough procedures. All these factors are detrimental for animal welfare, especially the excessive use of moving devices such as electric prods that, contrary to the function which it is used, generates panic among animals and induces mounting, back-up activity and more fatigued pigs, with delays in the slaughter speed. A worsen meat quality is also attributed to the use of this device for the higher incidence of bruised carcasses and PSE pork (Benjamin et al. 2001, Rabaste et al. 2007).

Pre-stunning facilities with inadequate designed could represent a source of skin damages too. The most critical areas are the passageways from the lairage pens until the entrance of single chute and the stunning race. In these steps, the close interaction of animals with handlers make pigs fearful with tendency to overcrowding, jamming, mounting and high vocalization (Faucitano, 2001). The abattoirs should be equipped with straight alleys and a minimal number of turns and corners to encourage the forward movement of pigs. An automatic push gates may help the staff to move animals, reducing interaction with the human and avoiding the use of electrical prods.

The design of race feeding the restraining conveyor has been shown to influence the amount of skin blemishes. An excessive width leads to frequent jamming against the race walls, resulting also in strong prodding to recreate the order and the speed chain (Faucitano, 2001). The intense and increasing noise of this point affects pigs' calm and consequently the handling procedure. Geverink et al. (1998) reported that the noise produced by the machinery, pressure hoses and pig and human vocalisations represented a source of stress which evoked huddling and escape behaviour inducing operators in using sticks (Fraqueza et al. 1998; Grandin 1998).

A massive use of electric goads is typical of the entrance into the stunning chute for separating pigs and driving them singly into the stun tunnel at constant speed. The electric goading and the limited space induce pigs climbing over the backs of other pen mates in search of protection within the group (Guise and Penny 1989; Lambooij and Engel 1991), slipping, falling (Dokmanovic et al., 2014) increasing skin bruises on the carcass, heart rate and negative changes in blood parameters and affecting post-mortem meat quality (Thorell, 2009). Grandin (2013) recommends stiff scrub brushes on the end of a stick as an electric prod alternative for moving pigs up a single-file race, Driessen et al. (2013) affirmed that when a restrainer conveyor is used, labyrinth system can reduce groups into single files. The use of sticks and rigid tubes must be avoided because they may cause deep bruising if improperly used (Szent István University, 2009). Other devices less invasive such as boards or paddles are always suggested in addition to a continuous training of stock people about the correct handling of pigs during pre-slaughter operations and an improvement of abattoirs structure.

Carcass and pork quality

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

Skin bruises

The presence of carcass blemishes are indicative of poor welfare and, depending on the severity, may cause carcass downgrading, up to 6% of its value (MLC, 1985), as well as additional costs for reduction of the slaughter line speed to remove damaged tissues and increased staff for carcass inspection. Further charges could be attributed to loss of market opportunities as severely bruised carcass or traits are rejected and can only be used for making lower value products (Faucitano, 2001).

The degree of damage on the skin of the carcass could be measured using different scales, either providing a general score based on the carcass appearance (1 = none to 5 = severe; MLC, 1985) or counting the bruises by anatomical location, usually head/shoulder, middle/loin and ham (ITP, 1996; Barton-Gade et al., 1996a), defining their shape and size to identify the source of infliction e.g., fighting, rough handling, overcrowding and poor facilities (Faucitano, 2001, ITP, 1996).

Small bruises are normally linked to biting during fighting and are often placed in the anterior (head/shoulder) (Weschenfelder et al. 2013). Conflicts among pigs can results in blemished hams due to the combination of aggressivity and the impossibility of avoiding an attack of a pig by another in high density pen (Faucitano, 2001, Geverink et al., 1996). Long and thin ones should be instead probably recunducted to climbing during overcrowding situations (loading, unloading and holding pen) and the moving along the slaughterhouse chutes, especially toward the stunning (Weschenfelder et al. 2013, Faucitano, 2001) and are located on the back. Rectangular and large dark brown marks are generally sticks-inflicted and concentrated in the loin region, contrary of goads which tends not leave marks on skin carcass, unless applied too widely (Dalla Costa et al., 2007, Faucitano, 2001).

The colour of the bruises too can give information on the time of infliction, with red bruises being the recenter (Faucitano, 2001) than yellows ones.

pH

Skin bruises are then linked to post-mortem pH of meat. High bruise scores on the carcass due to fighting in lairage or mounting behaviour results in higher ultimate muscle pH due to the effects of physical activity on glycogen levels at slaughter (Rabaste et al., 2007, Warriss 1996; Gispert et al. 2000). A positive correlation between the presence of bruises score and pHu are also found by Romero et al. 2013, Vimisio et al. 2013 in pigs and Strappini et al., 2010 in cattle. When animals are stressed, glycogen reserves can be depleted and hence a higher pH can be expected (McVeigh & Tarrant, 1982). Multiple and prolonged stress caused by improper handling, long transport, long fasting times and physical injuries can result in depletion of muscle glycogen stores before slaughter, reducing acidification and increasing ultima pH. McNally & Warriss (1996) reported that heavy bruised carcasses presented pH values over 5.8 compared to light bruised carcasses, suggesting a strong relationship between stress conditions, bruises and pH carcass values (Strappini et al., 2010). Values of final pH (pHu) higher than 6 are indicative of the DFD (dark, firm, dry) meat quality defect (Tarrant, 1989; Carragher and Matthews, 1996; Gregory, 1998).

DFD and PSE defects

In DFD, the high pH results in relatively little denaturation of proteins, water is strictly bound and little or no exudates is formed (Warriss, 2000). This is because there is little or no shrinkage of the myofilament and the oxygen penetration is reduced by the closed structure and results in a thin surface layer of bright red oxygenated myoglobin (MbO2) and under the purple colour the muscles (Adzitey and Nurul, 2011). This condition make appearing the meat darker, with a dry texture and a less pronounced taste, characteristics less appreciated in fresh cuts (Fisher, 2005). Furthermore, the high pH value, which is conducive to bacterial growth, results in shorter shelf-life (Fischer, 2005, Holmer et al., 2009, Faucitano et al., 2010).

Conversely the breakdown of glycogen just before slaughter lead to greater lactic acid rate in the muscle inducing a fast pH decline (lower than 6 at 45 min post-mortem) (Adzitey, 2011, Warriss and Brown, 1987), which if combined with high muscle temperature, may result in the PSE meat defect (Gregory, 1998). This sudden acidification provokes proteins denaturation and a consequently reduction in their water holding capacity and releasing of exudates. The shrinkage of the myofilament increases the light reflected from the meat and the haem pigments selectively absorbed green light, reducing the normal red colour. This makes meat paler (less red and more yellow), soft and exsudative (PSE) and with poor palatability for reduced tenderness and juiciness after cooking (Adzitey and Nurul, 2011, Fischer, 2005). PSE is a meat defect caused by a combination of different factors, such as stress-susceptible genes, rough handling shortly prior to slaughter and poor carcass chilling (Grandin 2000a). Temperatures above levels of 37.0-39.6 (Hannon *et al*., 1990) result in increased PSE pork and a reduced shelf-life due to increased chances of growth of microorganism (Lambooij, 2000), imposes negative economic implications to the pork industry.

The literature describes that PSE defect could be also affected by the genotype. The mutation known as the halothane gene (n) is responsible for genetic stress-prone pigs (Gispert et al., 2000) and a linkage between the halotane gene (n) and an increased incidence of PSE pork was shown by different studies (Fernandez et al., 2002, Channon et al., 2000). The halothane gene has been demonstrated having an additive effect with the other pre-slaughter stressors on PSE in meat (Channon et al., 2000). The highest incidence of serious PSE carcasses was observed in pigs slaughtered in a plant with the major n gene frequency. (Gispert et al., 2000).

The incidence of PSE pork could be reduced by controlling carcass chilling which is able to influence the metabolism of muscle *post mortem*. A rapid fall of post-slaughter temperatures reduces the speed of chemical and biochemical reactions and the rate of pH decline. Nevertheless, , an extreme fast chilling may produce tougher meat caused by cold shortening (Weschenfelder, 2013, Savell et al., 2005).

It is evident that pH values after slaughter greatly affect the final quality of meat (Gajana 2013, Hambrecht et al., 2004, Gispert, 2000). The rate of pH decline could adversely influence other pork characteristics as colour, water- holding capacity (WHC), shelf-life and other technological properties i.e. cooking loss, tenderness, juiciness, flavour (Gajana, 2012).

Colour

The colour impacts on meat sale because consumers use discoloration as a parameter of freshness and healthiness of pork meat (Muchenje et al.2011, 2008, Mancini and Hunt, 2005, Rosenvold and Andersen, 2003).

Meat colour is affected by elements such as feeding, age and physical activity of animal. The concentration of pigments in muscle, mainly myoglobin, defines the colour perception (Warner, 1994). Myoglobin store in the muscle changes between species, breeds and single muscles and it can present three different chemical forms (Gajana, 2012). Meat colour is usually detected by tricolorometric method where colour coordinates for reflecting lightness (L*), redness (a*) and yellowness (b*), are measured by a chromameter (Minolta) which evaluates the reflectance of the myoglobin and haemoglobin after exposure to oxygenation (Weschenfelder, 2013, Brewer et al., 2001).

Water-holding capacity

Water-holding capacity depends from muscle energy store at the moment of slaughter and from the rate of pH decline (Van der Wal *et al*., 1999; Barbut *et al*., 2008). It is defined as the ability of meat to retain its water which, in turn, influences the juiciness. Short-term acute stress at the time of stunning induces a fast *post-mortem* glycolysis and a storage of hydrogen ions and lactate meanwhile muscle temperature is high yet. Low pH values at high temperatures lead to denaturation of myosin and myofibrillar proteins (Warner *et al*., 1997; Joo *et al*., 1999) with a lower water-binding capacity (Offer *et al*., 1989) (Muchenje, 2011). The quick driving and coercion of pigs from lairage toward the stunning chute resulted in decreased water holding capacity 24 h *post-mortem* (Muchenje, 2011, Van der Wal *et al*., 1999) and consequently, in a higher drip loss. Practices as too long withdraw and excitement and forced exercise may lower the water binding capability of proteins even when the glycogen concentrations have been restored to normal during the *post-mortem* period (Muchenje, 2011). Loss of water from meat has negative economic impact on pig industry because it reduces the meat weight (Otto *et al*., 2007), water soluble nutrients and negatively affects technological properties as toughness, pork flavour and colour (Muchenje, 2011).

Drip loss

Drip loss is the inability of the muscle fibres to hold onto the natural juices of meat with a water loss from the shrinkage of muscle proteins in the form of drip, even in the absence of external forces (Weschenfelder, 2013, Fischer, 2007). This condition changes the shape of pork through shrinkage and results in firmness and poor juiciness (Muchenje, 2011). Drip loss measurement can be done by different methodologies, such as the bag (Honikel, 1998), filter paper (Kauffman et al., 1986) and EZ-Drip loss methods (Christensen, 2003) (Weschenfelder, 2013).

Some authors had found a significant correlation between genotype and this trait, with higher drip loss in pigs with halothane-sensibility regardless of the type of handling (Muchenje, 2011).

Cooking loss

Low water holding capacity (WHC) and low pH result also in high cooking loss (Aaslyng *et al*., 2003; Muchenje, 2007). Cooking loss is the percent weight difference between fresh and cooked meat samples (Muchenje, 2011, Honikel, 1998; Torley *et al*., 2000; Moelich *et al*., 2003). During cooking chemical reactions, such as protein denaturation and Maillard reaction shift physical meat characteristics involving also a loss of water, several essential minerals and vitamins that influence its final quality, acceptability (Chiavaro *et al*., 2009) and nutritional profile (Muchenje, 2011). Has been calculating that with cooking process, meat suffers losses from 20 to 40 % (Muchenje, 2011, Jonsall *et al*., 2001; Aaslyng *et al*., 2003; Muchenje, 2007). The amount of cooking loss is highly dependent on cooking method, cooking time/temperature and end-point temperature (Pearce et al., 2011, Aaslyng et al., 2003), but is correlated to *post-mortem* pH too (Torley *et al*., 2000; Muchenje, 2007). Since pre-slaughter stress influences pork acidity, its water holding capacity and structural muscle changes it may consequently affects also cooking loss Muchenje, 2011).

Porcine meat from RN-gene pigs is associated with high cooking loss (>25%) (Lundstrom *et al*., 1996; 1998; Jonsall *et al*., 2001) due to combination in meat of higher glycogen content and a lower protein binding water during cooking (Fernandez *et al*., 1991), Pugliese *et al*. (2005) confirmed that less cooking loss in pigs which are kept under free-range conditions. High cooking loss is synonymous of a less eating quality and implies financials losses for whole food industry (Muchenje, 2011, Aaslyng *et al*., 2003).

Flavour

Juncher *et al*. (2003) and O'Neill *et al*. (2003b) suggested that severe pre-slaughter stress could enhance more drip and cooking loss as well as warm-over flavours (WOFs) in PSE meat.

Among technological and sensorial meat quality parameters which can be compromised by incorrect pre-stunning conditions is included the smell , that is a fundamental component of product palatability, together juiciness and tenderness.

Juncher *et al*. (2003) found that following severe pre-slaughter stress pork can lead changes in aroma, with development of warmed-over flavours (WOFs).

This flavour defect is due to a rapid phospholipids oxidations and protein degradation that provoke the rancid-like flavour (Byrne et al., 2001, Byrne et al., 1999), even if they could be masked up by the acidic flavours associated with PSE meat (Muchenje, 2011, Gregory, 2007). Warmed-over flavour (WOF) is recognized as a major quality problem by the meat industry in marketing of pre-cooked, ready-to-heat and serve products (Byrne et al., 1999).

Tenderness

Pork tenderness could be indirectly affected by pre-slaughter handling, transport quality, type of stunning, freezing and thawing (Gregory, 2007, Channon 2000) as well as by breed, sex and age (Gajana, 2012). The cartilage and connective tissue content of meat are further elements that impacting on meat tenderness (Muchenje, 2011). Meat tenderness refers to the toughness or the ability of meat to resist fragmentation when being chewed (Muchenje, 2011) and represent the most important tasty characteristic. Warner–Bratzler shear force test is a well known method to measure tenderness observed sensorial and to determine meat acceptability (Gajana, 2012, Sanudo et al., 2003). Meat tenderness and texture are important factors for consumers since they lay down the trend consumption and purchase of meat. In order to achieve uniformity in tenderness and reduce technologic defects in pork, it's should improve and standardize *ante-mortem* handling of pigs and *post-mortem* manipulation procedures of carcasses along the chain (Gajana, 2012).

Aim

The aim of the thesis is clarify which are the factors that in pre-slaughter period can play adversely on pigs welfare and meat quality.

In particular, this work focused the attention on transport distance and climatic conditions, and on all these hazards which can negatively affect carcass and meat quality parameters during unloading at the slaughter plant and the moving of animals to the stunning cage.

Between carcass portions that could be damaged by inadequate pre-slaufhter handling, raw ham has to be mostly considered, because it is destined to DPO chain for dry-cured ham production, a very precious and high quality product of Italian food industry.

In order to indentify the factors more involved in carcass damages, the Risk Assessment (RA) was applied. It is a methodologyinstrument to identify these factors considering a wide scenario and not only a single steps of pre.slaughter procedures.

The RA approach used in the thesis is based on direct observation (mettere la defiizione). It is totally non invasive and much indicates to work in a scenarios where live animals are involved. If it is correctly applied, the RA could provide very useful information to identify critical points in the whole pre-slaughter management, solving carcass and meat quality damage problems.

The last goal of this thesis is remark the importance of the control and improvement of pre-slaughter practices which are very important for the pig meat industry economy.

CHAPTER 1

Risk characterization for carcass lesions during the pre-slaughter steps at unloading and stunning of Italian heavy pigs

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Abstract

Skin damage in pig carcasses are a welfare concern and can lead to rejection of valuable cuts by DPO consortia and food industry. Carcass lesions can occur during pre-slaughter period as a consequence of inadequate design facilities and rough incorrect handling. The aim of this work was to provide, through the application of RA (Risk Assessment), the identification of the main hazards which before slaughter play the major role in the appearance of skin damage in the ham, loin and shoulder-head of the pig carcass at the slaughterhouse. To this it was evaluated the occurrence of backward, slips, balks, overlaps, falls, overcrowds, back up, pushing and the use of rubber stick at unloading and during the moving to the stunning in 1680 Italian heavy pigs according to EFSA Guidelines on Risk Assessment. Frequency of pigs behaviors was recorded for each deck unloaded and related to damage score of the deck itself. For damage evaluation were considered the total carcass and the ham, loin and shoulder-head singularly. For the total carcass at unloading, the use of rubber stick showed the higher risk and frequency (1.97 and 18,75%). Backward, slips, balks, overlaps, falls and overcrowd ranged between 0.11 and 0.02 with low frequencies. Rubber stick reported the higher frequency (91.67%) and risk 46.29 toward the stunning and backward, back up, pushing, overcrowd, slipping and falling showed lower values (between 0.01 and 0.77). Similar results were found for ham, loin and shoulder-head where the stick was the parameter more correlated to lesions than animals responses. It was conclude that, in the observed scenario, the risk of total carcass lesions and for its single part was firstly related to the driving tool both at unloading and toward the stunning, there is a very light correlation with pigs behaviors.

Key words: brushes, pig handling, slaughter pig, electric prod, carcass

Introduction

The skin damage in pigs industry is a welfare and economic concern (Guàrdia et al., 2009) and is responsible for great losses for the producers due to the downgrading of the carcass and rejection of valuable cuts like ham and loin by highly sophisticated markets, the reduction of the production of dry cured hams (Candek-Potokar and Skrlep, 2012), which need to be completely unblemished and of the speed of dressing line to remove blemished tissues (Faucitano, 2001). Furthermore the carcass lesions are associated with poor meat quality: pigs with high score of skin damage tends to have higher levels of cortisol, creatine phosphokinase and lactate (Warriss et al., 1998) and the stressful handling associated with carcass lesions can predispose the occurrence of DFD or PSE meat (Rosenvold and Andersen, 2003).

Factors as stress susceptibility and aggressiveness can influence incidence of carcass bruises (Bolhuis et al. 2005) as well as loading and unloading practices and their duration, time of transportation and stocking density (Nanni Costa et al. 2002, Nanni Costa et al. 1999, Barton-Gade & Christensen 1998, Franqueza et al. 1998). Increase of skin damages carcasses was demonstrated also for time of lairage and fasting before slaughter (Guardià et al. 2009), mixing unfamiliar pigs (Brown et al. 1999) and use of moving device such as electric prod (Rabaste et al. 2007, Guise and Penny, 1998). However, in general, the main causes are related to aggression, harsh handling and poor design facilities (Faucitano, 2001), but it is not clear which are the main points where may occur these injuries and for which specific event.

Some studies tried to identify the critical steps during *ante mortem* period for skin damage on pigs (Romero et al. 2013 and Chevillon et al. 1997) and on culled cows (Strappini et al. 2013) with different approaches. However, there is not a standardized procedure to evaluate the real causes of carcass damage.

Risk Assessment (RA) is a systematic process consisting into identify and characterize potential hazards (e.g. to animal welfare or food safety), to estimate the probability and the magnitude of adverse effects resulting from exposure to those hazards and to determine the resulting risk (Dalla Villa et al. 2009).

The risk assessment approach includes four steps: hazard identification (factors that have the potential to create adverse effect or consequences), hazard characterization (quantitative estimate of the adverse effect), exposure assessment (qualitative or quantitative evaluation of the level, duration, and variability of exposure to the identified factors) and risk characterization (qualitative or quantitative estimation of the probability of occurrence and magnitude of known or potential adverse effects) (EFSA, 2009; Algers et al., 2009). In pig production, the RA has been carried out to evaluate meat quality (Guàrdia et al., 2005; Guàrdia et al., 2004), foodborne zoonoses (Fosse et al., 2008) and animal diseases (Berends et al., 1996), but never during pre-slaughter period in function of carcass lesions.

In this context, the aim of this work was to provide, through the application of risk assessment, according the European Food Safety Authority (EFSA, 2012), new information on the detrimental effects due to improper handling during all operations involved in the slaughter of heavy pig that can induce lesions in the whole carcass and single traits as ham, loin and shoulder-head.

Material and Methods

Risk Assessment Application

The study was conducted following the EFSA methodology on Risk Assessment for Animal Welfare (EFSA, 2012).

The scenario observed was a slaughterhouse of medium dimensions, speed chain of 280 pigs/hour and specialized in the slaughter of Italian heavy pigs for the production of Parma dry-cured ham. The plant was located in Lombardy and all supply farms are from Northern Italy.

Potential causes of lesions (hazards) were identified in the target population made of 12 deliveries of Italian heavy pigs (a total of 1680 animals, 140 for each journey) from the same commercial farm over the winter, spring and summer of 2014. At the farm, outside the boxes, the pigs were conveyed in an external passageway of concrete material, long 60 m and linked to a mobile ramp (length m 6.0, width m 0.7, solid side walls of m 1.0 and adjustable height), using to load pigs into the trucks. The pigs were loaded in the beginning of the morning after 10 hours of fasting.

The transports were carried out with double trailer lorries with three hydraulic decks each one. All compartments in the trailers were stocked according to the Standard Regulations Operating Procedure (Reg. 1/2005 CE, 2005) with a density of about 0.68 m^2/pig. The pigs were transported through rural and secondary roads and the journey time was about 30 minutes at an average speed of 60 km/h. At the slaughterhouse, the unloading was carried out by a fixed bay adjustable for the height and performed by the driver and the manager of the slaughter lairages, in around 20 minutes. After unloading, pigs were immediately moved to the stunning chute or into resting pens for a maximum time of 30 minutes.

When driven to the stunning, pigs were handled with rubber sticks by a slaughter operator until the single chute leading the stunning cage, where pigs were driven only by electric prod. Pigs were electrical stunned (head only-1,3A and 170V) and exanguinated horizontally for 3 minutes. After sticking, pigs were hanged by the left leg for 10 minutes before being immersed in a scalding tank for dehairing at 62°C for 5 minutes and the remained hair was manually removed after. The cleaned carcasses were evaluated for skin damages, assessed subjectively by the same trained operator, on the ham, loin and shoulder-head as Velarde et al. (2001) and Chevillon et al. (1997) by a four point scale (1=none to 4=extreme) according to the Danish Meat Reasearch Institute-scale (Barton Gade et al., 1996). Carcasses were then eviscerated, split, hot-boned and sectioned in different marketing pieces, before kept in chilling rooms (2–4 °C) for 24 hours.

Hazard measurement

The target of hazards for skin bruises was obtained merging events directly observed at the slaughterhouse and those reported in literature about lesions on the carcass. According to Dokmanovic et al. (2014) and Aiassa (2010) for pigs and Strappini et al. (2013) for bovine, operators handling and some pigs behaviors were observed and recorded together at unloading and during moving toward the entrance of stunning chute (Table 01 and Table 02).

Table 1. Descriptions of behaviors observed.

Behavior	Description
Slips	Pig's leg touching the ground
Falls	Pig’s body touching the ground
Back up	Pig moves rearward, opposite the direction of intended motion
Backwards	Pig makes a 180° turn and moves in the opposite direction
Overlaps	Pig mount another pig, with its front legs on the back of the other pig
Overcrowd	More pigs huddle and create a group
Balks	Pig refuse to walk or stops for greater than 2s
Pushes	Pig press the head against another/other pigs in front of him
Rubber stick	Handler uses rubber stick to encourage pigs to move

Data were recorded and evaluated considering the total number of events per deck of the truck, which was the experimental unit.

Data Analysis

The risk was estimated by multiplying the probability of occurrence (PO) for the Magnitude, according to EFSA (2012):

Risk = Probability of Occurrence x Magnitude

The probability of occurrence was considered multiplying the Distribution of Exposure by Distribution of Likelihood.

Probability of Occurrence = Distribution of exposure x Distribution of likelihood

For the Distribution of Exposure was used the frequency recorded of each event on total number of pigs observed, expressed as percentage, as used by Fosse et al.

(2008). As the Distribution of Likelihood was considered the ratio between the total number of each event and the sum of mean number of pigs with lesions at carcass, ham, loin and shoulder-head respectively for each deck.

Distribution of Exposure = Frequency of each event

Frequency of each event = Total number of each event/Total number pigs

Distribution of Likelihood = Total of each event/Sum of bruising score of total carcass

The Magnitude of the adverse effect, i.e. the potential adverse effect at the individual level, given that the animal is exposed to the hazard and experiences that adverse effect, was calculated multiplying Severity x Duration (EFSA, 2012; EFSA 2009), adjusting the levels in order to give weighting to the scores. As performed by Dalla Villa et al. (2009) that estimated Severity levels subjectively, a scale from 1 to 4 was chosen for assessing severity, based on the effect of the parameters observed on carcass lesions in the scenario.

Magnitude = Severity / 4

In the present study the duration was fixed to 1 for each hazard, because the direct observation of each events lasted always less or equal to 1 minute for the speed of the chain.

Table 2. Formulas used to estimate the final Risk (Dalla Villa et al. 2008)

Magnitudo = Severity/4 *Duration
Frequency = Total of each event/Total number of pigs
Distr. Of Likelihood = Total of each event/Total of mean bruised pigs
Distr. Of Exposure = Frequency
Prob. Of Occ. = Distr. Of Likelihood * Distr. Of Exposure
Risk = Magnitudo * Prob. Of Occurrence

A cluster analysis (3 groups) and a dendrogram graphic were performed with unloading and stunning variables correlation's with total carcass lesions in order to better understand the type of connection among the parameters observed (hazards) and the total score of bruises.

Results and Discussion

All factors potentially causes of lesions in carcass were analyzed into two groups, corresponding to the moment of observation, unloading and going to the stunning raceway for total carcass, ham, loin and shoulder-head respectively.

Carcass lesions

Table. 4. Classification of parameter for Risk of lesions on the total carcass

Parameters	Severity	Magnitudo	Frequency %	Distr. Likelihood	Prob. of Occ.	Risk
			Unloading			
rubber stick	2	0,5	18,75	0,21	3,94	1,97
backwards	1	0,25	6,49	0,07	0,45	0,11
slips	1	0,25	3,57	0,04	0,14	0,04
falls	2	0,5	1,73	0,02	0,03	0,02
balks	1	0,25	2,8	0,03	0,08	0,02
overlaps	2	0,5	1,96	0,02	0,04	0,02
overcrowds	1	0,25	2,2	0,02	0,04	0,02
			Toward Stunning			
rubber stick	2	0,5	91,67	1,01	92,59	46,29
backwards	2	0,5	11,96	0,13	1,55	0,77
back ups	2	0,5	6,9	0,08	0,55	0,28
pushes	2	0,5	1,96	0,02	0,04	0,02
overcrowds	2	0,5	1,73	0,02	0,03	0,02
slips	2	0,5	1,01	0,01	0,01	0,01

Distr=Distrubution; Prob=Probability.

For the total lesions of carcass at the unloading, the rubber stick was the only tool used by the handlers and the event with major frequency (18.75%), probably of occurrence (PO) of the adverse effect (3.94) and the higher risk (1.97) associated to lesions. The result agree with Chevillon et al. (1997) and Geverink et al. (1996), who reported a relationship between the use of stick and carcass lesions. The use of this device is just a routine and a reaction of handlers impatience for hesitation of pigs caused by novel sounds and environments connected to all transport's operations induce an high use of stick for moving on animals.

The transfer from the familiar pen to the novelty of the truck inner and the abattoir area, joined to the high physical activity induced by the costraint to go trought sloped ramps and along alleys by stockpersons, may cause fear in pigs, evoking reluctance to move on or try to reverse (Dalmau et al. 2009, Faucitano 2001).

Moreover is known that is practice of transport workers and abattoir employees make excessive use of sticks and/or goads to force and move pigs quickly the last metres prior to the stunning to mantain the rhythm of slaughter. (Dalmau et al. 2009, Faucitano 2001).

Pigs behaviors as backwards, slips and balks showed frequencies between 6.49 and 2.80 %, a PO ranged 0.45 and 0.08 and a negligible risk values, probably due to an indirect connection with skin bruises. Overlaps, falls and overcrowd showed the lowest frequencies, PO and risk values not relevant for lesions, unlike found by Weshenfelder et al. (2012) and Torrey et al. (2008), who justified greater skin damage by the great frequency of overlaps, slips and falls in this step. The PO of adverse effect was too low because these actions non strictly induce bruises.

Going to the stunning chute, the rubber stick was again the parameter with the highest frequency (91.67 %) and highest risk to cause lesions in carcass (46.29). It is well known that this tool, as well as electric prod, increase incidence of blemished carcasses (Rabaste et al. 2007, Guise and Penny 1989, Faucitano et al. 1998) and its overuse immediately before slaughter could decrease animal welfare and the glycogen stores in the muscles with a worsen quality of meat due to the occurrence of PSE (pale, soft, exudative) (Rabaste et al., 2007). This data reflect the trend of abattoir employees that, for impatience and the need to respect the slaughter speed, make an improper use of sticks and goads, especially near the stunning race (Faucitano, 2001) inducing panic in pigs and potential deep bruising in carcass (Szoucs et al. 2008).

The frequency of backwards along the alleys until stunning raceway gate was higher than at unloading (11.96 and 6.49, respectively), probably due to the fear of animals for novel, increased noise combined with the presence of employees with tools (stick) in a closer space as the restrainer pen: is just documented that, in reduced area and in presence of stress factors, pigs try to escape, changing the direction (Rabaste et al, 2007). The PO for backwards was calculated equal to 1.55 and the estimated risk of lesions 0.77. This behavior, not directly correlated to skin damage, could induce handlers that the use stick or rigid tube often too much and harshly to drive and separate pigs to the final single chute (Rabaste et al., 2007) maintaining constant the speed chain (Faucitano, 2001), but may causing deep bruises (Szucs et al. 2008).

Back up, observed only inside the restraining pen, had a frequency of 6.90 %, due to the very limited area for movement where can entry only 5-6 pigs. Moreover, the high use of the stick by the handler may had reduced this behavior of animals, that scared, usually went quickly on and entered inside the single chute. As consequence, the PO and the connected risk on carcass lesions were low (0.55 and 0.28).

Pushes, overcrowds, slips and falls showed the lowest frequencies probably explained by the fact that, in this scenario, the animals were quite calm. The risk of lesions was almost nonexistent, maybe because the events are more linked to animal welfare issue than directly to lesions one.

Lesions on ham, loin and shoulder-head

For the ham, at unloading the rubber stick remained the main hazard of lesions with the highest PO (3.94) and risk (1.97), reporting the same values than for total carcass. Pigs behaviors also reported a similar trend of the whole carcass with the backwards that was the second events connected to lesions (PO=0.48, risk=0.12) and the other behaviors with lower values, ranged between 0.15 and 0.04 for the PO and 0.04 and 0.02 for the risk, without any apparent connection with damage to this trait.

A similar situation occurred along the passageways toward the stunning, with the highest PO and risk found by the stick: 94.42 and 47.21, respectively. The behavioral parameters showed a low PO and risk values minimal and not relevant for lesions to the ham. However, it's true that during fighting's , that could happened in lairage of the plant, pigs removed are more susceptible to lesions in the rear of the body (Turner et al. 2006) and could be responsible of the lesions found in the hams.

For the loin area, at the unloading the rubber sick resulted the main hazard of skin lesions with PO and risk equal to 3.67 and 1.83 respectively. Backwards showed about the same numbers of the total carcass and the ham (PO=0.44 and risk=0.11), slips, balks, falls, overcrowds and overlaps ranged from 0.13 and 0.04 of PO and 0.03 and 0.02 of risk. For the moving to the stunning, the rubber stick recorded was the highest value (87.68) of PO as well as the risk (43.84), , followed by backward (1.49 and 0.75) back up, pushes, overcrowds and slips that had PO values among 0.50 and 0.01 and risk ranged from 0.25 to 0.01. These results not agree with Faucitano (2001), who reported that typical behavioral responses of pigs before slaughter make hard to handle them and create lacerations in skin, loin too.

At unloading and toward the stunning, also for shoulder-head the use of rubber stick was the major event linked to lesions (PO=87.68 and Risk=47.67). This data is probably due to the fact that the frequency of this parameter, as the other, was recorded when observed independently if it caused effectively lesions and in which part of the carcass. In fact the use of driving tools in this part of pigs' body was never seen in this scenario, also because its use toward this part could induce fear in pigs, who scared, wouldn't go on the pathway, but rather try to escape. Although the results, its possibly that the lesions recorded in this area are due to pre-slaughter fighting (Faucitano, 2001) or pigs' agitation, that jamming in the close passageways, injured themselves with contact of the concrete walls of alleys.

For animals behavior, backwards was in both two steps the factor more connected with lesions to this area, with highest values of PO and Risk, 0.45 and 0.11 at unloading, 1.67 and 0.84 to the stunning respectively; lesions could be caused by impacts of animals with the facilities during the backwards. Back up reported PO and Risk values highest of ham and loin (0.58 and 0.29) maybe explained with the fact that, once forced to move, pigs tended to overcrowd, jamming each other and beating with structures, especially against the gate of stunning chute. The same reason could explain the values of PO and risk, even if low, of pushes, overcrowds and slips (PO=0.05, 0.04 and 0.01 and Risk=0.02, 0.02 and 0.01, respectively) usually responses to anxiety and attempt to escape provoked by the need of operators to dissolve the group and reduce it to a single file (Faucitano, 2001).

Table. 5. Classification of parameter for Risk of lesions on ham, loin and shoulder-head at unloading.

Parameters	Severity	Magnitudo	Frequency%	Distribution of likelihood			Probably of Occurrence			Risk		
	ham, loin and shoulder-head			ham	loin	shoulder-head	ham	loin	shoulder-head	ham	loin	shoulder-head
rubber stick	2	0,5	18,75	0,21	0,20	0,21	3,94	3,67	3,94	1,97	1,83	1,97
backwards	1	0,25	6,49	0,07	0,07	0,07	0,48	0,44	0,45	0,12	0,11	0,11
slips	1	0,25	3,57	0,04	0,04	0,04	0,15	0,13	0,16	0,04	0,03	0,04
balks	1	0,25	2,80	0,03	0,02	0,03	0,09	0,08	0,10	0,02	0,02	0,02
falls	2	0,5	1,73	0,02	0,03	0,02	0,03	0,03	0,04	0,02	0,02	0,02
overcrowds	1	0,25	2,20	0,02	0,02	0,02	0,04	0,05	0,04	0,01	0,01	0,02
overlaps	2	0,5	1,96	0,02	0,02	0,02	0,04	0,04	0,05	0,02	0,02	0,01

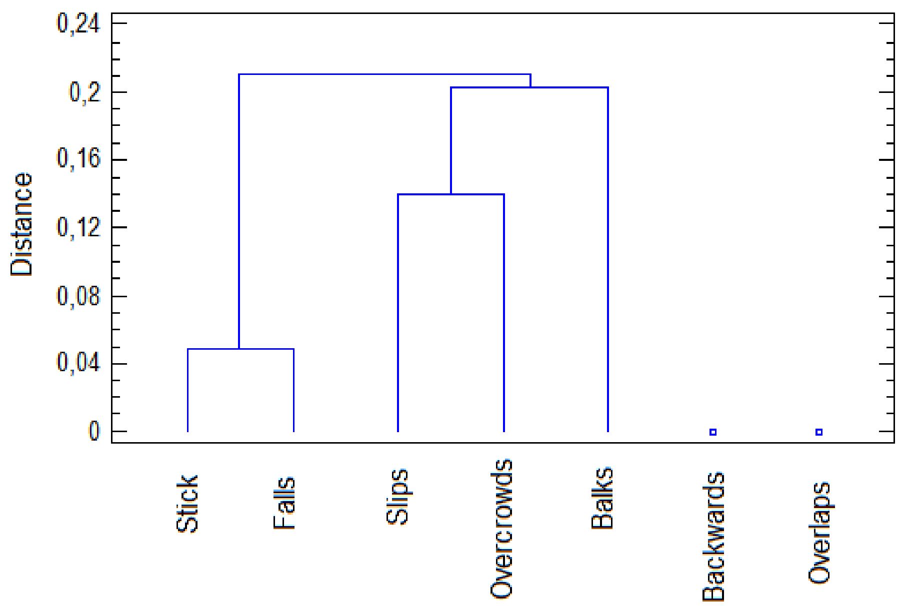
Table.6. Classification of parameters for Risk of lesions for ham, loin and shoulder-head toward the stunning.

Parameters	Severity	Magnitudo	Frequency%	Distribution of likelihood			Probably of Occurrence			Risk		
	ham,loin and shoulder-head			ham	loin	shoulder-head	ham	loin	shoulder-head	ham	loin	shoulder-head
rubber stick	2	0,5	91,67	1,03	0,96	1,04	94,42	87,68	95,34	47,21	43,84	47,67
backwards	2	0,5	11,96	0,13	0,12	0,14	1,55	1,49	1,67	0,77	0,75	0,84
back ups	2	0,5	6,90	0,08	0,07	0,08	0,55	0,50	0,58	0,27	0,25	0,29
pushes	2	0,5	1,96	0,02	0,02	0,02	0,04	0,04	0,05	0,22	0,02	0,02
overcrowds	2	0,5	1,73	0,02	0,02	0,02	0,03	0,03	0,04	0,02	0,02	0,02
slips	2	0,5	1,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01

Considering the risk characterization a sort of classification of the potential hazards, the cluster analysis gave a clearer representation of the linkage of the variables with lesions in general, as Arandom et al. (2012) who evaluated the relation among stress behaviors of pigs and blood parameters and transport time.

At unloading the use of stick is clearly connected to some pigs behaviors, as slips, falls, overcrowds and balks that are probably induced to it. Otherwise backwards and overlaps results in a single cluster each one because may be explained only by the fear of pigs of the new external environment (Figure 1).

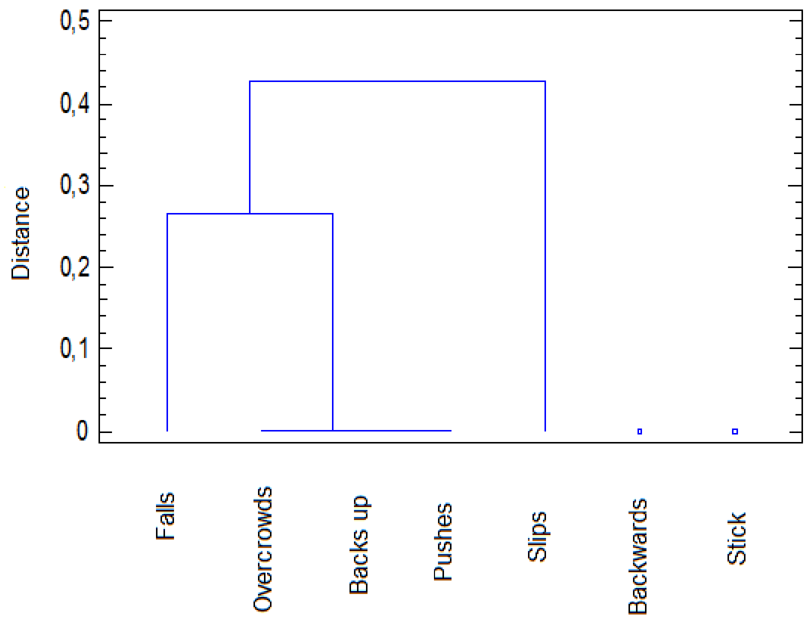
Figure 1. Dendogram of unloading hazards and total carcass lesions



The movement toward the stunning raceway (Figure 2) showed that all pigs behaviors, excepted the backwards, were connected together and, probably, one the cause of the other, due to the well-known stress and anxiety typical of this phase. Backwards not clustered with other pigs' parameters with which has no relation, but is linked to lesions perhaps the hits of pigs during backwards versus different surfaces of the plant, in the attempt to avoid to go on.

The use of rubber stick results in a single cluster indicating that operators' activity not depended from animals' responses, but tend to be routine behavior even if often excessively and aggressively acted that could be the direct cause of skin lesions in pigs body.

Figure 2. Dendogram of hazards toward the stunning and total carcass lesions



Conclusions

In the observed scenario, the risk of carcass lesions was mainly related to the use of rubber stick. The operator handling confirmed as an important hazard for the presence of bruises of the carcass during pre-slaughter phases, especially near the stunning where the animals management result more difficult and severe.

These results suggest that improving staff's training and providing better conditions during the driving of pigs at slaughterhouse could be an effective device to reduce the incidence of skin lesions and the consequent economic losses, as well improve animal welfare. It's well known that flags and paddle are more recommended tools for driving pigs, at contrary of sticks, goads and prod. However, all steps of pigs handling prior to slaughter should be taken into account, including loading at farm and transport, because lesions may be caused also in these critical phases.

Further studies should be done to identify the real causes of lesions and the moment of their infliction to reduce carcass damages and increase pigs welfare.

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CHAPTER 2

Effect of Transport Distance and Season on Some Defects of Fresh Hams Destined for DPO Production

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Simple Summary: Transport to the slaughterhouse is a stressful event for pigs. Travel duration and conditions can negatively affect animal welfare and carcass quality. Some defects in fresh hams are strictly connected to pre-slaughter transportation. Journeys with short (<37 km) and long (>170 km) distances may increase damage in fresh hams and decrease Denomination Protected Origin

(DPO) Parma dry-cured ham production.

Abstract: Pre-slaughter handling is related to defects in fresh hams that result in exclusion from the DPO (Denomination Protected Origin) Parma chain, including hematomas, lacerations, microhemorrhages and veining. To determine the effects of transport conditions on hams, we collected data on defects in 901,990 trimmed fresh hams from heavy pigs provided by 3,650 batches from slaughterhouse during 2012 and 2013. For all batches, transport distance (1–276 km) season and year of delivery were considered. A decrease of all defect occurrences was observed for increasing distance up to 170 km ($P < 0.05$). Above 170 km, however, all defects frequencies increased ($P < 0.05$). Season showed an effect on the incidence of defects, with an increasing of hematomas and lacerations in winter and autumn respectively ($P < 0.05$) and the highest percentage of veining and hemorrhages in spring ($P < 0.05$). Summer had the lowest incidence of defects on fresh hams. We concluded that the incidence of the examined defects and the subsequent rejection for DPO Parma ham production is lower in fresh hams transported 38–170 km during the summer.

Keywords: pigs; transport; distance; season; DPO Parma ham; pre-slaughter handling; defect

1. Introduction

Pre-slaughter is known to be a critical period for pigs [1,2], because several factors, *i.e.*, inadequate transport conditions, inappropriate handling [3] and length of the journey [4], can seriously affecting animals welfare both physically and psychologically. Additionally, carcass quality can be adversely affected by inadequate pre-slaughter conditions, such as rough practices of loading and unloading and inappropriate driving style of trucks and pigs that may lead to skin wounds, hemorrhages and hematomas [2]. Increased incidence of blood-splashed and skin damage was found in pigs handled roughly or driven by electric prods during pre-slaughter [5]. These injuries represent a significant economic loss, especially if they are located on the most valuable cuts such as hams, loins and shoulders. According to Von Borrell (2005) [6], transport is considered to be a major stressor might have deleterious effects on product quality. Previous research indicates that pig welfare is affected by both long and short journeys [7]. Mota-Rojas *et al.* [8] found that the percentage of bruised carcasses increases with journey duration, while Barton Gade and Christensen [9] reported an increased risk of skin damage during short transports (2–3 h), and Gispert *et al.* [10] found higher skin damage scores in short transports (<2 h) *vs.* long transports (>2 h).

The effect of the season on pig welfare and carcass quality have been also reported [11,12]. The frequency of skin blemishes, hematomas and blood splashing was shown to increase in winter, probably caused by slips and falls due to slippery icy floor [13–15].

Raw hams from heavy pigs (160–170 kg live weight) reared in Northern Italy are primarily processed to DPO (Denomination Protected Origin) dry-cured hams under the control of several consortia, of which Parma Consortium is largest, branding about 10 million dry-cured hams per year (PQI 2014, PQI 2013, Prosciutto di Parma.it) [16,17]. The Parma Quality Institute (PQI) [16] inspects fresh hams destined for the DPO dry-curing process for the presence of an inappropriate physical characteristics like lean-fat ratio or physical injuries, such as hematomas, microhemorrhages, muscle lacerations and subcutaneous veining caused by pre-slaughter handling; these lesions lead to exclusion of the cuts from the DPO Parma chain. A study of more than 100,000 fresh hams monitored by PQI in 2013 found that 4.3 % of these were rejected for DPO dry-cured ham production due to hematomas, microhemorrhages, muscle lacerations and subcutaneous veining.

Extended to the annual production of fresh hams destined to DPO Parma dry-cured ham equal to 16 million, the total number of fresh hams rejected for pre-slaughter defects could reach 689,000 with an estimated economic loss around 7.6 million euro per year for the whole Parma Consortium and Italian Food Industry.
The aim of this study was to evaluate the effects of transport distance, in connection to the slaughter season, on the incidence of defects observed on fresh hams destined for processing to DPO dry-cured ham.

2. Experimental Section

2.1. Data Sampling

This study was carried out in 2012 and 2013 on data collected from 901,990 trimmed fresh hams of heavy pigs provided in 3,650 batches supplied by 411 farmers and slaughtered over 396 days in a single plant located in Northern Italy. Each batch contained 7–151 pigs with an average of 124 animals per batch. All pigs were 9 months old with live weights of kg 171.1 ± 6.1, as required by the Parma dry-cured ham Consortium [18].

2.2. Pre-Slaughter Conditions and Abattoir

Transportation distances from farm to slaughterhouse ranged from 1 to 276 km and average shipping speed based on motorway proximity and road conditions was estimated at 60 km/h. The deliveries were carried out using double trailer lorries with three hydraulic deck. The lorries had natural and mechanical ventilation system, with fans placed on the left side of the trucks. Stocking density during transport was estimated ranging 141–251 kg/m^2 and the journey duration ranged 0.25–5 h. The loading was done using the mobile ramp commonly present at the piggery (length m 6.0, width m 0.7, with solid side walls of m. 1.0 and adjustable height), while unloading at the slaughterhouse was carried out by a platform (length m 9.3, width m 2.7, solid side walls m 1.0) with adjustable height at the level of the lower deck. The vehicle was always unloaded before the rear trailer. Lairage time ranged between 30 min to 1.5 h. During this period, pigs were not mixed with unfamiliar animals. Averages and ranges of seasonal temperature and relative humidity during 2012 and 2013 in the area where pigs were transported are shown in Table 1.

Table 1. Temperature (°C) and Relative Humidity (%) in the slaughterhouse area during 2012 and 2013.

	Temperature (°C)						**Relative Humidity (%)**					
	2012			**2013**			**2012**			**2013**		
	Average	Range		Average	Range		Average	Range		Average	Range	
Winter	3	−2	9	4	0	8	81	58	100	85	69	100
Spring	17	11	23	16	10	21	70	44	100	73	50	100
Summer	24	17	32	23	16	30	66	39	100	70	42	100
Autumn	10	6	15	10	7	15	83	65	100	86	68	100

Pigs were driven with plastic sticks or rubber boards to resting pens, where they remained 0.5–4.0 h, with only a few batches resting overnight. After resting, pigs were showered and driven through a single chute to the stunning cage. Stunning was accomplished by electrical tongs (head only; 170 V, 1.3 A). Carcasses were then exsanguinated horizontally for 3 min and hanged vertically for 10 min before being immersed into the scalding tank. Carcasses were hot-boned and kept in the cooler (2–4 °C) overnight. The next day, hams were trimmed to the commercial shape, classified for damages and selected according to market criteria.

2.3. Defects Observed

Data on defects such as hematomas, lacerations, microhemorrhages and superficial veining related to pre-slaughter practices were recorded on trimmed fresh hams. Hematomas are lumps formed by blood clots beneath the skin, typically associated with ruptured blood vessels, that can be produced by impacts against either the handling facilities or improperly used handling prods [3]. Lacerations are similar to dark hematomas distributed in the medial side of the ham and are caused by muscle tears, which may be related to slipping on wet floors during driving. Microhemorrhages are characterized by pinpoint bleeding in the muscles due to capillary rupture and are generally attributed to rough handling, electrical stunning and vertical exsanguination [19–22]. Superficial veining is a subcutaneous venous lattice affecting the medial, or sometimes the entire, surface of the thigh. This ham defect appears several hours after slaughter, is particularly noticeable during the dressing process and is still evident at the end of the processing and seasoning [23]. The incidence and severity of the veining defect was found to be associated with the prolongation of pre-slaughter procedures (loading, transport and lairage time), with the use of CO_2 stunning methods and with the increase of time between the separation of ham from the hot carcass and chilling from 15 min to 60 min [23]; however, the causes remain largely unexplained [19].

Trimmed hams were classified on the basis of the presence of defects, according to PQI photographic standards, by two trained slaughterhouse operators and recorded in an electronic database according to batch and producer identity. Defects were calculated as percentage per batch.

2.4. Statistical Analysis

Batches were classified by distance between supplier and slaughterhouse using the FASTclust procedure of Statistical Analysis System (SAS 9.3 Cary, NC, USA, 2009).

Data were assigned to four clusters based on the smallest Euclidean distance from the initial seed in the cluster. The number of batches assigned to each cluster and the respective mean distances, standard deviations (SD) and minimum (min) and maximum (max) distances are reported in Table 2. Slaughter days were grouped into seasons and were monitored over two years to evaluate defect incidence change from year to year. The distribution of batches and pigs between season and year is reported in Table 3. Defect incidences approximated a Poisson distribution and were log transformed by the GLIMMIX procedure (SAS 9.3 Cary, NC, USA) prior to statistical analysis. The model included cluster, season and year and their respective interactions as fixed effects, and farm within day of slaughter as random effects. The ILINK option was used to back-transform least squares means, and differences between least squares means were evaluated by Tukey-Kramer's test ($P < 0.05$).

Table 2. Distance and transport conditions for batches and pigs based on cluster.

Cluster (Distance)	1 (11–37 km)	2 (38–86 km)	3 (89–170 km)	4 (199–276 km)
No. of batches	1,573	990	347	740
No. of pigs	195,596	119,213	42,554	93,632
Average No. pigs/batch	124	120	123	127

Table 2. *Cont*.

Cluster (Distance)	1 (11–37 km)	2 (38–86 km)	3 (89–170 km)	4 (199–276 km)
Distance:				
-average (km)	21	50	121	237
-standard deviation (km)	10	9	18	12
-min (km)	1	38	89	199
-max (km)	37	86	170	276
Transport condition:				
-type of vehicle:				

single vehicle	298	246	74	112
double trailer	1,275	744	273	628
-estimated duration (h)	0.5	1–1.5	2–3	3–5
-average stocking density (kg/m²)	213	206	207	218

Table 3. Distribution of batches and pigs based on season and year.

Cluster (Distance)	1 (11–37 km)	2 (38–86 km)	3 (89–170 km)	4 (199–276 km)
Season				
Winter				
No. of batches	384	200	103	172
No. of pigs	47,765	24,581	12,881	21,759
Spring				
No. of batches	401	266	87	190
No. of pigs	49,563	31,662	10,842	24,181
Summer				
No. of batches	415	256	71	176
No. of pigs	50,496	30,659	8,458	22,608
Autumn				
No. of batches	373	268	86	202
No. of pigs	47,772	32,311	10,373	25,048
Year				
2012				
No. of batches	780	433	202	373
No. of pigs	96,406	52,193	26,148	46,808
2013				
No. of batches	793	557	145	367
No. of pigs	99,190	67,020	16,406	46,824

3. Results and Discussion

In 2012, the incidence of hematomas, lacerations and microhemorrhages observed in trimmed hams were 4.3%, 1.7% and 1.5%, respectively. In 2013, hematomas increased to 6.2%, while lacerations and microhemorrhages showed very similar incidences equal to 1.7% and 1.6%, respectively. During the two years, PQI, detected in the whole Parma chain (around 4200 farms and 130 slaughter plants) about twice as much the incidence of these defects [16].
The results of variance analysis and the least-squares means of defects frequency by sources of variation are reported in Table 3. Distance, season and year showed to have a significant effect on the incidence of defects under study. Significant interactions between distance × season and between distance x year were found for hemorrhages, and for hemorrhages and veining, respectively. Moreover, there was a significant season x year interaction for hemorrhages, veining and laceration. Incidence of defects decreased with increasing transport distance from Cluster 1 (11–37 km) to Cluster 3 (89–170 km) ($P < 0.05$). The higher incidence of defects associated with short transport distances may be due to the lack of time to lie down and recovery from loading stress. Also, a reduction of standing pigs and increasing journey duration could explain this result. Lambooij (2007) showed that the percentages of slaughter pigs standing during transport decreased with journey time, which leads to a reduction in risk of slips, falls and overlaps [2]. Additionally, these results could be the consequence of truck drivers working hurriedly to accomplish all planned transports for a given day, as well as the effect of the lower quality of rural roads, which represent the major part of the route in short journeys. Nevertheless, extending of transport distance above 170 km (Cluster 4) results in an increased incidence of defects, which reaches and exceeds the frequency recorded for Cluster 1. There were no differences in hematomas and veining between clusters 4 and 1, while lacerations and hemorrhages incidences were significantly higher (P <

0.05) in Cluster 4. Thus, the incidence of defects reported herein was not proportional to the increase of transport distance. Mota-Rojas *et al.* [8] observed an increase in pig carcass bruising prolonging the journey from 8 to 23 h. Gallo *et al.* [24] identified similar trends in slaughter beef transported for 36 h. The incidence of defects on fresh hams was also influenced by season of transport (Table 4). The percentage of hematomas was greatest ($P < 0.05$) in winter, decreasing in spring and summer ($P < 0.05$) and reaching the lowest incidence in autumn ($P < 0.05$). There are no differences between winter, spring and summer for lacerations, while the incidence of this defect was greater ($P < 0.05$) in autumn. Gosàlvez *et al.* [15] reported a higher incidence of bruised carcasses in pigs transported in winter and in autumn. Scheeren *et al.* [13] hypothesize that the greater proportion of skin damage in winter is linked to the tendency of pigs to stand during transportation to avoid making contact with the cold floor and walls of the trailer. Dalla Costa *et al.* [25] found that pigs were more difficult to handle in winter and need more coercion, which led to more handling-induced bruises. The higher incidence of lacerations observed in autumn could be related to the increase of live weight in this season, due to a restarting of feed intake for the decrease of temperature with respect to the summer. The average live weights recorded by the slaughterhouse during 2012 and 2013 confirms that in autumn there was an increase of more than one kg with respect to the summer (169.1 *vs.* 170.6 kg in 2012 and 169.4 *vs.* 170.9 kg in 2013). This seasonal increase of live weight of heavy pigs was also shown by the national slaughter statistics of heavy pigs [26].

The incidence of hemorrhages was greater ($P < 0.05$) in spring and in autumn while the veining defect was greater in spring. These results cannot easily be attributed to differences in seasonal conditions. The lowest occurrences of lacerations and hemorrhages were found in summer. Warmer temperatures recorded during this season probably lead to a more careful handling in order to reduce the risk of transport mortality [15,25,27]. It is well known that high environmental temperature is a risk factor for transport mortality [28,29].

A significant source of variation in the incidence of raw ham defects was found in studies over several years. The higher incidence of all defects was found in 2013, especially for hematomas and veining. Crisis-invested DPO dry-cured ham production became more important in 2013 with respect to the previous year. It is probably that the reduction of employees for reducing costs lead to an overworking of staff involved in transport and in pre-slaughter handling with a consequent reduction of its quality. The detrimental effect to animal welfare due to overworking of employees involved in animal handling was highlighted by Grandin [30].

Table 4. Results of variance analysis and least-squares means of defects incidence by sources of variation.

Sources of variation	Hematomas (%)	Lacerations (%)	Hemorrhages (%)	Veining (%)
	P / Least squares mean	P / Least squares mean	P / Least squares mean	P / Least squares mean
Distance	**0.0079**	**<0.0001**	**<0.0001**	**<0.0001**
1 (11–37 km)	4.57 a	1.48 b	1.32 b	11.04 a
2 (38–86 km)	4.44 ab	1.31 c	1.34 b	11.14 a
3 (89–170 km)	4.08 b	1.30 bc	1.08 c	9.24 b
4 (199–276 km)	4.78 a	1.91 a	1.77 a	11.50 a
Pooled SEM(*)	0.11	0.06	0.05	0.22
Season	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Winter	6.53 a	1.32 b	1.19 b	12.05 b
Spring	5.88 b	1.33 b	1.74 a	15.23 a
Summer	3.58 c	1.28 b	0.96 c	8.70 c
Autumn	2.87 d	2.13 a	1.71 a	8.18 c
Pooled SEM(*)	0.12	0.06	0.05	0.24
Year	**<0.0001**	**0.0359**	**<0.0001**	**<0.0001**
2012	3.70 b	1.42 b	1.24 b	9.73 b
2013	5.37 a	1.55 a	1.48 a	11.74 a
Pooled SEM(*)	0.08	0.04	0.04	0.04
Distance × season	**0.7421**	**0.3412**	**0.0352**	**0.5894**
Distance × year	**0.1526**	**0.7083**	**0.0137**	**0.0007**

Season × year	0.1926	0.0056	<0.0001	<0.0001

(*) SEM: Standard Error of Mean; Means in the same column without the same letter differ significantly (P < 0.05).

Interaction between distance and season (Table 5) affected the incidence of hemorrhages (P < 0.05). The defect increased markedly when distance increased above 170 km in spring and in autumn (P < 0.05). Differences between seasons were observed for less than 37 km distance (Cluster 1), with summer showing the lowest incidence of hemorrhages (P < 0.05). Above this distance, the lowest incidences of the defect were found in summer and in winter (P < 0.05).

Table 5. Effect of interaction between distance and season on the incidence of hemorrhages.

	Cluster (Distance)				Pooled SEM
	1 (11–37 km)	2 (38–86 km)	3 (89–170 km)	4 (199–276 km)	
Hemorrhages (%)					
Winter	1.36 ab x	1.14 ab y	0.87 b y	1.49 a y	0.0931
Spring	1.55 b x	1.71 b x	1.58 b x	2.16 a x	0.1096
Summer	0.98 ab y	1.00 ab y	0.65 b y	1.33 a y	0.0912
Autumn	1.48 b x	1.67 b x	1.52 b x	2.26 a x	0.1527

Least square means within a row with different letters (a–b) differ (P < 0.05); Least square means within a column with different letters (x–y) differ (P < 0.05).

Interaction between distance and year (Table 6) affected the frequencies of hemorrhages and veining. They increased both in 2012 and 2013 when distance increased above 170 km. The lowest incidences of these defects (P < 0.05) were found for cluster 3 (89–170 km) in 2012 only.

Table 6. Effect of interaction between distance and year on the incidence of hemorrhages and veining defects.

	Cluster (Distance)				Pooled SEM
	1 (11–37 km)	2 (38–86 km)	3 (89–170 km)	4 (199–276 km)	
Hemorrhages (%)					

2012	1.32 a	1.27 a	0.91 b	1.57 a x	0.0688
2013	1.33 b	1.42 b	1.28 b	1.99 a y	0.0796
Veining (%)					
2012	10.39 a x	10.63 a	7.63 b x	10.64 a x	0.2838
2013	11.73 y	11.68	11.17 y	12.42 y	0.3366

Least square means within a row with different letters (a–b) differ (P < 0.05); Least square means within a column with different letters (x–y) differ (P < 0.05).

Interaction between season and year (Table 7) affected the incidences of lacerations, hemorrhages and veining. No differences between years were observed for lacerations but these differences appeared for hemorrhages in summer and autumn and for veining in spring, summer and autumn. These results confirmed the effects of the season on the incidences of raw ham defects. With regards to the influence of the year, as stated before, its effect appears related to a reduction of the quality of animal handling.

Table 7. Effect of interaction between season and year on the incidence of lacerations, hemorrhages and veining defects.

	Season				**Pooled SEM**
	Winter	**Spring**	**Summer**	**Autumn**	
Lacerations (%)					
2012	1.19 b	1.31 b	1.16 b	2.23 a	0.0824
2013	1.47 b	1.35 b	1.41 b	2.04 a	0.0873
Hemorrhages (%)					
2012	1.28 b	1.71 a	0.71 c x	1.53 b x	0.0711
2013	1.11 b	1.76 a	1.30 b y	1.90 a y	0.0802
Veining (%)					
2012	11.40 b	17.55 a x	6.72 c x	6.60 c x	0.3040
2013	12.73 a	13.06 a y	11.27 ab y	10.14 b y	0.3369

Least square means within a row with different letters (a–c) differ (P < 0.05); Least square means within a column with different letters (x–y) differ (P < 0.05).

4. Conclusions

The study has examined the effects of travel distances from 1 to 276 km, showing that both short and long journeys may have adverse effects on fresh ham defects. The higher incidence of defects associated with short transport distances may be due to the lack of time to lie down and recovery from loading stress, but probably also to hauliers working quickly to accomplish all planned transports for a given day. Additionally, these results could be related to the lower quality of rural roads, which represent the major part of the route in short journeys. Extending the transport distance above 170 km results in an increased incidence of raw ham defects; the incidence of defects reported herein was not proportional to the increase of transport distance. This research highlights the need for Italian pig industry to improve pre-slaughter handling in order to increase the quality of raw hams for DPO production, reducing the economic losses related to inadequate practices before slaughter and increasing the welfare of animals to make it as good as possible. However, additional research is necessary to understand seasonal influences on frequencies of defects on the fresh hams destined for the DPO process.

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Conflicts of Interest

The authors declare no conflict of interest.

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CHAPTER 3

The effect of the stress immediately prior to stunning in different breeds on pro-, macroglycogen, lactate and pork meat quality traits

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Abstract

Muscular glycogen plays an important role on the extent of tissue pH decline, following slaughter. Two glycogen fractions were examined—proglycogen and macroglycogen—which are metabolized with different priority. Little is known about the effects of pre-slaughter handling or differences between breeds on glycogen fractions and pork meat quality traits. Therefore, the aim of the study was to evaluate the levels of lactate, pro-, macro-, and total glycogen, and meat quality traits in two different muscles across three different breeds of pigs, in the presence or absence of physical, pre-slaughter stress. Twenty-eight pigs of the Italian Large

White, Italian Duroc, and Pietrain breeds were subjected to rough (RPH) or gentle (GPH) pre-slaughter handling. RPH group pigs were subjected, before stunning, to a fast driving, which was supported by the use of electric prods, whereas GPH group pigs were driven slowly, without electric prods. Pre-slaughter handling showed no significant effects across breeds (P>0.05) on the levels of proglycogen and macroglycogen within the *longissimus dorsi* muscle; however, glycogen levels were lower in pigs subjected to RPH. In *semimembranosus* muscle, the level of macroglycogen was lower in RPH group pigs (P<0.01); handling groups showed no significant effect on proglycogen levels (P>0.05). In *longissimus dorsi* muscle, the pH-24, drip loss, cooking loss, total loss, and shear force were not affected by handling (P>0.05). We conclude that physical stress, as a result of rough handling before slaughter, has potential to reduce the quality of pork meat.

Key words: Breeds; Glycogen; Meat quality traits; Pigs.

Introduction

The store of muscular glycogen, at the point of slaughter, plays a decisive role on the successive rate and extent of post mortem glycogen metabolism in pig muscles (Pösö and Puolanne, 2005). Glycogen synthesis and storage research (Alonso *et al*., 1995) has led to a renewed interest on the two constituent fractions, i.e. proglycogen and macroglycogen, which had been identified about 50 years prior (Wismer-Pedersen and Briskey, 1961), and more recently described in greater detail (Lomako *et al.*, 1993; Alonso *et al*., 1995). The proglycogen fraction is acid-insoluble, has a molecular weight (MW) of approximately 400 kDa, and a low ratio of glucose units to protein; the macroglycogen fraction is acid-soluble, has a MW of about 10^4 kDa, and a high ratio of carbohydrate to protein content (Lomako *et al*., 1993). These two forms are metabolized with different priorities under aerobic and anaerobic conditions (Asp *et al*., 1999; Graham *et al*., 2001).

Short-term stress, immediately before stunning for slaughter, increases the decline rate of pH and temperature in early *post mortem*, and consequently has a detrimental effect on pork meat quality traits (D'Souza *et al*., 1998; Henckel *et al*., 2000; van der Wal *et al*., 1999). In this situation, the muscular glycogen store is subjected to a rapid degradation, both *in vivo* and early *post mortem* (Henckel *et al*., 2002), but the role of the glycogen fractions in accelerating *post mortem* glycolysis and their relationships with pork meat quality are not well known.

The purpose of the present study was to investigate the effect of short-term, physical stress prior to stunning on proglycogen, macroglycogen, and lactate levels, and meat quality traits, by examining two muscles of pigs belonging to three different breeds.

Material and methods

The experimental protocol complied with the rules approved by the Ethic Committee of University of Bologna. The physical stress conditions applied before stunning were carried out in accordance with the Council Regulation (EC) procedure number 1099/2009 (European Council, 2009).

Animals and experimental design

A total of 28 pure bred, unrelated, castrated pigs were used; these included 10 Italian Large White (ILW), 10 Italian Duroc (IDU), and 8 Pietrain (PI) pigs. The selection criteria included pigs that were clinically healthy and were non-carriers of the recessive T allele (or n allele) of the g.1843C>T polymorphism of the ryanodine receptor 1 (*RYR1* or halothane) gene. Pigs were collected when at ~30 kg live weight, from the experimental research farm of our Department. They were randomly distributed amongst 8 pens; two pens per breed, with equal numbers of animals per pen. Pigs were fed *ad libitum* for 115 days using a commercial feed pellet, containing 16% crude protein (0.8% lysine), and 14.1 MJ/kg digestible energy. All pigs had *ad* *libitum* access to water via a nipple drinker. At the end of the 115 day growing period, all pigs were slaughtered within a single day, at the same abattoir. Mean live weights and the standard deviation were 114.0 ± 20.05 kg for ILW, 123.0 ± 12.57 kg for IDU, and 115.2 ± 9.24 kg for PI. Subjects of each breed were allocated randomly to two groups (5 ILW, 5 IDU, and 4 PI per group): gentle pre-slaughter handling (GPH, or non-stressed) and rough pre-slaughter handling (RPH, or stressed).

Pre-slaughter condition

The farm was located 127 km from the abattoir; transport time was approximately 2 hours, and the pigs were slaughtered immediately upon their arrival. The physical stress treatment for pigs of the RPH group began approximately 5 minutes before slaughter. These pigs were subjected to a fast driving, consisting of a 25-m run, supported by the use of electric prods, using shocks no longer than one second. Pigs in the GPH group were driven slowly for the same distance, without the use of electric prods. All animals were stunned by electronarcosis (V 220, Amp 1.3) before slaughter.

Sampling and meat quality analyses

Tissue pH, 45 min after slaughter (pH-45), was determined in the muscles *longissimus dorsi* (LD) at the last rib, and in the *Semimembranosus* (SM) on the left side of carcass. After 24 hours at a temperature of 0–4°C, pH was measured again (pH-24) in the LD muscle, in the same position. For practical reasons, the hams were sectioned before 24 hours had passed, so it was not possible to obtain a pH-24 reading for SM. pH was measured using a pH-meter equipped with a glass electrode (model 5232, Crison, Italy). In conjunction with the pH-45 measurement, approximately 10 g of sample tissue from LD and SM was collected from each animal, immediately frozen, and stored in liquid nitrogen for subsequent analysis of proglycogen, and macroglycogen, according to Adamo and Graham (1998). After slaughter, blood samples were collected and plasma samples were used to determine the concentration of lactate (Kit Randox).

Instrumental colour, cooking loss, drip loss, total loss, and shear force were evaluated in LD from the left side of the carcass. The objective CIE colour (L*a*b* system) was measured at 24 and 72 hours after slaughtering (Minolta Camera Ltda., Japan). Drip loss was evaluated in 10 g meat samples, which were weighed and suspended in inflated bags, at 0–4°C, for 48 hours. Thereafter, the samples were weighed again. Drip loss was determined as the difference between final and initial weight, and expressed as a percentage (Honikel, 1998). To measure cooking loss, samples were cooked in a water bath until the sample temperature reached 75°C (Honikel, 1998). Cooking loss was calculated as the difference between the sample weight before and after cooking, expressed as a percentage. After cooking, the samples were allowed to stand to reach room temperature (~15°C), then cut into 1 × 1 × 2 m pieces, and placed in a Warner-Bratzler apparatus (model 1221, Instron), perpendicular to the direction of the muscle fibres. Total loss was calculated as the sum of drip loss and cooking loss, and expressed as a percentage of the initial weight of the sample.

Statistical analyses

Data were analysed by the mixed-model procedure (PROC MIXED) of SAS (Version 9.2). The model included the fixed effects of pre-slaughter handling group (RPH or GPH), breed (ILW, IDU, PI), and their interactions. Where the interactions were significant, the means were compared by Tukey-Kramer test at a significance level of P=0.05. Carcass weight was used as a covariate, but was not significant and removed from the model.

Results and discussion

Based on molecular diagnostic test, all pigs were homozygous g.1843CC for *RYR1*. In our samples, the initial pH values (pH-45) in both muscles were greater than 6.0; the final pH levels (pH-24) in LD were higher than 5.3. This finding confirms that these were not PSE meats (Adzitey and Nurul, 2011).

The effect of pre-slaughter handling, across breeds, on pH-45, pH-24, lactate, pro-, macro-, and total glycogen are shown in Table 1. In LD and SM, pH-45 was significantly lower in the RPH group than in the GPH group (P<0.05), in agreement with previous studies (Dokmanovic *et al*., 2014; Peres *et al*., 2014; Hambrecht *et al*., 2004; Henckeç *et al*., 2000; van Der Walt *et al*., 1999).

Lactate levels in LD were significantly higher in the RPH group than in the GPH group (P<0.001), also in agreement with the results of previous studies (D'Souza *et al*., 1998; Hambrecht *et al*., 2004; Hambrecht *et al*., 2005). Despite higher levels of lactate recorded in the RPH group, we found no significant difference between final pH levels in the LD muscle. This is probably related to the short intensity and duration of physical efforts before slaughter; the same result was found in studies that evaluated similar intensities of pre-slaughter stress (Dokmanovic *et al*., 2014; Peres *et al*., 2014; Rabaste *et al*., 2007).

The lack of an observed stress effect on lactate levels in SM (P>0.05) may be due to the lower activity in this muscle of glycogen debranching enzyme (GDE), which is essential to control the rate of glycogenolysis and glycolysis (Kylä-Puhju *et al*., 2005). The GDE activity in LD and SM muscles of pigs was studied by Yla¨-Ajo *et al*. (2007), and they found lower activity of GDE in SM, which may be a limiting factor in lactate production. The difference in GDE activity between these two muscles is due to differences in cooling rates and metabolic speed (Kylä-Puhju *et al*., 2005).

Table 1. Effect of pre-slaughter handling and breed on pH, proglycogen, macroglycogen, total glycogen and lactate.

Variable	Pre-slaughter handling, Pre-s.		Breed			Pre-s	Breed	Interaction	R-MSE
	Gentled	Rough	ILW	ID	PI				
Longissimus dorsi, LD									
pH-45 minutes	6.43[a]	6.27[b]	6.44	6.36	6.25	0.04	ns	ns	0.21
Lactate, µmol/g	37.01[b]	50.89[a]	39.91	43.60	48.34	0.005	ns	ns	12.04
Macroglycogen, µmol/g	11.23	8.01	9.10	11.79	7.97	ns	ns	ns	4.38
Proglycogen, µmol/g	67.53	61.89	65.79	67.82	60.51	ns	ns	ns	27.44
Total glycogen, µmol/g	78.76	69.90	74.90	79.62	68.48	ns	ns	ns	26.92
pH-24 hours	5.51	5.50	5.55	5.51	5.47	ns	ns	ns	0.11
Semimembranosus, SM									
pH-45 minutes	6.46[a]	6.29[b]	6.46	6.35	6.31	0.01	ns	ns	0.17
Lactate, µmol/g	32.65	39.12	31.55	41.67	34.45	ns	ns	ns	10.37
Macroglycogen, µmol/g	16.71[a]	9.72[b]	16.76	12.02	10.87	0.001	ns	ns	4.88
Proglycogen, µmol/g	75.72	66.01	82.27	64.63	65.69	ns	ns	ns	20.17
Total glycogen, µmol/g	91.37	76.80	99.03	76.65	76.56	ns	ns	0.04 °	22.94

ns, not significant. ILW, Italian Large White. ID, Italian Duroc. PI, Pietrain. R-MSE, Root mean square error.

°=Interaction effect are showed on Figure 1; [ab] Different letters in the same row denote significant (P<0.05) differences.

In LD, for all breeds, significant effects of different stress treatments upon the levels of pro-, macro-, and total glycogen were not observed (P>0.05). This is probably due to the low motor action of this muscle (Young *et al.*, 2009). In SM, which has the higher motor action, the pigs of the RPH group showed a significantly lower content of macroglycogen than the GPH group (P<0.01). Macroglycogen is a source of energy in aerobic metabolism (Asp *et al.*, 1999), and is degraded during aerobic, low-intensity exercise (Essén-Gustavsson *et al.*, 2005). It follows, that the increased movement of animals subject to physical effort before slaughter is responsible for the decrease in macroglycogen levels. The lack of a significant pre-slaughter handling effect on proglycogen of both examined muscles (P>0.05) is expected, because proglycogen is largely degraded anaerobically (Sterten *et al.*, 2010; Yla¨-Ajo *et al.*, 2007; Rosenvold *et al.*, 2003). In the present study, the intensity of physical effort that RPH group pigs were encouraged to was not enough to significantly alter the levels of lactate in SM muscle, between the two groups of pigs.

A significant difference in SM glycogen levels was observed between different breeds and their administered stress treatments (P<0.05, Table 1). ILW pigs from the GPH group had significantly higher total glycogen levels than IDU pigs from the RPH group (Figure 1). Moreover, the total glycogen levels in these two groups were similar to the others. The effect of stress upon pigs is influenced by their genetic background (Terlouw, 2005), and the variation in glycogen content between breeds is due to the type of muscle fibres (Terlouw and Rybarczyk, 2008; Thompson *et al.*, 2006).

In both muscles, the levels of pro-, macro-, and total glycogen tended to be lower in animals subjected to RPH; little difference between breeds was observed within these parameters. Furthermore, the effect of handling varied between the two muscle types, being most evident in SM.

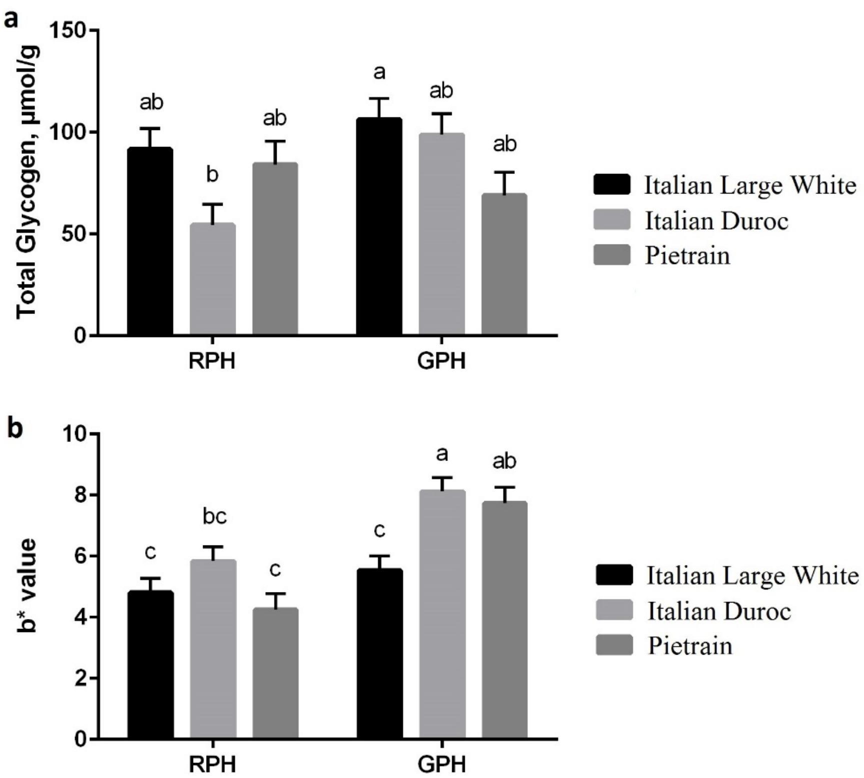

Figure 1. Interaction between pre-slaughter handling (RPH, Rouge. GPH, Gentle) and breed. a) total glycogen of *Semimembranosus.* b) b* 24 hours value in *Longissimus dorsi*. [abc] Different letters denote significant (P<0.05) differences.

The effect of pre-slaughter handling and breed on instrumental colour, drip loss, cooking loss, total loss, and shear force, in LD are shown in Table 2. Except for b* at 24 hours, there was no observed effect of stress on carcass meat quality traits (drip loss, cook loss, total loss and shear force; P>0.05); this agrees with the results of Peres *et al*. (2014).

As initial pH and lactate levels effect pork colour (Peres *et al*., 2014), it is also expected that stress will, too. However, in this study, breed was responsible for the main differences in pork colour.

The L* value, measured 24 and 72 hours after slaughter, was higher in PI than in ILW pigs (P<0.01); IDU showed values similar to both PI and ILW (P>0.05). The lack of a significant pre-slaughter handling effect on L* values is due to similar final pH levels in both the RPH and GPH groups (Table 1). Lower final pH values are responsible for greater damage to proteins, due to the approach of the isoelectric point; this increases the amount of free water and causes greater scattering of light and consequently higher L* values (Brewer *et al*., 2001). At the final pH (pH-24), the L* values observed (less than 60) do not indicate the occurrence of PSE meat (Adzitey and Nurul, 2011).

When animals physically exert themselves, there is an expected increase in levels of myoglobin (Lawrie and Ledward, 2012), and consequently, an increase in a* values. This was not significantly observed in our study (P>0.05), nor in a similar study by Dokmanović *et al*. (2014). However, the effect of pre-slaughter handling stress upon b* values varied according to the breed. When measured 24 hours after slaughter, the values in RPH groups were lower than those in GPH groups, for Italian Duroc (ID) and Pietrain (PI). without effect of handling group in Large White (Figure 1). When measured 72 hours after slaughter, no significant difference was observed in b* values between handling groups, but the ILW breed showed significantly lower values when compared to the 24 hour values (P<0.01). It seems that pigs subjected to more intense physical exertions, tend to have lower b* values, exhibited as more yellow colour of the meat in the RPH group. Similar results were found by Hambercht *et al*. (2005), who studied the effect of long duration transport in pork quality.

In general, handling stress had little influence upon parameters that indicate meat quality; any influence observed was largely restricted to instrumental colour. These results could be due to the low intensity and short duration of pre-slaughter stress that the RPH group pigs were exposed to. In addition, measurements were taken from a postural muscle (LD), which is less affected by stress and movement than locomotor muscles like the SM (Correa *et al*., 2014). The results suggest that the effect of pre-slaughter handling upon pork colour of LD varies by breed.

Table 2. Effect of pre-slaughter handling and breed on color, drip loss, cooking loss, total loss and shear force in *Longissimus dorsi*.

Variable	Pre-slaughter handling, Pre-s.		Breed			Pre-s	Breed	Interaction	R-MSE
	Gentled	Rough	ILW	ID	PI				
L* 24 hours	54.31	53.27	51.10[b]	53.61[ab]	56.65[a]	ns	0.001	ns	2.67
a* 24 hours	9.17	8.45	8.64	9.16	8.63	ns	ns	ns	1.93
b* 24 hours	7.14[a]	4.97[b]	5.17[b]	6.98[a]	6.00[ab]	<0.001	0.003	0.033 °	1.03
L* 72 hours	55.75	56.84	53.45[b]	56.41[ab]	59.00[a]	ns	0.017	ns	3.74
a* 72 hours	4.15	3.40	3.64	4.15	3.53	ns	ns	ns	1.67
b* 72 hours	11.47	10.71	10.16[b]	11.71[a]	11.41[a]	ns	0.004	ns	1.00
Drip loss, %	3.25	3.41	2.70	3.55	3.74	ns	ns	ns	1.14
Cooking loss, %	18.41	16.97	18.80	17.92	16.35	ns	ns	ns	3.57
Total loss, %	21.06	19.78	20.96	20.82	19.47	ns	ns	ns	4.03
Shear force, kgf	2.44	2.27	2.47	2.23	2.30	ns	ns	ns	0.34

ns, not significant. ILW, Italian Large White. ID, Italian Duroc. PI, Pietrain. R-MSE, Root mean square error. °=Interaction effect are showed on Figure 1; [ab] Different letters in the same row denote significant ($P<0.05$) differences.

Conclusions

The effects of pre-slaughter handling in pork meat quality indicators were limited to instrumental colour. Additionally, rough handling affected initial pH values and lactate levels within the muscles. The levels of pro-, macro-, and total glycogen also tended to be lower in animals subjected to RPH. We conclude that RPH, in the conditions studied, is both a stress factor and a welfare concern that has the potential to negatively affect meat quality.

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GENERAL CONCLUSIONS

In a context of increasing attention towards animal welfare and excellent productions, this thesis focused on the examination of the impact of pre-slaughter management on pig's behavioral and physiological responses, as well as on pork quality. This thesis evidences how stockpeople handling, the transport practices and in general pre-slaughter stress conditions influence the welfare of animals before death, the carcass appearence and the technological quality of meat.

The conclusion of the first work is that during unloading at slaughter plant of pigs and the driving toward the stunning, the operators handling is the main cause of lesions on carcass and on hams. Handlers'behaviour affected damages score due to an excessive and harsh use of driving tools, that in the studied scenario is represented exclusively by the rubber stick. The use of this device induced panic in pigs which hutting against facilities and the disorder of agitated animals make the handlers frustrated and more stimulated in using moving devices too frequently in order to contain animals and to feed properly the speed chain.

Animals behaviour responses are at the same time an indicator of poor welfare and a risk factor for skin bruises with a probable reduced carcass important traits quality as ham, loin and shoulder. Risk assessment application resulted a good method to monitor pre-slaughter practices in order to identify carcass damage causes.

The second work of the thesis underlines how travel conditions and distances significanlty affect the presence of defects in ham causing the rejection by DPO Parma Consortium. Too short and too long transportation distances both increase the frequency of damages in raw hams, specifically heamatomas, lacerations, microhaemorrhages and veining. The incidence of defects and carcass damages in general is connected also to the season of transport, with a tendency to greater heamatomas and lacerations in cooler months and major blood-splashed defects (microhaemorrhages and veining) in warmer seasons.

The third and last work of this thesis in fact shows that pro- macro- and total glycogen levels tend to be lower in precious muscles as *longissimus dorsi and semimembranosus* following ante-mortem rough handling and strong physical effort independently from stress breed prone. Even if in the study there wasn't found relevant changes in quality indicators as colour, drip and cooking loss and share force, the influence also on pH and lactate confirm, as other studies, that the handling manner is a real potential cause of worseing of quality meat degree as well as of pigs welfare.

Further and deeper studies should be performed to better understand which are the critical points of pre-slaughter period in order to eliminate the risk factors in general. The risk assessment could be a good approch for this object because is a non invasive method that give a final more complete view of the whole process. If more applied in slaughter pig field could be standardazied and adopted by all pig industry as a control routine practice with a general improvement of both the state of pigs and the quality of carcass and its single traits.

Surely trainig programs for operators and better handling practices at the abattoir are the base for an improvement of animals conditions before slaughter and limiting carcass and meat downgrading for damages.

To guarantee the best travel conditions considering the lenght of journey and the period of the year improves also animal welfare and reducing ham defects and the economical losses due to their refusal by DPO Consortia.

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