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THE IMPACT OF SWINE WELFARE ON SOME QUALITATIVE TRAITS OF MEAT AND LONG CURED ANIMAL DERIVED PRODUCTS

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DISSERTATION

THE IMPACT OF SWINE WELFARE ON SOME QUALITATIVE TRAITS OF MEAT AND LONG CURED ANIMAL DERIVED PRODUCTS

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ABSTRACT OF DISSERTATION

The present dissertation collects the results of three different research trials which, although exploring different aspects of animal welfare and animal derived products quality, have the common aim to understand the effects of swine welfare (both at farm level and during transport) on the main fresh and dry-cured meat characteristics.

In the first trial, two experiments were carried out in order to compare the effects of illumination regimes differing in light duration (14 vs. 8 hours/day at 70 lux, trial A) or light intensity (80 vs. 40 lux over a 12-hour lighting period, trial B) on meat and ham quality of Italian heavy pigs (Body Weight at slaughter of about 160Kg). Pigs subjected to a longer photoperiod (trial A) showed a tendentially higher ($P < 0.1$) BW at slaughter and a higher ($P < 0.01$) saturation degree of subcutaneous fat of the raw thighs, while cured hams deriving from pigs subjected to the higher illumination intensity (trial B) showed a higher ($P < 0.05$) relative content of polyunsaturated fatty acids. However, these differences didn't significantly affect ham quality, as determined by chemical and sensorial analysis. Our results support the conclusion that within a moderate range of light intensity and given an appropriate dark period for animal rest, an increase of light duration or intensity above the minimum mandatory levels has no negative impact on carcass composition, meat or long-cured hams quality.

The second trial was designed with the aim to investigate the effects of water restriction on growth traits, animal welfare and meat and ham quality. Two groups of liquid-fed Italian heavy pigs were compared: one having a permanent supply of fresh water by means of nipple drinkers, and the other having no water supply except that delivered with food. Overall, the parameters analyzed as concerns growth rate, behavioural traits, blood, as well as carcass, fresh meat and cured hams quality showed no significant differences ($P > 0.05$) between the experimental groups. Water consumption data combined with the observed drinking behaviour seem to indicate that a high amount of the water delivered from the drinkers (approximately 80%) was actually not ingested by the animals, but wasted. Nevertheless, even though a low number of visits to the drinker was recorded for both groups, it's noteworthy that liquid feeding did not suppress drinkers use or drinker manipulation in both groups. Therefore, water restriction does not appear to be an applicable method to obtain a reduction of water waste (and of subsequent manure production). A proper installation and maintenance of the drinkers, together with the provision of adequate enrichment material, could instead reduce both water waste and exploratory activities directed towards the drinkers, without preventing the pigs from having fresh drinking water permanently available.

The third trial was carried out in Canada and was focused on heat stress during commercial transport of market-weight pigs (115±10Kg BW) during summer. Its aim was to assess the effectiveness of water sprinkling pigs in reducing heat stress response

in terms of pigs' blood lactate, carcass and meat quality. Over 12 weeks, pigs were transported to the slaughter plant (2 hour trip) with two pot-belly trailers, one of which was equipped with a water sprinkling (WS) system. Animals were sprinkled for 5 minutes in the stationary truck both at the farm (immediately after the end of loading) and at the slaughter plant (immediately before unloading). Animals transported in the WS truck showed significantly lower exsanguination lactate levels ($P<0.05$) and greater pH value of the *Longissimus dorsi* and *Semimembranosus* muscles at 1hour *post-mortem* ($P<0.01$and $P<0.05$, respectively). The truck compartment where animals were located during transport determined considerable variations in signs of heat stress and meat quality, probably depending on the microclimate of the compartment. The results of this study showed that sprinkling pigs at ambient temperatures greater than 20°C may improve animal welfare and pork quality, particularly in pigs transported in compartments located in the front and in the rear of the middle deck.

This body of research supports the general conclusion that swine welfare could be effectively improved in different scenarios through simple and cost-effective means. At farm level, simply improving the installation and maintenance of illumination and water distribution systems could improve animal welfare, without negatively affecting the quality of the main animal-derived DPO products, whereas during transport, an appropriate sprinkling protocol could effectively reduce the heat stress experienced by the animals and improve meat quality.

RIASSUNTO DELLA TESI

Questa tesi raccoglie i risultati di tre differenti ricerche che, sebbene indaghino su differenti aspetti del benessere animale e della qualità dei prodotti di origine animale, hanno come obiettivo comune la comprensione delle relazioni tra il benessere del suino (sia a livello di allevamento, sia durante il trasporto) e le principali caratteristiche della carne fresca e stagionata.

La prima ricerca si compone di due diverse prove sperimentali, ed ha lo scopo di confrontare gli effetti sul suino pesante (peso medio alla macellazione: 160Kg) di programmi di illuminazione che differiscono per durata del fotoperiodo (14 vs. 8 ore di luce al giorno ad una intensità di 70lux, prova A) o per intensità luminosa (80 vs. 40 lux per una durata di 12 ore, prova B), in termini di qualità della carne e dei prosciutti stagionati. Secondo i risultati della prova A, i suini sottoposti ad un fotoperiodo prolungato hanno mostrato un peso alla macellazione tendenzialmente più elevato ($P>0.1$) e un più elevato grado di insaturazione del grasso sottocutaneo proveniente dalle cosce non stagionate ($P<0.01$), mentre i prosciutti stagionati derivanti da animali sottoposti ad intensità luminose più elevate (prova B) hanno mostrato un più elevato contenuto di acidi grassi polinsaturi nel grasso sottocutaneo. Ad ogni modo, come mostrano i risultati dell'analisi sensoriale e dell'analisi chimica, queste differenze non hanno modificato la qualità dei prosciutti in maniera significativa. Questi risultati supportano la conclusione che, entro un range di intensità luminose moderate e dato un periodo di buio di durata appropriata per consentire il riposo degli animali, un aumento della durata del fotoperiodo o dell'intensità luminosa al di sopra dei livelli minimi prescritti dalla legge non ha alcun impatto negativo né sulla composizione della carcassa né sulla qualità delle carni o dei prosciutti stagionati.

La seconda ricerca è stata condotta con lo scopo di studiare, in suini pesanti che ricevono un'alimentazione liquida, gli effetti del razionamento idrico sui parametri produttivi, il benessere e la qualità delle carni e dei prosciutti stagionati. Sono stati messi a confronto due gruppi sperimentali, dei quali uno aveva costantemente a disposizione l'acqua di abbeverata grazie ad abbeveratoi a succhiotto installati all'interno dei box, mentre il secondo gruppo non riceveva altra acqua oltre a quella che veniva somministrata insieme all'alimento. Nel complesso, I parametri analizzati per quanto riguarda l'accrescimento, il comportamento, i valori ematici, così come la qualità della carcassa, delle carni fresche e dei prosciutti stagionati, non hanno mostrato differenze significative fra i due gruppi sperimentali ($P > 0.05$). I dati relativi al consumo idrico combinati con il comportamento di abbeverata sembrano però indicare come una elevata percentuale (all'incirca l'80%) dell'acqua erogata dagli abbeveratoi non sia stata in realtà ingerita dagli animali, bensì sprecata. Ciononostante, anche se il numero di visite all'abbeveratoio osservate è stato ridotto in entrambi i gruppi sperimentali, è degno di nota come l'alimentazione liquida non abbia mai soppresso l'uso o la manipolazione degli abbeveratoi in nessuno dei due gruppi sperimentali.

L'utilizzo della restrizione idrica non appare pertanto un metodo attuabile per perseguire una riduzione degli sprechi (e di conseguenza della quantità di liquami prodotta). Si auspica invece che una corretta installazione e manutenzione degli abbeveratoi, assieme ad un incrementato uso degli oggetti di arricchimento ambientale, possa limitare sia gli sprechi di acqua che le attività esplorative dirette dagli animali verso gli abbeveratoi, consentendo nel contempo agli animali di avere l'acqua di abbeverata costantemente a disposizione.

La terza ricerca, che è stata condotta in Canada, è stata incentrata sullo stress da caldo durante il trasporto di suini al macello (peso di macellazione: 115±10Kg) nel periodo estivo. Lo scopo della sperimentazione è stato quello di valutare l'efficacia di un sistema di nebulizzatori (o doccette) installato all'interno dei camion e azionato quando il camion era stazionario, nel ridurre lo stress da caldo negli animali trasportati. La risposta allo stress è stata valutata in termini di lattato ematico, qualità della carcassa e delle carni fresche. Nell'arco di 12 settimane, i suini sono stati trasportati al macello (durata del viaggio: due ore) utilizzando due camion identici, dei quali uno era equipaggiato con un sistema di doccette all'interno dei compartimenti. Il sistema è stato azionato per cinque minuti nel camion stazionario, sia in allevamento (immediatamente prima della partenza) sia al macello (immediatamente prima dello scarico). Gli animali che hanno subito il raffrescamento hanno mostrato un lattato ematico più basso al dissanguamento ($P < 0.05$) ed un valore di pH più elevato nei muscoli *Longissimus dorsi* e *Semimembranoso* ad un'ora *post-mortem* (rispettivamente, $P < 0.01$ e $P < 0.05$). ($P<0.01$). La posizione degli animali nei diversi compartimenti del camion ha determinato variazioni considerevoli negli effetti delle doccette sullo stress da caldo e sulla qualità della carne, verosimilmente in dipendenza del microclima del compartimento stesso. I risultati dello studio hanno mostrato che l'utilizzo delle doccette, in particolare quando le temperature sono al di sopra di 20°C, può migliorare il benessere degli animali e la qualità delle carni, con effetti più accentuati nei compartimenti frontale e posteriore del piano intermedio del camion.

I risultati di questo percorso di ricerca supportano la conclusione generale che esistano mezzi semplici ed economicamente efficaci attraverso i quali il benessere del suino possa essere migliorato a diversi livelli della catena produttiva. A livello di allevamento infatti, la semplice ottimizzazione dell'installazione e della manutenzione dei sistemi di illuminazione e di distribuzione dell'acqua è in grado di aumentare il benessere dei suini senza avere impatti negativi sulla qualità delle carni fresche e dei prosciutti stagionati. Durante il trasporto invece, un protocollo adeguato per raffrescare gli animali può efficacemente ridurre gli effetti dello stress da caldo migliorando nel contempo la qualità della carne.

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CONTRIBUTION OF AUTHORS

This thesis consist of a collection of papers of which the candidate is an author or co-author. All papers in this thesis are either already published or under review for publication. Such a collection is aimed to have a cohesive, unitary character making it the report of a single program of research. For consistency and convenience, all manuscripts presented here follow the same format. However, copies sent to their respective journals follow their specific requirements.

Manuscripts based on the thesis:

- Sardi L., Nannoni E., Grandi M., Vignola G., Zaghini G., Martelli G. (2012). Meat and ham quality of Italian heavy pigs subjected to different illumination regimes. *Berliner und Münchener Tierärztliche Wochenschrift* 125(11/12), 463-468.
- Nannoni E., Martelli G., Cecchini M. , Vignola G.; Giammarco M.; Zaghini G., Sardi, L. (2013). Water requirements of liquid-fed heavy pigs: effect of water restriction on growth traits, animal welfare and meat and ham quality. *Livestock Science* 151, 21-28.
- Nannoni E., Widowski T, Torrey S., Fox J., Rocha L.M., Gonyou H., Vanelli Weschenfelder A., Crowe T., Martelli G., Faucitano L., The effects of water sprinkling on exsanguination blood parameters and carcass and meat quality variation in pigs transported during summer. Under review for publication in the *Meat Science* journal (revised version submitted on February 13th, 2013).

The work presented here (animal preparation, sample and data collection and analyses, preparation of the manuscripts) was performed by the candidate, under the supervision of Prof. Luca Sardi, Prof. Giovanna Martelli and Dr. Luigi Faucitano.

Ulysses Reunited with Penelope, after Primaticcio
Courtesy of the Artist Lindee Climo and Mira Godard Gallery

45'' X 48''
Oil on linen 1994
Private Collection
Artist Lindee Climo
Mira Godard Gallery, Toronto, Canada

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LIST OF SYMBOLS AND ABBREVIATIONS

The most commonly used abbreviations, symbols and acronyms are listed below.

ADG Average Daily Gain

BF Biceps Femoris

BW Body Weight

CP Crude Protein

DE Digestible Energy

DM Dry Matter

FCR Feed Conversion Rate

F-o-M Fat-O-Meater

Hb Haemoglobin

HCT Haematoctrit

LD Longissimus Dorsi

MUFA Monounsaturated Fatty Acids

N:L Neutrophil-to-Lymphocyte ratio

PDO Protected Designation of Origin

PUFA Polyunsaturated Fatty Acids

RBC Red Blood Cells

RMSE Root Mean Square Error

SD Standard Deviation

SFA Saturated Fatty Acids

SM Semimembranosus

TBARS Tiobarbituric Acid Reactive Substances

WB Warner-Bratzler (Shear Force)

WBC White Blood Cells

WHC Water-holding capacity

CHAPTER 1:

PREAMBLE

Animal welfare science advanced quickly in recent years, and many notable new developments occurred. The massive increase in public concern for animal welfare was accompanied by a parallel increase in the scientific study of animal welfare, which in turn provided research-based knowledge for political action (*i.e.*, new legislation) to improve animal welfare. Furthermore, animal producers, corporate customers, civil organisations, governments and inter-governmental organizations are developing voluntary, welfare-based quality assurance schemes for farmed livestock, which are being used to encourage or require the adoption of animal welfare standards in food production, and to assure the public that such standards are followed. Additionally, when considering animal welfare in a broad sense, the advent of new biotechnology, which makes it possible to engineer animals to suit our own needs, raises new animal welfare concerns, since any genetic modification carries the risk of compromising fitness and the capacity of animals to cope.

The debate over animal welfare is complex and needs to be understood while establishing new policies and procedures, especially considering how animal welfare, business profit, product quality and environmental sustainability might be aligned. From a production standpoint, it is reasonable to expect that substantial changes will occur only when it is recognized that there is profit in taking good care of animals.

Therefore, research in animal welfare should be focused in two main areas. Firstly provide, whenever possible, sound scientific confirmation to the general assumption that good welfare is good for meat quality, and secondly determine which management improvements might be put into practice in a cost-effective manner in order to efficiently improve the welfare of the farmed animals.

Within this framework, if the relationship between animal welfare at slaughtering and fresh meat quality is well established, and has been extensively investigated, the same cannot be said of the relationship between swine welfare (both on farms and during the pre-slaughter period) and the quality of the main animal-derived processed products. Furthermore, research in meat quality often focused either on the effects of different genetic lines or on the effects of pre-slaughter events (such as transport, lairage and handling), and rarely took into account the effects of different housing and management systems. The effect of different rearing techniques on meat quality is difficult to assess and can give inconsistent results, since other factors (such as pre-slaughter handling) can play an important role.

The aim of this body of research is to understand, in a broad sense, the relationships between housing and management techniques, animal welfare and meat quality. The choice of the management modifications proposed was based on their applicability at a reduced cost from a practical standpoint. Different scenarios will be taken into account, in the context of different producing systems: on-farm and in-transit animal welfare, fresh and dry-cured meat quality.

CHAPTER 2:

LITERATURE REVIEW

2.1 Ethics in animal production and animal welfare policies

In the 1900s, pigs rearing moved gradually from small-scale family production (with the family having a close, personal relationship with the pig and the killing being a troubling scene for the most sensitive members of the family) to a large-scale commercial production. In this framework, concern over the welfare of pigs did not disappear but changed in important ways. Instead of sympathy for individual animals based on a close interpersonal connection, animal welfare became a more abstract concern (Fraser, 2008a). The development of large-scale swine production metamorphosed pig production from small, extensive (outdoor), labour-dependent enterprises into large, intensive (indoor), capital-dependent, production systems and stimulated debate concerning its impact on animal/human health, environmental effects and concerns for the ethical care of animals (Kittawornat and Zimmerman, 2010).

While people are much more sensitive to the welfare of animals, few are prepared to abandon animals as a source of food and to close the chapter of animal agriculture in human history (Pascalev, 2006). Even if most nations recognize that animals have at least some rights and deserve a humane treatment and this is expressed in animal welfare laws and policies that protect animals in farms, research laboratories and in the wild, criticism of intensive animal production does not focus on the exploitation of animals as such but on the scarce living space allowed per animal, the barren environment in which the animals are kept, and the high production levels and concomitant behavioural and welfare problems of the animals (Blokhuis et al., 2000).

Following the “Brambell Report”(1965), where the concept of mental suffering was introduced, and the Amsterdam Treaty (EC, 1997), where animals were acknowledged as “sentient beings”, law and policies started to recognize the necessity to improve the protection and the respect for animal welfare. This resulted in the development of husbandry practices and rules for housing, transporting, breeding and killing farm animals that aim to minimize the suffering and enhance animal wellbeing.

> "The absence of a neocortex does not appear to preclude an organism from experiencing affective states. Convergent evidence indicates that non-human animals have the neuroanatomical, neurochemical, and neurophysiological substrates of conscious states along with the capacity to exhibit intentional behaviors. Consequently, the weight of evidence indicates that humans are not unique in possessing the neurological substrates that generate consciousness. Nonhuman animals, including all mammals and birds, and many other creatures, including octopuses, also possess these neurological substrates."
>
> Cambridge Declaration on Consciousness (2012)

Despite the huge interest in animal welfare, actual improvements in animal farming condition, especially under intensive farming systems, might be deemed to be minimal. Most of EU citizens believe that animal standards have been improved over the last ten years, but the large majority of public (77%) deems that further improvements are needed (Eurobarometer, 2007). The common belief is actually that animal scientists are failing society. The greatest need for animal science is not new discovery, but a better understanding of how animals best fit into strategies for sustainable production for the living environment (Webster, 2005). There is also a huge need of information on how to implement programmes to improve animal welfare at the practical level (Grandin, 2010).

There are different reasons why animal welfare policies fail. First of all, there are different conceptions of animal welfare, which are based on values and world-views that have deep roots in our culture and that are not resolved by scientific research. Actions designed to ensure high standards of animal welfare are not likely to achieve widespread support unless they take account of the different conceptions of animal welfare. In addition to being based on good animal welfare science, they will need to make a reasonable fit to the major value positions about what constitutes a good life for animals (Fraser, 2008b).

According to Grandin (2012), there are three kinds of really bad welfare policies: ones that are too vague, ones that have unintended bad consequences and may make animal welfare worse, and ones where policy makers get information from sources on only one side of the issue. Vague policies are so generalized and ambiguously written that making consistent enforcement is impossible. Words like “properly”, “adequate,” and “sufficient” should not be used in welfare guidelines or legislation unless these terms are defined, otherwise they could result in different interpretation from different inspectors. Policies with unintended bad consequences include sudden application of new laws, without considering the necessary period of transition, which often requires years of sustained work. Lastly, policies that fail to look at both sides of the issue are the result of the tendency for some people (both the animal advocates and the agricultural lobbyists) to rely on very biased literature. Policy makers should keep in close contact with people who actually work in the field in order to avoid the most extreme and abstract ideologies on animal issues, which usually come from people who have lost touch with what is actually happening to the animals and have never visited farms or slaughter plants. On the other hand, people who work in the “trenches” can become desensitized to suffering. The most effective managers for maintaining high standards of animal welfare are involved enough to care but not so involved that they become desensitized to suffering.

2.2 Challenges for livestock production

Livestock is currently one of the fastest growing agricultural subsectors in developing countries. This growth is driven by the rapidly increasing demand for livestock products, this demand being driven by population growth, urbanization and increasing incomes in developing countries (particularly in Africa and Asia). Human population is expected to reach 9.15 billion in 2050, with a range of 7.96-10.46 billion (FAO, 2009). Meeting the substantial increases in demand for food will have profound implications for livestock production systems over the coming decades.

While crop production growth will come mostly from yield increases rather than from area expansion, the increase in livestock production will come about more as a result of expansion in livestock numbers in developing countries, particularly ruminants (although in developed countries carcass weight growth is also expected to contribute) (Thornton, 2010). Besides the need for new pastures, increasing competition for land in the future will also come from biofuels, driven by continuous concerns about climate change, energy security and alternative income sources for agricultural households. The prices of food-feed crops are therefore likely to increase in the coming decades, dramatically reversing past trends. Depending on the economic choice of different actors belonging to the biofuel productive chain, and even governments and other institutions, the change in land use patterns will occur at different levels of intensity (Rathmann et al., 2010).

Furthermore, increasing livestock numbers in the future will clearly add to the demand for water, particularly in the production of livestock feed. In this context, more research is needed to ensure that livestock production in the future contributes to sustainable and productive use of water and natural resources (*e.g.*: precision agriculture and farming). In this framework, whole-system and life-cycle (‘cradle to grave’) analyses, by assessing the full range of costs and benefits, will become increasingly important as “sustainability indicators” (Heller & Keoleian, 2003; Thornton, 2010).

Confined livestock production in industrialized systems are the source of much of the world’s poultry and pig meat production, and such systems are being established in developing countries, particularly in Asia, to meet increasing demand. Parallely, developed countries will see a continuing trend in which livestock breeding focuses not only on production and productivity, but also on new, “societally important” traits such as product quality, increasing animal welfare, food safety, disease resistance and reducing environmental impact (Kanis et al., 2005).

There is conflicting evidence as to the potential for adding value to animal products through higher welfare standards. There are common questions regarding welfare-branded, organic and local food, for example, particularly in times of considerable economic uncertainty. Identifying situation where animal welfare can be increased along with profits, and quantifying these trade-offs requires integrated assessment frameworks that can handle the various and often complex relationships between animal welfare, management and performance (Lawrence & Stott, 2009).

2.3 Profiting from animal welfare: achieving the change

There is a vast wealth of information available about what management practice, activities and processes lead to improvements in animal welfare. However, the knowledge generated by science needs to be implemented on the ground by those people who have direct control over animal lives (farmers, transporters and abattoir staff). Beyond these professional figures, there are many other groups interested in improving animal welfare through human-behaviour change: farm advisors and sales representatives, farm assurers and standard-setting bodies, legislators, animal welfare charities and campaigners, veterinary surgeons and animal health technicians, animal welfare scientists, retailers and in some cases the purchasers of the final products (Whay and Main, 2010).

2.3.1 Demand-side solutions

In the last years, the focus on protecting animals and improving animal welfare through legislation has begun to be replaced by a 'mixed model' of legislative minimum standards supplemented by 'market-led' initiatives. Consequently there has been a growing focus on 'demand-side' solutions especially to improving animal welfare through stimulating 'demand' for high-welfare products (Lawrence and Stott, 2009).

A number of studies have recorded a willingness to pay for higher animal welfare expressed by both consumers and citizens (Lagerkvist & Hess, 2011), but the views expressed in response to questions by citizens (who take an ethical position on animal welfare issues) are not always reflected in actual purchasing behaviour generating market demand (Krystallis et al., 2009). Moreover, research suggests that improved farm animal welfare is strongly associated by consumers with other (often unrealistic) food attributes, such as food quality, safety, taste, nutrition and environmental impact, and that people's willingness to pay also reflects beliefs about these attributes (Edwards, 2005; Grunert et al., 2004).

Retailers demonstrate a great interest and use different strategies regarding the promotion of animal welfare provenance as a means of differentiating their products. The sale of premium products is often used to enhance public image and encourage more high-spending customers into stores. For this reasons, a myriad of Quality Assurance (QA) schemes for high-welfare products is emerging, but they cost money both to cover the cost of the inspection process and to address welfare problems as they arise. Most of them seek to increase retailers/producers incomes, either by giving an assurance of added value and thereby commanding a higher price, or through the assertion that by improving animal welfare farmers can reduce costs associated with lack of 'fitness' in their animals. It is reasonable to claim that farmers can profit from improvements to welfare through reduction of disease and injury (if the cost of prevention and treatment is not too high). However, many elements of improved husbandry as perceived by the public (and the animals), such as increased space allowances and enriched environment, can only be achieved at a cost to the producer (Webster, 2005).

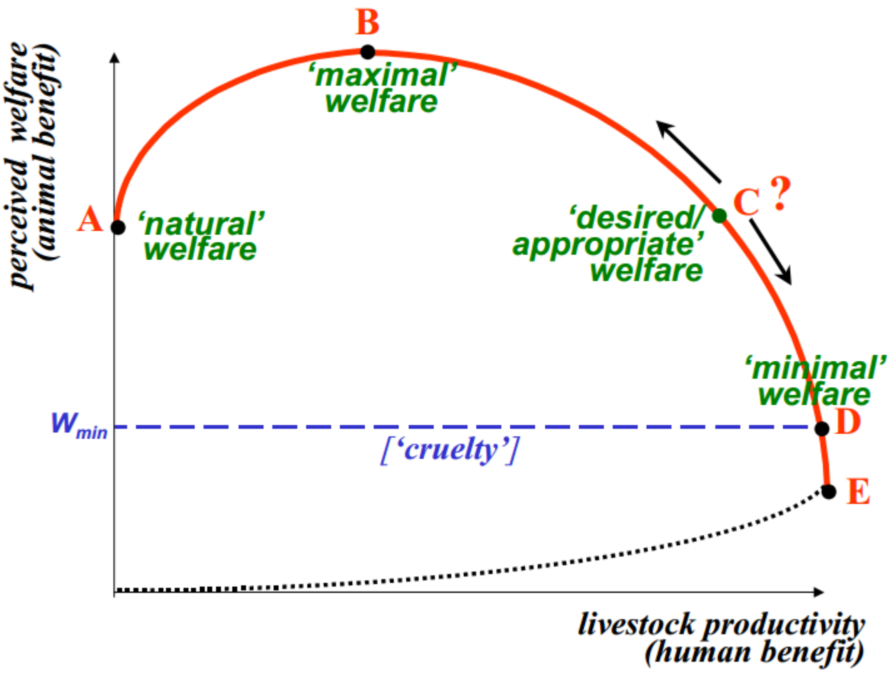
It is therefore completely unrealistic to assume that QA schemes can achieve significant improvements in animal welfare unless the customers are prepared to pay a significant premium. This, unfortunately, means that the extent to which such 'market-led' solutions will be effective in the long-term remains unclear due to issues such as the risks of market failure or fragility of consumer preferences in the context of economic recession (Lawrence and Stott, 2009). In time however, these niche markets tend to be saturated, the premium shrinks and the added value to the farmer disappears. So far as the animals are concerned, the problem with the niche market is that only a small minority of animals stands to benefit (Webster, 2005).

2.3.2 Supply-side issues: how much welfare can farmers afford to deliver?

On the other hand, if improvements in animal welfare must ultimately be mediated through changes in producers' strategic management decisions and day-to-day husbandry, the drivers determining farmer decision-making must be understood.

Over a decade ago, the agricultural economist, John McInerney, proposed that there is a non-linear relationship between welfare and productivity (Figure 2.1). The assumption is that as humans start to use animals, improvements in welfare and productivity coincide due to the inputs of feed, housing, protection from predators etc. that is afforded them. However, as levels of productivity increase, welfare may show no further improvement and then be increasingly impaired by the higher metabolic demands or environmental constraints placed on the animals.

Figure 2.1: Conflicts between animal welfare and productivity (McInerney, 2004).



The shape of this curve indicates also that initial welfare improvements can be gained at very little cost, but moves towards ‘high’ welfare standards become increasingly expensive.

When considering the converse question of how improvements in welfare affect performance and profitability, a non-linear relationship is also apparent depending on the extent to which improvements influence biological function. At the physiological level, animals subjected to repeated or prolonged stress show endocrine changes, which affect adversely many productive processes including synthesis of lean tissue and milk, feed intake and efficiency of feed use, reproductive efficiency in production of eggs or live offspring. There are also complex interactions between welfare and disease (stress can impair immune function, making animals more susceptible to disease) and, in the eyes of consumers at least, between welfare and food quality/safety (FAWC, 2011).

There are consequently strong, self rewarding drivers to improve welfare on farm when this is considered from the perspective of biological function. This is particularly clear in the case of basic requirements like good health, good nutrition and a suitable thermal environment, but is also true for freedom from fear and stress. Despite these relationships, good physical welfare is not always delivered in practice. This may be because of lack of knowledge, or other priorities for available time, labour and finance.

In some cases, the relationship between welfare attributes and the productive performance of farm animals is less clear. For example, the financial benefits of appropriate enrichment to meet behavioural needs may sometimes be negligible, but in other situations can reduce the risk of costly outbreaks of injurious behaviours such as tail biting in pigs. Where welfare is seen from the perspective of naturalness and as being synonymous with extensive outdoor systems, perceived improvements in welfare may not be reflected in greater production efficiency. Thus the banning of restrictive housing systems which do not allow animals to express normal behaviour, such as stalls for pregnant sows or cages for laying hens, impose costs in terms of greater capital investment, labour demand and management skill (FAWC, 2011). In situations where the cost of welfare improvement is not sufficiently rewarded by performance improvement, it is necessary to determine whether there is a market for animal welfare, which will compensate for these costs by means of a product price premium. For market forces to effect welfare improvement, there needs to be effective transfer along the supply chain of both information and reward.

In order to bring about a change in producers behaviour to address an animal welfare problem, different approaches should be used (Whay and Main, 2010):

- Producers whose animals are in the poorest welfare: this group is only susceptible to enforcement as a means of promoting change. In this case legislation and codes of practice should set minimum standards and ensure compliance before products can reach the market-place.
- The majority of producers are likely to make changes with a combination of encouragement (e.g. subsides) and enforcement, but are unlikely to actuate changes for themselves so they need external contact to initiate and, to some extent, sustain the process.
- The last group includes the producers which are self-motivated and self-actuating. They are rewarded by being able to produce premium-priced products and accessing niche market opportunities. This group does not require intervention as such, but will benefit from receiving access to new knowledge, scientific findings and exchange information with their peers.

2.4 Defining animal welfare

As has been often remarked, the term animal welfare emerged in society to express ethical concerns about the quality of life experienced by animals. The term is therefore a socially constructed concept, and not one that expresses a scientific concept. This is the reason why, in these first pages, the term “welfare” was used without defining it, as though its interpretation was clear and unambiguous. In fact, this is far from the case. A broad definition of welfare would include the notions of the animal in complete mental and physical health, the animal in harmony with the environment, the animal being able to adapt without suffering to an artificial environment provided by human beings, and that somehow the animal’s feelings should be taken into account (Duncan, 2005).

Scientists have provided many definitions of good animal welfare yet there is no consensus as to how to precisely define animal welfare. The inability of specialists to agree on a complete definition of welfare is in part due to the fact that there are so many different factors that could be used to determine an animal’s welfare. According to Fraser (2003), three main views about the welfare of farm animals developed and constitute now “value frameworks” which are closely connected to an individual’s world view and convictions:

- One view emphasizes the biological functioning of the animal in the sense of health, growth and productivity. In 1993, Mc Glone stated “an animal is in a poor state of welfare only when physiological systems are disturbed to the point that survival or reproduction are impaired.”. This first view is commonly heard among those who are involved in animal production.
- A second view emphasizes the “affective states” of animals – pain, suffering, and other feelings and emotions. This second view is commonly heard among humanitarians concerned with animal welfare. According to Duncan (1993) “neither health nor lack of stress nor fitness is necessary and/or sufficient to conclude that an animal has good welfare. Welfare is dependent on what animals feel”; before him, Dawkins (1988) stated “to be concerned about animal welfare is to be concerned with the subjective feelings of animals, particularly the unpleasant subjective feelings of suffering and pain”.
- A third view is that animals should be allowed to live in as natural circumstances as possible, where they can express their normal behavior. Kiley-Wortington (1989) stated “If we believe in evolution ... then in order to avoid suffering, it is necessary over a period of time for the animal to perform all the behaviors in its repertoire because it is all functional...”. This third view, emphasizing natural living, is common among consumers of animal products.

Scientists tend to bring to animal welfare assessment much the same three values frameworks outlined above. These three views of animal welfare are by no means mutually exclusive. Actually the different aspects of the concept of animal welfare have always to be taken into consideration in the studies on animal science. This means that, whatever the definition, all the biological components, concurring in determining the welfare level, have to be studied and linked together: the emotional state of the animal, its biological functioning and its ability to show normal patterns of behaviour (Carenzi and Verga, 2009).

The Five Freedoms, as developed and updated by the Farm Animal Welfare Council (FAWC, 2009) combine elements from the three approaches to welfare explained above and are a very useful framework to identify the main welfare problems as well as a starting point to identify the main welfare components (Velarde & Dalmau, 2012).

These freedoms, which represent ideal states rather than actual standards for animal welfare, include:

1. Freedom from Hunger and Thirst – by ready access to fresh water and a diet to maintain full health and vigour.
2. Freedom from Discomfort – by providing an appropriate environment including shelter and a comfortable resting area.
3. Freedom from Pain, Injury or Disease – by prevention or rapid diagnosis and treatment.
4. Freedom to Express Normal Behaviour – by providing sufficient space, proper facilities and company of the animal's own kind.
5. Freedom from Fear and Distress – by ensuring conditions and treatment which avoid mental suffering.

A variety of parameters (or variables) needs therefore to be selected, assessed objectively and then summarized in a "welfare score". When selecting variables to be included in animal welfare assessment, concerns about objectivity and scientific respectability may arise. In fact, although each variable may be scored objectively, values play a key role in the selection, weighing and interpretation of variables (Fraser, 2003).

2.5 Assessing animal welfare

When assessing animal welfare, monitoring systems and legislation largely rely on examination of inputs, 'what' or 'how much' of different resources are given to animals (i.e. space allowance, floor type, pen design, etc.). These parameters are easy to define, to measure and have a high inter and intra-observer reliability. However, these measures have often been criticized for potentially low validity due to their indirect nature and complex interactions with other resource and management conditions. Thus, input measures are a poor guarantee for good animal welfare, as animals may experience the same situation or handling procedure differently depending of their genetic background, temperament, or previous experiences.

Also in agreement with the OIE recommendations (2006), the most recent assessment protocols (*e.g.* Welfare Quality®, 2009) place their emphasis on animal-based measures (also called "outcome" or "performance" measures) rather than on the resource and management in an attempt to estimate the actual welfare state of the animals. Such physiological, health and behavioural measures have inherent advantages over input measures. Since welfare is a condition of the animal, outcome measures are likely to be the most direct reflection of their actual welfare state. They permit to evaluate the welfare by directly observing the animal, regardless of how and where it is kept. Secondly, animal-based measures permit to compare the welfare of animals from different farms or slaughterhouses (Velarde & Dalmau, 2012). Figure 2.2 shows some examples of animal- and design- criteria, Figure 2.3 shows the measures of the Welfare Quality ® assessment protocol.

Figure 2.2: Examples of directly observable criteria for assessing animal welfare for all species of animals (adapted from Grandin, 2010)

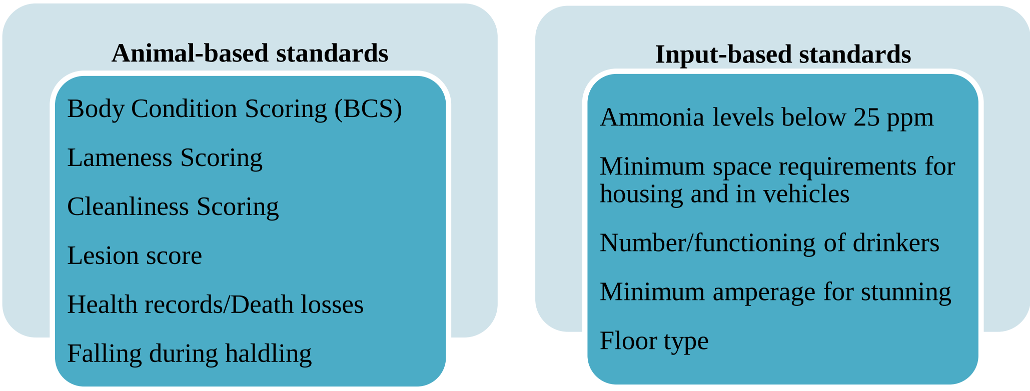


Figure 2.3: Synthesis of the measurements of the Welfare Quality® assessment protocols for growing-finishing pigs on farm (adapted from Welfare Quality®, 2009).

Welfare principles	Welfare criteria		Measures
Good feeding	**1**	Absence of prolonged hunger	Body Condition Score
	2	Absence of prolonged thirst	Water Supply
Good housing	**3**	Comfort around resting	Bursitis, absence of manure on the body
	4	Thermal comfort	Shivering, panting, huddling
	5	Ease of movement	Space allowance
Good health	**6**	Absence of injuries	Lameness, wounds on the body, tail biting
	7	Absence of disease	Mortality, coughing, sneezing, pumping, twisted snouts, restal prolapse, scouring, skin condition, ruptures and hernias
	8	Absence of pain induced by management procedures	Castration, tail docking
Appropriate behaviour	**9**	Expression of social behaviours	Socia behaviour
	10	Expression of other behaviours	Exploratory behaviour
	11	Good human-animal relationship	Fear of humans
	12	Positive emotional state	Qualitative Behavior Assessment (QBA)

Legend: ▢ Animal-based measurement, i.e. measurement that is taken directly on the animal
▢ Resource-based measurement, i.e. measure which is taken regarding the environment in which the animals are kept
▢ Management-based measurement, i.e. measure which refers to what the animal unit manager does on the animals and what management processes are used.

It is clear from the above-mentioned examples that input-based standards work well for specifying baseline minimum conditions for acceptable levels of welfare. However, to detect a problem with a poorly-designed environment, animal-based measures such as huddling, injuries, sleeping posture or body cleanliness should be used (Grandin, 2010). As concerns animal-based measurements it is worth noting that, if for auditing purposes simple and directly observable measurements should be used, when welfare assessment is applied in research and veterinary diagnosis, a wide array of animal-based measurements can be used, including for example blood parameters, extended behavioural observations and variation in physiological parameters.

2.6 The welfare of growing-finishing pigs

The growing-finishing period (*i.e.* the period from 4-8 weeks after weaning until pigs reach a market weight) in commercial swine production represents the phase of production with the longest time and the greatest opportunities for improvements in pig performance, health and welfare. The most direct influences on pig welfare through the grow-finisher stage are the quality and quantity of human interaction, the housing system, managements practices, facility design, genotype and the health of the pigs (Gentry et al., 2008).

2.6.1 Housing and management

Housing is usually a long-term condition for farm animals and thus results in a chronic state of an individual, be it stressed or not. Under intensive farming systems, growing-fattening pigs are confined to a limited, stimulus-poor space for economical, ergonomical and health reasons (Millet et al., 2005). Despite the good health status of the animals, when confined in pens pigs are no longer able to express their full range of species-specific behaviours and may experience chronic poor welfare if facilities are not properly designed and managed.

In indoor piggeries, high levels of ammonia and dust in the atmosphere can be irritating and suppress appetite and growth in the pigs (von Borell et al., 2007). Donham (1991) found several air contaminants (dust, ammonia carbon dioxide, and microbes) to be correlated to pneumonia and pleuritis. Pigs kept indoors in hot climates have few ways of keeping cool, since they have no possibility to wallow or move to the shade. According to Huynh et al. (2005b), clear physiological changes occur in fattening pigs starting at approximately 22°C.

Stressors such as high ambient temperature, regrouping and restricted floor space can reduce feed intake and weight gain, and were proved, when presented together, to have an additive effect (Hyun et al., 1998). Mixing unfamiliar animals can by itself promote aggression and fighting, and significantly depress productivity (Tan et al., 1990).

Research in a number of livestock industries has shown that interactions between stockpeople and their animals can limit the productivity and welfare of these animals, through increasing their fear of humans. Studies in the dairy and pig industries have shown the potential of cognitive-behavioural training programs to improve the attitudes and behaviour of the stockpeople towards their animals (Hemsworth, 2003).

As concerns pen design, floors should be comfortable for pigs to walk, stand and lie on and should not contribute to injury or distress. Accommodation for fattening pigs may be fully-slatted, partly-slatted, minimally bedded with scraped dunging area or deep bedded with straw or strawdust. Although there are national differences, housing with fully or partly-slatted flooring (typically on concrete slats with 17-20 mm slot spacing) predominates within the EU (EFSA, 2007). Slatted floors have benefits in terms of separating the animals from their excrement, thus leading to improvements in pen hygiene and reduced labour requirements. However, pigs show a preference for resting on solid rather than slatted floors (Aarnink et al., 1994). Quality of manufacture of slats is also important in terms of minimizing injuries, the most important factors being the absence of sharp or jagged edges, and the provision of non-slip, non-abrasive surfaces. Some Authors found injuries to the foot, and leg weakness problems, to be reduced if pigs are housed on solid rather than slatted floors (Jørgensen, 2003; KilBride et al., 2008), and that the prevalence of hock bursitis was higher in pigs kept on both partially slatted and totally slatted floors and lower in pigs finished on deep straw than in pigs kept on sparse straw bedding (Mouttotou et al., 1998).

Space allowance (area per animal) is another important aspect. The observed effects of space allowance on indicators of poor welfare, including performance, show that pig welfare is impaired if space allowance is too small. This could be because of difficulties in coping with space restriction per se, or with the impact of small space allowance on other relevant aspects of their housing conditions, such as aggression level, possibility to perform thermoregulatory behaviour, or ability to separate the lying from the dunging area (EFSA, 2005).

Adequate access to food for all group members is a prerequisite for good welfare, especially when pigs are subjected to feed restriction. Welfare problems arising from poor feeder space availability can lead to feed deficiencies or to competition for feed, with increased aggressive behaviour (SVC, 1997).

With respect to the water needs, insufficient availability of drinkers in relation to the number of animals, incorrect positioning or inadequate maintenance of drinkers precluding a correct flow of water are common management errors. As a rule, water flow rates vary from 500 ml/min for weaned pigs to 1000 ml/min and more for finisher pigs. Pigs usually adapt to a slow flow rate by increasing drinking time. On the other hand, when drinker flow-rate is higher than the recommended level, pigs increase water spillage. The effects of incorrect positioning of drinkers (*e.g.* low height), and increased flow rate are additive with incorrect positioning showing the more marked effect on water wastage (Li et al., 2005). As concerns drinker number, Turner et al. (1999 and 2000) suggested providing one drinker per 20 animals kept at 14-18°C with adequate water flow-rate. However, some production strategies include restricted water access. Such voluntary water restrictions primarily regard pigs on wet feeding systems and are aimed at saving water and reducing the final volume of animal waste.

When there is too little sensory input, because of social isolation, a barren environment or too little light duration and/or intensity, pigs are likely to show abnormal behavioural and physiological responses. Flashing lights can be disturbing to pigs and poor welfare is also associated with light of a wavelength or intensity that does not allow the pig to discriminate the behaviour of other pigs (i.e. light intensity below 10 lux). Also continuous loud noise can negatively affect pigs' bevaviour, because grunting and noises are widely used in communication between pigs and are an important stimulus in the pig's environment (EFSA, 2007).

2.6.2 Environmental enrichment

Environmental enrichment (presence of bedding, toys, human interaction, etc.) may be beneficial to growing-fattening pigs by allowing them to express their natural behaviour. Behavioural abnormalities can occur in captive animals as strategies to cope with the barren, restrictive environment and are usually elicited by frustration, stress, or lack of control over the environment. The most common type of behavioural abnormalities are stereotypies, which are repetitive invariant behaviours, with no obvious function (*e.g.* Mason, 1991).

Intensive rearing conditions do not allow pigs to express their exploratory behaviour. Pigs have a strong motivation to perform manipulative or “rooting” behaviour with the snout, and in natural environments will spend significant proportions of daylight hours performing this behaviour. In barren environments without access to substrates, pigs often redirect this rooting behaviour towards pen fitting or pen-mates, contributing to increased levels of belly-nosing, tail-biting and aggression (Petersen et al., 1995; Beattie et al., 1995).

Provision of bedding can have benefits both in terms of improved physical comfort and in terms of increased environmental complexity (*i.e.* stimulating rooting behaviour) (O’Connell, 2009), but is not suitable in fully-slatted systems because the mixing of straw with manure can complicate the effective removal of manure using automated systems.

At present, the use of chains and car tyres is still fairly widespread on farms, but these objects are usually not recommended for long-term use, as they can quickly lose their novelty factor. Even though straw appears to have the highest potential to reduce undesired harmful behaviours, research on point-source enrichment objects (such as ‘toys’ or substrate dispensers) is still needed to individuate functional and easy-to-use alternatives (van de Weerd et al., 2009).

2.6.3 Peculiarities in Italian pig production: Heavy Pigs

Italy is among the countries in the world that pay closest attention to the protection of food products. The production of Protected Designation of Origin (PDO) dry-cured hams such as Prosciutto di Parma and Prosciutto di San Daniele implies that animals comply with specific requirements in terms of genetics, weight, diet and life conditions. Besides pig farms, rules are imposed also to slaughterhouses and processing factories. There are not only rigorous European Community regulations governing these aspects but also strict Production Guidelines whose compliance is assured by official certifying bodies (Consortium of Parma ham, 1992; Consortium of San Daniele ham, 1996).

Heavy pigs intended for dry-cured ham production must be at least 9 months old and weight 160Kg (± 10%) at slaughter. They represent about 90% of the whole Italian pig production. Rearing heavy pigs requires, because of the prolonged production cycle, particular attentions in terms of animal welfare.

First of all, Italian heavy pigs must have wider living spaces with respect to other pigs reared in the rest of Europe. It is worth noting that present legislation makes no additional provision regarding space allowance for pigs weighing more than 110 kg. Secondly, facilities must be properly designed (in terms of floor type, environmental enrichment, feeder space, number of drinkers, thermal comfort) in order to avoid chronic stress conditions. Lastly, since heavy pigs are traditionally fed on a restricted liquid diet and reared on totally slatted floor (i.e. without rooting material), a certain degree of oral dissatisfaction can lead, especially when environmental enrichment is not provided, to increased, stereotyped exploratory activity, which can eventually be redirected towards pen structures or pen-mates (Scipioni et al., 2009).

Raw thighs intended for dry-cured ham production must meet specific requirements. Since the quality of the raw matter, together with the processing method, directly affects the sensory and chemical profile of the dry-cured meat, any variation, in particular with respect to fatty acid composition or muscle traits, can affect the typicality and consistency of the dry-cured hams.

Thighs are acceptable for Parma or San Daniele production when the iodine number and the linoleic acid content in the subcutaneous fat do not exceed 70 and 15% respectively, according to the rules set by the two Consortia. These parameters for fat composition were introduced to limit the content of polyunsaturated fatty acids that reduce the consistence of fats in the ham and increase their oxidability. As concerns the quality of meat, fresh legs of pigs affected by full-blown myopathies (PSE, DFD, evidence of the after-effects of phlogistic or traumatic processes, etc.) that have been certified by a veterinarian at the slaughterhouse are excluded from protected production.

The role of green muscle traits such as pH, proteolytic enzymes, fat content and morphology as related to origin crossbreed has been investigated to understand their effects on pork muscle at various stage of the process, including salting, drying, and maturing under controlled temperature and humidity conditions (Virgili and Schivazappa, 2002). Knowledge of the effects of these traits on the sensory and chemical parameters of the dry-cured hams can provide an useful tool to improve the sensory quality of end products by selection and control of raw matter.

2.7 The welfare of pigs during transport

During the time between leaving the farm and slaughter, animals are subjected to removal from the familiar surroundings, loading and unloading from vehicles, and transport. Transportation is a novel situation for pigs and, as such, is capable to provoking apprehension. Potential stressful factors to which animals are exposed include unfamiliar noises and smells, vibrations and sudden speed changes of the truck, variations of environmental temperature, lower individual social space, food and water deprivation. Such stressors elicit both behavioural and physiological responses which can also contribute to a reduction in carcass yield and meat quality (as reviewed by Bench et al., 2008). Animal losses during transport to the slaughter plant and poor meat quality are important issues for all sectors of the pig industry. Food safety and quality concerns include the increased potential for pathogen spreading and shedding, shrink loss, dark firm dry (DFD) or pale soft exudative (PSE) meat, and increased carcass trim due to bruising.

Many of these factors are associated with pre-slaughter handling. In pigs, physical exercise and psychological or emotional stress not only trigger responses through both the voluntary and autonomic nervous systems but also cause a pronounced metabolic acidosis (Hamilton et al., 2004). In severe cases, acidosis is associated with significant physical impairment, and eventually death.

Mortality during transport is an end-point measure of poor welfare. When a journey results in the death of pigs, it would be safe to assume that the welfare of all pigs on that load may well be compromised (Marchant-Forde and Marchant-Forde, 2009). Even though with large seasonal variations (Ellis and Ritter, 2006), up to 1% of all pigs transported in the United States either die or become nonambulatory during transport to the packing plant. This latter group includes injured animals and non-ambulatory, non-injured animals (NANI). NANI or “fatigued” pigs exhibit symptoms of an extreme stress response (open-mouth breathing, skin discoloration, muscle tremors), together with metabolic acidosis (high blood lactate and low blood pH levels) and a significant elevation of body temperature. Most fatigued pigs will fully recover if held in a low-stress environment; however, a number do not recover from this condition and will eventually die (Ellis and Ritter, 2006).

2.7.1 Loading and unloading

Loading and unloading are recognised as being major stressors for pigs (Warriss, 1998a). Alterations in blood acid-base levels from resting to post-handling have been shown to be proportional to the intensity and duration of the stressful stimulus and normally result in a change to an acidotic state. Hamilton et al. (2004) suggested that a

2-h rest after low-intensity handling may be adequate for blood acid-base status to return to normal; however, if pigs are handled more intensely, then more time is required for blood acid-base levels to return to resting values.

Pigs which have been previously subjected to sympathetic handling and provision of a more varied environment in the weeks before slaughter, or pigs that had experience with leaving their home pen and some of the transport conditions are easier to handle and better able to cope with the stressors they inevitably encounter during the preslaughter period (Abbott et al., 1997; Gevernik et al., 1998).

Both aggressive handling and driving pigs long distances during loading adversely affects rectal temperature and blood-acid balance (Ritter et al., 2009). Rough handing often involves the use of electric prods, which causes pain and fear, is an aversive method for pigs and should be avoided (e.g. EFSA, 2011). The use of electric prods increases the occurrence of behaviours that may lead to injury and bruises (slipping, falling, overlapping) and results in higher blood lactate concentrations (Correa et al., 2010).

Loading often includes driving the pigs up a ramp to get them onto the truck. Depending on the truck internal design, pigs may be required to negotiate one or more ramps. As number of ramps and ramp steepness increase, the physical effort required from the pigs to negotiate them increases. Most guidelines indicate that ramps for pigs should not be steeper than an angle of 20 degrees (*e.g.* EFSA, 2011). However some vehicles have hydraulically operated tail-lifts or decks that do not require the animals to climb or descend slopes and can make loading and unloading easiest and quickest (Brown et al., 2005).

2.7.2 Transport

Loading density is one of the most easily-manipulated and regulated variables in the transportation of pigs. Animals should be able to stand in their natural position ad all must be able to lie at the same time (Lambooij, 2000). According to Warriss (1998b), at stocking densities above about 250 kg/m^2 there may not be enough room available for all the pigs to lie down, leading to continual disturbance of recumbent animals by those seeking a place to rest. Recommended loading densities are often adjusted to the different transport conditions (weather, road type, distances, pig breed and size) among the different countries. If the space allowed is not appropriate, market pigs can experience higher mortality (due to heat stress), injuries and lower meat quality (Bench et al., 2008).

Transport distance and duration may vary greatly. Conditions on the transport vehicle can affect the stress experienced by the animals, which has an impact on how long animals can be transported before their welfare is compromised. According to Pérez et al. (2002), pigs may adapt to travel and recover from the stress of pre-transit loading if conditions are good. Because the most intense stresses in pig transport are at loading and unloading, short journeys can result in a more intense stress response, particularly if the driving, loading density and ventilation are not appropriate. Research produced conflicting results on the effects of loading densities on meat quality parameters, but such outcomes should be cautiously compared across studies given the interactive effects of genetics, handling, distance transported and loading densities.

Barton Gade and Christensen (1997) observed that giving more space (0.42 and $0.50m^2$/pig) did not result in more pigs laying down during short transport (2 hours), and may cause disturbance and difficulties for pigs maintaining their balance, compared to lower space allowances: with a stocking density of 0.35 m^2 pigs showed minimal movement and began to sit and then to lie down as the journey progressed. On the other hand, for long-haul transportation (25 hours), Lambooij and Engel (1991) suggested that the loading density should be limited at about 232 kg/m^2 (~0.47 m^2/pig) for animal welfare and meat quality reasons. Recommendations to allow the long-haul transport of pigs only under superior conditions are reflected in current EU guidelines in which transport duration limits ("short distances", i.e. 8 hours or less and "long distances", i.e. up to 24 hours) are determined by the type of vehicle and whether it is "basic" or "higher standard" (Bench et al., 2008).

Vehicle design is crucial in terms of animal welfare. For example, the ability to form pens within the truck can help minimizing mixing of unfamiliar pigs. Furthermore, the vehicle needs adequate ventilation and insulation to prevent exposing pigs to thermal extremes. Keeping temperatures down during summer months is usually achieved by providing ventilation openings. During the winter, most trucks are designed so that ventilation slots can be closed off to minimize air flow. However, truck microclimate can vary considerably depending on the position within the truck, with the compartments located in the front of the lower decks being the most vulnerable during summer (Brown et al., 2011b) because of the proximity to the engine and the reduced ventilation. In the hot season, animals may benefit from active ventilation devices, which may also be combined with water sprinkling systems to increase evaporative cooling in hot weather. (Brown et al., 2011b; EFSA, 2011).

Floor type should be anti-skid and anti-noise, and straw or wood shavings should be used as bedding especially during winter. Lastly, since pigs are susceptible to travel sickness, vehicle design and driving style should minimize vibrations (Bradshaw et al., 1996).

2.8 The impact of animal welfare on meat quality

According to Gregory (1993), the relationships between welfare and product quality are not commonplace, because processes within the animal intervene between substrate and product which reduce farming practices to a common level that is subordinate to the animal's metabolism. Nevertheless, he pointed out four ways in which compromised welfare can be linked to product quality:

1. product quality which is influenced by acute stress;
2. ante-mortem trauma (bruising, haemorrhages, lacerations, skin blemishes, broken bones) occurring in parts of the animal which are edible;
3. disease states which leave lesions or taints in the edible product;
4. product quality which is dependent on the long-term cumulative effects of exercise, lack of exercise or poor husbandry conditions.

From such a classification it is clear how some practices or processes which compromise welfare can lead to immediately recognizable negative effects on product quality. This is the case of trauma and diseases, which leave evident sequelae in the edible parts.

On the other hand, there are processes which occur in the muscular tissue as a result of the stress experienced by the animals in the pre-slaughter period. This is the case of the two main problems concerning meat quality in pigs, PSE (pale-soft-exudative) and DFD (dark-firm-dry) meat. These defects, which are a serious concern for the meat industry, are due to alteration in the post-mortem muscle metabolism and can reduce consumer acceptability, shelf life and yield of meat.

Lastly, there are fewer evidences to suggest that improved welfare deriving from improved management and housing systems can benefit product quality. This kind of effect of welfare on meat quality has been studied with particular interest in alternative housing systems (*e.g.* outdoor housing, organic farming and application of environmental enrichment). The issue with these new systems (or, in the case of intensive farming, with modifications in the management system) is whether they indeed improve the welfare of farmed pigs and whether, given the fact that they replace systems which already produce high-quality meat, they affect production characteristics and meat or carcass traits (Millet et al., 2005).

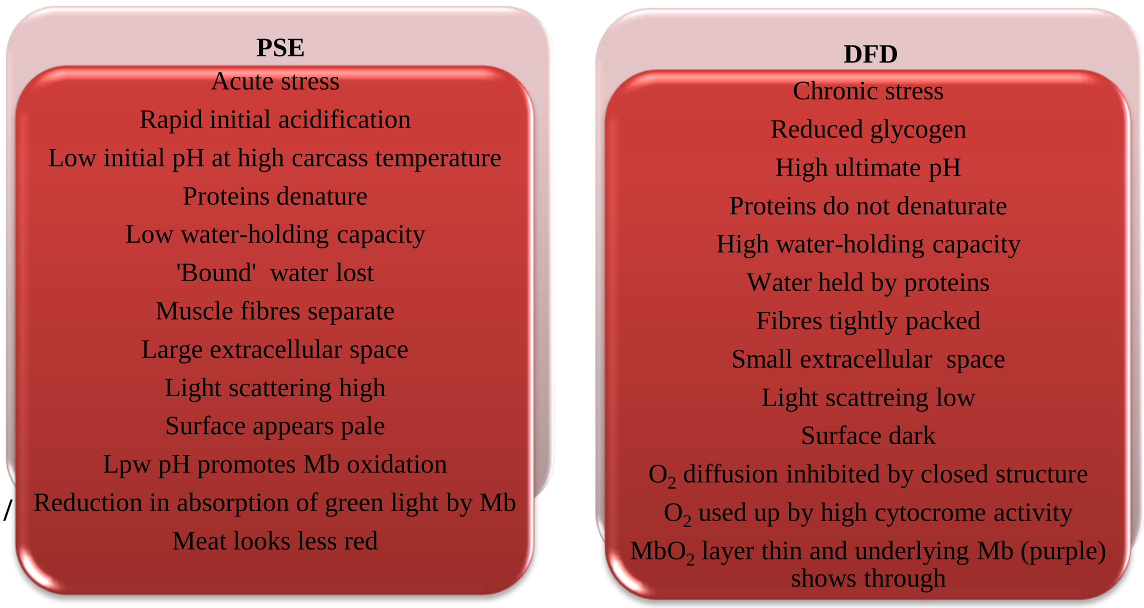
2.8.1 Effects of pre-slaughter handling

Under normal circumstances, there is a gradual decline in muscle pH post-slaughter. DFD and PSE meat are defined in connection with the pH of meat at a specific time after slaughter. PSE is said to have occurred when the pH of meat is < 6 at 45 minutes after slaughter, whereas DFD is when the ultimate pH post mortem measured after 12-48 hours post mortem (depending on the species) is ≥ 6.

Events leading to PSE and DFD meats are summarized in Figure 2.4. If pigs are stressed prior to slaughter, muscle glycogenolysis is increased by adrenergic mechanisms resulting in increased muscle temperature and increased rate of muscle pH decline post-slaughter. Under these conditions, myosin denatures and shrinks. The consequence is an increase in light-scattering properties and a reduction in water-holding capacity (WHC): meat becomes pale, soft and exudative.

The other extreme, which is of lesser importance to the matter of pork quality, is DFD meat. Long-term pre-slaughter stress significantly deplete muscular glycogen reserves Low glycogen concentrations at slaughter, will lead to a slow pH fall and a high ultimate pH, which exposes meat to bacterial spoilage and reduces its shelf-life.

Figure 2.4: Summary of events leading to PSE and DFD meat (adapted from Warris, 2000). Mb=myoglobin, MbO_2=oxymyoglobin.



There is abundant literature about the effects of pre-slaughter stress on meat quality. Stress-susceptible genotypes are much more prone to develop PSE meat (Murray and Jones, 1994). The propensity of muscles to become PSE or DFD can be influenced by metabolic type as reflected in their fibre type composition: red, oxidative fibres (like the m. *adductor* of the pig) have a relatively low concentration of glycogen, therefore tend to be more prone to producing DFD meat. In contrast, white, glycolitic fibres (like the pig m. *Longisssimus dorsi*), have a high glycogen content and glycolitic capacity and are more prone to the PSE condition (Klont et al., 1998).

As concerns stressing factors, the type of acute stress which can lead to PSE pork is that often occurring in the period immediately before stunning. Large plants with high line speeds may require considerable use of coercition to deliver pigs to the slaughter point (enclosed race systems, restraining conveyors, use of electric goads) and lead to high levels of stress in the animals, resulting in poorer meat quality. Examples of chronic stress that can produce DFD meat are long periods without food (fasting), fatigue caused by long transport under poor conditions, or the fighting that occur when unfamiliar pigs are mixed together (Warriss, 2000).

Prevention of PSE and DFD meat relies on specific handling procedures to avoid stress. These include container transport to reduce loading and unloading stress, the use of controlled temperature vehicles, reducing mixing of unfamiliar animals. The practice of spraying pigs with cold water (10-12°C) during lairage reduces fighting in lairage and the prevalence of DFD (Warriss, 2000). Besides, by cooling pigs it also has the potential to reduce the prevalence of PSE (Long and Tarrant, 1990).

2.8.2 Effects of welfare-oriented pig production systems

Several parameters can be changed in alternative or welfare-oriented housing systems compared with conventional husbandry of fattening pigs (e.g. genotype, feeding strategies, space allowance, outdoor access, provision of environmental enrichment). However, most alternative systems allow animals to display their species-specific repertoire (i.e. engage in social contact and exploratory behaviour). There are two main paths through which the housing system can affect meat quality: differences in pre-slaughter stress and physical training (Millet et al., 2005).

As concerns pre-slaughter stress, it is known that barren environments affect the behavioural development of pigs so that they are less able to cope with the environmental challenges. Foury et al. (2011) observed that pigs reared in enriched systems had lower levels of blood and urinary stress indicators and lower carcass damage scores at slaughter when compared to pigs reared in a conventional system. Environmental enrichment has therefore the potential to exert a major influence on final product quality through the modification of the physiological response to stressors. According to Beattie et al. (2000), environmental enrichment during rearing had a small but significant effect on meat quality, with pork from pigs reared in barren environments being less tender and having greater cooking losses than pork from pigs reared in enriched environments. On the contrary, Day et al. (2002) observed that environmental enrichment had minimum effects on growing-finishing pigs and that, unexpectedly, pleasant handling (*i.e.* stroking) did make groups of animals more difficult to handle during routine husbandry tasks such as weighing. A significant number of studies in outdoor pigs however reported reduced muscle pH and/or increased drip loss, suggesting greater susceptibility pre-slaughter stress (Edwards, 2005).

Increased physical exercise can be important for animal welfare, as muscle tone and bone strength can be adversely affected by restricted movement. However, Gentry et al. (2002) studied the effect of increased (10X) space allowance and spontaneous exercise on meat quality of finishing pigs reared on slatted floors, and they observed no improvements in pig performance, pork loin measures, or muscle characteristics. It can also be expected that alternatively housed pigs, which have more opportunity for exercise, will have a reduced incidence of exhaustion during loading, which could lead to a reduction in the development of DFD meat. Besides, lactate formation following physical stress is significantly lower in trained versus untrained pigs, which could be a positive factor in relation to the incidence of PSE meat (Millet et al., 2005). Stern et al. (2003) found most meat quality traits to be similar between indoor and outdoor pigs in the summer. On the contrary, Enfalt et al. (1997) observed in outdoor-reared pigs lower pH, higher drip loss, higher shear force and impaired tenderness, juiciness and overall acceptance. In general, conflicting results are reported as concerns meat juiciness, tenderness and flavour. Primary attributes of meat quality have not been shown to be consistently influenced in a favourable way by welfare-oriented productions (Edwards, 2005).

The influence of the production systems on the product quality has been extensively reviewed (Edwards, 2005; Lebret, 2008; Bonneau and Lebret, 2010). On the whole, the Authors concluded that both feeding and rearing systems influence growth performance and carcass composition in pigs, through the relative growth deposition of fat and lean tissues. However, the effects of welfare-oriented rearing conditions on meat quality are rather inconsistent, thereby increasing the variability of meat quality as a result of interaction effects between different factors (genotype, feeding strategy, seasonal effect on physical activity and metabolism, pre-slaughter handling of animals…).

By contrast, improved eating quality of pork and dry-cured products can be achieved using slow growing-fat local pig breeds reared in extensive finishing conditions which allow high intramuscular fat and micronutrients deposition. In these particular conditions (*e.g.* heavy pigs intended for dry-cured ham production in the Mediterranean area), the positive genotype x environment interaction results in actual higher eating quality (Bonneau and Lebret, 2010).

2.8.3 Consumer perception and information

The market has played a key role in selectively driving up welfare standards, as supply chain actors employ animal welfare criteria to create additional value on particular products. Yet, it is worth noting that, apart from a few very specific products or product ranges, farm animal welfare is rarely a stand-alone selling point for food (Buller, 2010). Figure 2.5 summarizes the properties a welfare-improved product should have to be promoted at the retail level.

Figure 2.5: (adapted from Gregory, 1993)

Properties a welfare-improved product must enjoy before it can be promoted at the retail level

- *Recognizable:* A marketable product label has to be attached to the item which describes the perceived welfare improvement.
- *Quality Assured:* The perceived welfare improvement should not seriously harm the quality of the product, the quality image of the supermerket company or the quality image of the adjacent alternative products.
- *Guaranteed:* Can the supermarket guarantee that the label describe swhat the product actually is? Is it possible to "police" the welfare improvement ore rely on the supplier to do so?
- *Profitable:* The return from the product has to outweight the edditional effort and cost of marketing it.

From the consumers' perspective, animal welfare is a typical credence attribute. This means that consumers in many cases are not able to verify themselves the actual level of animal welfare when such claims are made (EC, 2009). However, welfare-oriented rearing conditions, such as outdoor production, are often favourably perceived by consumers which are prone to find free-range pork more palatable, more nutritious and safer than the conventional one, whenever they are informed on the origin of the meat (Edwards, 2005). This is why specifications on the production systems having a claim of higher quality often include increased space allowance, enriched environment and/or outdoor access. Such conditions have very little or inconsistent actual effects on pork quality, as discussed before (Bonneau and Lebret, 2010). There is, therefore, a gap between the actual and perceived differences in quality. Where consumers have (perhaps unrealistic) expectations about the better taste of welfare-oriented products, a disconfirmation of this expectation may raise a potential barrier to further demand of similar products (Grunert et al., 2004).

Moreover, we need to acknowledge that the market is not necessarily a universal panacea for improving welfare standards, the main pitfalls being: its dependence upon consumers' willingness and ability to pay, its essentially hedonistic and non-cumulative nature, its avoidance of non-market-friendly aspects of the production process (notably transport and slaughter) and its selective use of scientific evidence. Lastly, many people believe that farm animal welfare is something that should be governed by regulatory means, with food chain actors assuring that animal products on sale should come from systems that conform to welfare standards (Buller, 2010). A more effective approach may be to acknowledge consumers' concerns and then engage consumers in discussions about the costs and constraints related to accommodating their interests, especially when these conflict (Croney, 2011).

Toma et al. (2010), analyzed some determinants of welfare friendly consumer behaviour in different countries and observed that access to information was the strongest determinant, followed by perceived responsibility of consumers and education, which also had strong influence, then by labelling with lower impact and ending with children in the household, with the lowest influence on behaviour.

Therefore, an EU harmonized labelling scheme could avoid segmentation of the internal market and produce the desired effects (enable consumers to make informed purchasing decision and make it possible for producers to benefit from market opportunities), provided that consumers are adequately informed on the meaning of the label, and that the information provided is readily understandable (EC, 2009).

PREFACE TO CHAPTER 3

Even though minimum levels of environmental illumination for pigs (in terms of light duration and light intensity) are established by the European legislation, at present there is a scarcity of studies considering the effects of different light regimes on swine welfare, and in particular its effects on meat and ham quality. The minimum illumination level specified by legislation (40 lux or above, for at least 8 hours per day) enables better observation by stockpeople and can therefore contribute to better welfare, but its benefits for pigs are not well stated. When defining optimal illumination regimes, the animals' preferences and performances should be taken in consideration in order to attain an effective improvement of animal welfare.

In the light of previous findings showing that increased light duration and light intensities can improve growth parameters and behavioural traits, this chapter, which consist of two experimental trials, will focus on the effects of different light regimes on productive parameters, fresh meat and dry-cured ham quality of Italian heavy pigs. It is hoped that data gathered from this trial will contribute to a first step towards specifying improved illuminance levels which could effectively improve pig welfare without affecting meat and ham quality.

Research paper based on the chapter:

- Sardi L., Nannoni E., Grandi M., Vignola G., Zaghini G., Martelli G. (2012). Meat and ham quality of Italian heavy pigs subjected to different illumination regimes. *Berliner und Münchener Tierärztliche Wochenschrift* 125(11/12), 463-468.

CHAPTER 3:

MEAT AND HAM QUALITY OF ITALIAN HEAVY PIGS SUBJECTED TO DIFFERENT ILLUMINATION REGIMES

3.1 Abstract

In order to attain a good level of welfare, pigs require a sufficient environmental illumination. Therefore, minimum levels for light duration and light intensity are set up by the European legislation. Two independent and separate trials were carried out aiming to determine whether an increase above the minimum mandatory levels of lighting duration (14 vs. 8 hours/day at 70 lux: trial A) or of light intensity (80 vs. 40 lux over a 12-hour lighting period: trial B) could modify carcass traits, meat and cured hams quality of Italian heavy pigs (body weight at slaughtering of about 160 kg). Slaughtering parameters, fresh meat quality and fatty acid composition of raw thighs and cured hams were assessed. Pigs receiving the longer photoperiod showed a tendency ($P < 0.1$) toward a higher slaughtering body weight and a higher saturation degree ($P < 0.01$) of subcutaneous fat of the raw thighs, while cured hams deriving from pigs subjected to the higher illumination intensity showed a higher ($P < 0.05$) relative content of polyunsaturated fatty acids.

Our results indicate that, within a moderate range of light intensity and given an appropriate dark period for animal rest, neither a prolonged photoperiod nor a higher light intensity caused any negative effect on the carcass traits, meat or quality of long-cured hams.

3.2 Introduction

Under current EU legislation on pig protection (EC, 2008), animals must be kept in light with an intensity of at least 40 lux for a minimum period of eight hours per day. This provision, aimed at preventing the baseless practice of rearing pigs in darkness to reduce aggression between animals, clearly reflects the existence of a need of pigs in terms of lighting intensity and duration which must be fulfilled in order to allow their explorative and social activities and thus ensure the attainment of an appropriate level of animal welfare. However, literature dealing with pig requirements in terms of environmental illuminance, and in particular with the relationship between illumination regimes and meat quality, is not abundant and therefore, research on light intensity, duration of the light period and type of lighting should be considered a priority, as was recommended in the EFSA's latest opinion on the welfare of fattening pigs (EFSA, 2007).

It is worth to note that most researches have focused on studying the behavioural implications and the welfare assessment (Van Putten and Elshof, 1984; Baldwin and Start, 1985; Taylor et al., 2006), rather than seeking to assess production traits and the quality of carcasses and meat derived from pigs reared under different illumination regimes. No literature is to our knowledge available with respect to the impact of the light-programme on long-cured meat products.

In this framework is noteworthy that endopeptidase activity, which affects ham firmness, may be influenced by the photoperiod, as demonstrated by the fact that seasonal changes of cathepsin B activity in hams were found to follow a circannual rhythm, reaching a maximum predicted value in mid January and a minimum in July (Virgili and Schivazappa, 2002; Virgili et al., 2002). An excessive proteolysis level throughout the curing period has been associated with excessive softness of dry-cured hams that is usually accompanied by other defects like stickiness on chewing, dark colour, astringent or metallic aftertastes, depots of tyrosine crystals and formation of white films on the cut surface (Parolari et al., 1994; Virgili et al., 1998).

Previous findings evidenced that increasing illumination levels above the mandatory minimum both in terms of light intensity and in terms of light duration could determine an improvement of animal welfare (Boccuzzi, 2010). A longer photophase determines some positive effects both on growth parameters and behavioural traits (Martelli et al., 2005), whereas a higher light intensity reduces aggressive behaviours of heavy pigs (Martelli et al., 2010) in comparison with animals reared according to the minimum mandatory level for light intensity and duration. Therefore, the aim of the present studies was to supplement the knowledge obtained through the above mentioned studies by giving further original information concerning the influence of these conditions on carcass traits and the quality of meat and long-cured hams.

3.3 Materials and Methods

The experiments were carried out in the facilities of the Faculty of Veterinary Medicine of the University of Bologna, Italy, in observance of current Italian legislation, implementing European Council Directive 2008/120 (EC, 2008) on swine protection. The experiments were approved by the local Ethic Committee.

3.3.1 Animals, housing and feeding

Two separate and independent studies (trial A and B) were carried out. In both trials pigs were reared until reaching a body weight (BW) of approximately 160 kg and a minimum age of 9 months, according to the rules established for Parma Ham production (Consortium for Parma Ham, 1992). In both trials, pigs were kept in collective pens on a totally slatted floor, with a floor space of 1.20 m^2 per pig. Each pen was equipped with a bite drinker and a collective stainless steel feeder (0.3 m wide x 3.5 m long). Environment was enriched by providing steel hanging chains. Pens were located in temperature-controlled rooms (22°C) equipped with a forced-air ventilation system. Water was available *ad libitum*. A commercial feed was offered as wet (meal to water ratio = 1 : 3) and rationed at 9% of the metabolic BW ($BW^{0.75}$) of pigs up to a maximum of 2.8 kg dry matter per pig, per day. Lighting was entirely artificial and it was supplied by neon tubes (OSRAM LUMILUX, cool white, luminous flux 3350 lm, light colour 840, rated colour temperature 4000 K) placed at 280 cm above the floor. Luminous intensity was measured at pig-eye level using a luxmeter device (model HD 8366, Delta Ohm, Italy). During the period of darkness light intensity was 1.5 lux.

The experimental trials were carried out as follows (the experimental design of both trials is summarized in Figure 3.1):

- <u>Trial A (Light duration):</u> 56 hybrid castrated male pigs with an initial average body weight of about 113 kg were used. They were allotted to two experimental groups, each containing four replications of seven pigs:
    - group A1 (Control group), which was exposed to an 8-hour light phase, corresponding to the minimum mandatory level for light duration, followed by a 16-hour dark period (8L:16D) per day;

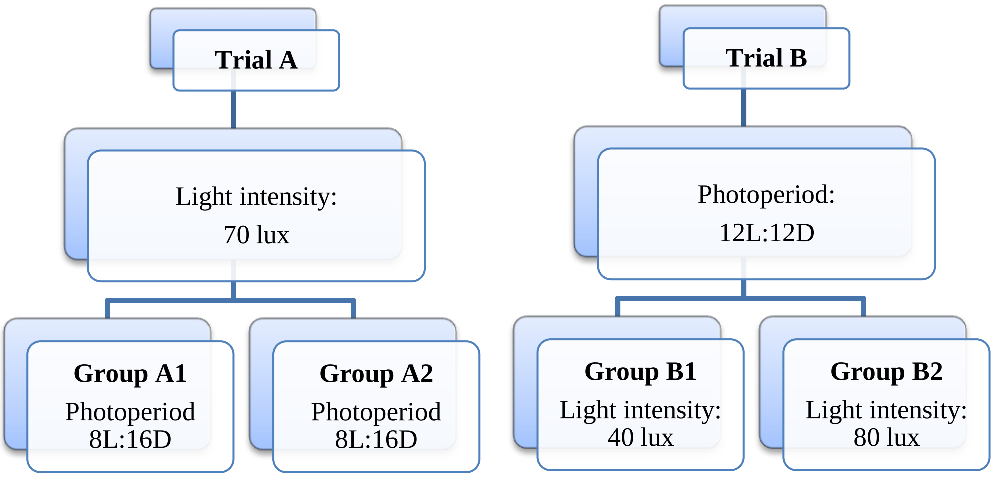
- group A2, which was exposed to a 14-hour light period followed by a 10-hour dark period (14L:10D) per day.

Light intensity was 70 lux for both groups.

- Trial B (Light intensity): 80 hybrid castrated male pigs with an initial average body weight of about 75 kg were used. They were allotted to two experimental groups, each containing eight replicates of five pigs:
  - group B1(Control), which was exposed to a light intensity of 40 lux, corresponding to the minimum mandatory light intensity;
  - group B2, which was exposed to a light intensity of 80 lux.

The duration of the photoperiod was the same for both groups (12 hours; 12L:12D).

Figure 3.1: Experimental design of the two trials



3.3.2 Carcass traits, meat and fat quality

When pigs reached the BW of about 160 kg, they were transported to a commercial slaughterhouse (the journey lasted about 1 hour). Slaughtering took place after 12-hour fast and was preceded by electrical stunning. Thereafter, the dressing out percentage was calculated and the lean meat yield of carcasses was assessed by Fat-o-Meater (FOM-SFK, Copenhagen, DK).

At 45' post mortem, the pH value of the *Semimembranosus* (SM) muscle was measured by means of a portable pH meter (model 250A, Orion Research, Boston, MA). Thereafter, each carcass was dissected into the main commercial cuts (tight, loin, and fat cuts), whose weights were recorded. At 24 hours post mortem, a second measurement of the pH value was taken from the SM muscle. The colour of the lean portion of the thighs (SM muscle) was assessed, at 24 hours post mortem, according to the CIELAB System (CIE, 1976), using a Minolta Chromameter CR-200 (Minolta Camera Co., Ltd.,Osaka, Japan) (Figure 3.2). Drip loss and cooking loss were evaluated in samples taken from the *Longissimus dorsi* muscle according to the method described by Honikel (1998) (Figures 3.3 and 3.4).

Figure 3:2: The portable pHmeter (on the left) and the colorimeter (on the right) that were used during the experimental trials**.**

Figure 3.3: Drip loss determination: a 3-cm thick slice was weighted and placed inside an airtight container on top of a wire mesh. After refrigeration at 4°C for 24h, the sample was carefully dabbed and weighted to obtain drip loss percentage.

Figure 3.4: Cooking loss determination: standard-sized samples (5x5x3 cm) were cooked in a pre-heated waterbath (86°C) until they reached the core temperature of 74°C, then refrigerated to 4°C and weighted.

Samples of subcutaneous fat (outer and inner layers) were taken in the overhanging area of the *Biceps femoris* (BF) muscle in order to determine the fatty acid composition by gas chromatograph (HRGC8560 Series Mega 2 gas chromatograph; Fisions Instruments, Milan, Italy). Total lipids were extracted from each sample of subcutaneous fat by means of the chloroform/methanol (2 : 1, v/v) method described by Folch et al. (1957) and measured gravimetrically. The Iodine number was assessed according to the AOAC method (AOAC, 2000). Fatty acids were esterified using 5% methanolic hydrogen chloride. The fatty acid methyl esters were separated by gas chromatography using a Supelco SP-2330 capillary column (Supelco, Bellefonte, PA, USA). Injector and detector temperatures were kept at 220°C and 280°C, respectively. The column was programmed as follows: 140°C for 1 min; the temperature was then raised to 220°C (3°C/min) and held constant for 15 min. Fatty acids were identified by comparing the retention times of the peaks with those of known standards. Results were expressed as weight percentages of total fatty acids.

3.3.3 Ham yield and quality

Hams were cured over a 18-month period according to Parma Ham production rules (Consortium for Parma Ham, 1992). In particular, the processing scheme is based on a 25-day dry salting period at 1–3°C followed by a rest period of 90 days at 1–4°C and airdrying and primary ripening at 15– 20°C for 90 days at 60–90% relative humidity. Pork fat is then smeared on cut surfaces; final ripening takes place at 17–18°C for 160 days followed by postripening at 17–18°C for at least further 120 days (Parolari et al., 1994). Thighs were weighed before and after trimming, after salting and at the end of the curing period in order to calculate the weight losses after the different phases of the curing process. In each trial 28 samples of *Biceps femoris* muscle were taken from seasoned hams (fourteen for each group) and analysed for moisture (AOAC, 1995), crude protein, sodium chloride content (AOAC, 2000) and proteolysis index (Careri et al., 1993). Colour was assessed in cured hams both in samples of the SM muscle and in samples of subcutaneous fat according to the CIELAB System (CIE, 1976), using a Minolta Chromameter CR-200 (Minolta Camera Co., Ltd., Osaka, Japan). Subcutaneous fat samples (outer and inner layers) were taken in the overhanging area of the *Biceps femoris* muscle and analysed by gas chromatography as described above for fat from the raw thighs.

Cured hams were deboned and a sample-slice (including BF and SM muscles) was taken transversally from the caudal portion of the ham to the middle of the femoral bone impression (Figure 3.5). The slice was sensorially evaluated by a panel of trained experts, who subjectively rated hams (on a 1-to-10 scale) for each of the following parameters: lean firmness, lean colour homogeneity, lean colour bi-tonality, marbling, ham fatness, fat firmness. Besides, a total score was attributed to each ham on the basis of the total impression the panelist got while evaluating an ham (1-to-10 scale: 10 = optimal characteristics, 1 = very bad quality).

Figure 3.5: Dry cured ham sampling for the sensorial evaluation

3.3.4 Statystical Analysis

Data of each trial were separately analysed using the SAS package (SAS, 1999). Normality of data was assessed by the Kolmogorov-Smirnov test (UNIVARIATE procedure) and the data obtained were submitted to analysis of variance (GLM procedure) using duration of photoperiod or light intensity level as the main effect. In the case of pre-existing differences between groups, analysis of covariance was used. The experimental unit used was the individual (pig or ham). For nonparametric data (sensory evaluation), the Mann-Whitney test (NPARWAY procedure) was used. The significance level for all statistical tests was set at $P < 0.05$.

3.4 Results

Slaughtering parameters and carcass traits are shown in Table 3.1. Pigs belonging to group A2, which were subjected to a longer photoperiod than pigs of group A1, showed a tendential ($P < 0.1$) improvement of body weight at slaughter. No significant differences were noted between the experimental groups of each trial with respect either to lean meat percentage, calculated by F-o-M, or lean and fatty cuts yield. Similarly our result did not reveal any significant difference among the experimental groups in both trials with respect to the qualitative traits of meat (colour, pH, drip loss and cooking loss; Table 3.2).

Table 3.3 shows the fatty acid composition of subcutaneous fat of uncured (raw) thighs. Some differences between the experimental groups were detected in trial A: the fat of pigs from group A1 (8L:16D at 70 lux) was significantly ($P < 0.01$) richer in polyunsaturated fatty acids and therefore significantly ($P < 0.01$) poorer in saturated fatty acids than the fat of pigs from group A2 (14L:10D at 70 lux). In detail, palmitic acid was significantly higher in group A2 ($P < 0.01$) than in group A1, whereas linoleic, linolenic and arachidonic acid were significantly higher in group A1 ($P < 0.05$; $P < 0.01$; $P < 0.01$, respectively) than in group A2. The Iodine number was thus significantly higher ($P < 0.01$) in group A1 than in group A2.

Table 3.1: Slaughtering parameters and carcass quality

	First trial (light duration)			Second trial (light intensity)		
	A1 (8h)	**A2 (14h)**	**SEM[a]**	**B1 (40lux)**	**B2 (80lux)**	**SEM[a]**
Pigs n°	28	28		40	40	
Live weight (kg)	157.5*	163.1	1.68	160.73	161.8	1.94
Carcass weight (kg)	131.3	135.6	1.45	134.34	134.58	1.73
Dressing out (%)	83.4	83.2	0.18	83.54	83.13	0.19
Lean Meat (%)	49.2	49.3	0.4	47.49	47.11	0.33
Loin[b] (%CW[c])	23.3	23.6	0.17	23.69	23.31	0.19
Thight (%CW)	23.2	23.6	0.14	23.97	24.12	0.10
Lean Cuts (%CW)	60.2	60.9	0.31	61.4	60.69	0.43
Fat cuts (%CW)	32.2	31.8	0.33	31.67	32.28	0.43
Lean/Fat cuts	1.89	1.93	0.03	1.96	1.92	0.03

In the same trial * P < 0.1
[a] SEM = Standard Error of the Mean
[b] Loin with neck and ribs
[c] CW = Carcass Weight

Table 3.2: Meat quality

	First trial (light duration)			Second trial (light intensity)		
	A1 (8h)	**A2 (14h)**	**SEM[a]**	**B1 (40lux)**	**B2 (80lux)**	**SEM[a]**
Pigs n°	28	28		40	40	
pH 45'	6.53	6.48	0.03	6.74	6.84	0.03
pH 24h	5.89	5.85	0.03	5.75	5.82	0.02
L[b]	40.25	40.35	0.29	40.46	40.39	0.5
Hue[b]	0.19	0.18	0.01	0.23	0.22	0.01
Chroma[b]	14.7	14.55	0.32	12.32	12.09	0.38
Drip Loss[c] (%)	2.07	1.81	0.12	3.14	3.62	0.14
Cooking Loss[c] (%)	20.85	21.15	0.61	20.17	20.89	0.76

[a] SEM= Standard Error of the Mean
[b] According to the CIE L*a*b* system, 3 parameters of the colour are measured: L*=lightness (range: 0(black) to 100 (white); a*=red-green shift (range:-50(green) to +50(red)); b*= yellow-blue shift (range:-50(blue) to +50(yellow). Hue and chroma are calculated as follows: Hue= arctan(b/a).; Chroma=$\sqrt{(a^2+b^2)}$
[c] Analysis were performed on 20 samples (LD muscle) for each trial

Table 3.3: Fatty acid composition of subcutaneous fat of uncured thighs

	First trial (light duration)			Second trial (light intensity)		
	A1 (8h)	A2 (14h)	SEM[a]	B1 (40lux)	B2 (80lux)	SEM[a]
Thighs (n.)	14	14		20	20	
C 14:0 (%)	1.34	1.44	0.03	1.4	1.35	0.03
C 16:0 (%)	23.36***	24.65	0.25	23.37	23.56	0.17
C 16:1 (%)	1.84	2.13	0.08	2.24	2.16	0.07
C 18:0 (%)	12.42	12.83	0.19	12.01	12.63	0.19
C 18:1 (%)	42.06	41.5	0.35	45.84	46.56	0.28
C 18:2 (%)	16.1	15.09**	0.24	12.3	11.19	0.34
C 18:3 (%)	0.91	0.81***	0.02	0.65	0.56	0.03
C 20:4 (%)	0.84	0.72***	0.03	0.71	0.65	0.04
SFA[b] (%)	37.20***	39.02	0.34	37.1	37.55	0.32
MuFA[c] (%)	44.91	44.4	0.36	49.42	50.61	0.41
PuFA[d] (%)	17.89	16.58***	0.26	13.48	11.84	0.53
Iodine number	67.45	64.82***	0.53	66.59	64.83	0.53

In the same trial ** P < 0.05 , *** P < 0.01
[a] SEM = standard error of the mean
[b] SFA = Saturated Fatty Acids
[c] MuFA = Monounsaturated Fatty Acids
[d] PuFA = Polyunsaturated Fatty Acids

Ham weights and ham weight losses during the dry-curing process are shown in Table 3.4. The weight of the thighs before trimming was significantly higher (P < 0.01) in group A2 than in group A1 and these differences remained significant, although at a different threshold (P < 0.05), also during the following phases of the curing process (weight after trimming, weight after salting and final weight).

Table 3.4: Ham weights and weight losses during the curing process

	First trial (light duration)			Second trial (light intensity)		
	A1 (8h)	A2 (14h)	SEM[a]	B1 (40lux)	B2 (80lux)	SEM[a]
Thighs (n.)	28	28		20	20	
Pre-trimming weight	15.00***	15.88	0.17	16.18	16.21	0.22
Trimmed weight (kg)	12.37**	12.97	0.15	12.53	12.5	0.16
Weight after salting	11.52**	12.11	0.14	12.01	11.95	0.16
Final weight (after 18 months) (kg)	8.93**	9.42	0.11	9.16	9.07	0.14
Weight loss after trimming(%TW[b])	17.53	18.38	0.24	22.39	22.96	0.41
Weight loss after salting (%TW)	6.93	6.64	0.11	4.11	4.36	0.13
Weight loss of cured hams (%TW)	27.81	27.39	0.27	26.99	27.42	0.35

In the same trial ** P < 0.05
[a] SEM = Standard Error of the Mean
[b] TW = Trimmed Weight

With respect to the chemical composition of the cured hams (Table 3.5), the only significant difference found ($P < 0.05$) was a lower sodium chloride content in group A2 versus group A1. As concerns the relative fatty acid composition of the cured hams (Table 3.6), no significant differences were noted between the experimental groups in trial A, whereas a higher content ($P < 0.05$) of polyunsaturated fatty acids (mainly tied to a relatively higher level of linoleic acid) was found in group B2 (higher light intensity) when compared to B1. No significant differences between groups were detected with respect to the sensory analysis of cured hams (Table 3.7).

Table 3.5: Chemical composition and colour (lean and fat portions) of cured hams

	First trial (light duration)			**Second trial (light intensity)**		
	A1 (8h)	**A2 (14h)**	**SEM[a]**	**B1 (40lux)**	**B2 (80lux)**	**SEM[a]**
Samples (n.)	14	14		16	16	
Moisture (%)	59.89	59.81	0.25	60.82	59.83	0.26
Crude protein (%)	28.65	28.86	0.14	27.86	28.25	0.09
Sodium chloride (%dm)	5.73	5.36**	0.23	6.07	5.87	0.06
Proteolysis index (%dm)	28.08	26.92	0.48	25.81	26.33	0.3
Fat Colour						
L	74.97	75.42	0.38	72.58	73.5	0.52
Hue	-1.35	-1.17	0.1	-1.42	-1.32	0.02
Chroma	8.8	8.63	0.11	7.56	7.69	0.19
Meat colour (*Semimembranosus* muscle)						
L	39.72	39.54	0.35	36.37	37.39	0.41
Hue	0.38	0.37	0.01	0.41	0.44	0.02
Chroma	11.88	11.44	0.22	9.63	9.29	0.22

In the same trial ** $P<0.05$

[a] SEM = Standard Error of the Mean

Table 3.6: Fatty acid composition of subcutaneous fat of cured hams

	First trial (light duration)			Second trial (light intensity)		
	A1 (8h)	**A2 (14h)**	**SEM[a]**	**B1 (40lux)**	**B2 (80lux)**	**SEM[a]**
Samples (n.)	14	14		14	14	
C 14:0 (%)	1.62	1.54	0.04	1.59	1.64	0.03
C 16:0 (%)	23.66	23.77	0.25	23.64	24.08	0.25
C 16:1 (%)	2.62	2.83	0.08	2.67	2.63	0.11
C 18:0 (%)	9.66	10.06	0.19	10.91	9.75**	0.26
C 18:1 (%)	46.01	46.12	0.22	47.78	47.21	0.32
C 18:2 (%)	14.68	13.98	0.32	12.02**	13.21	0.25
C 18:3 (%)	0.74	0.74	0.32	0.65	0.7	0.02
C 20:4 (%)	0.73	0.66	0.02	0.63	0.7	0.03
SFA[b] (%)	35.09	35.53	0.36	36.24	35.56	0.36
MuFA[c] (%)	48.75	49.04	0.21	50.46	49.83	0.36
PuFA[d] (%)	16.16	15.38	0.35	13.31**	14.61	0.27

In the same trial ** $P < 0.05$
[a] SEM = Standard Error of the Mean
[b] SFA = Saturated Fatty Acids
[c] MuFA = Monounsaturated Fatty Acids
[d] PuFA = Polyunsaturated Fatty Acids

Table 3.7: Sensory analysis of cured hams

	First trial (light duration)			Second trial (light intensity)		
	A1 (8h)	**A2 (14h)**	**SEM[a]**	**B1 (40lux)**	**B2 (80lux)**	**SEM[a]**
Samples	14	14		16	16	
<u>Lean Portion</u>						
Lean firmness (points)	5.84	5.93	0.16	5.43	5.71	0.17
Colour homogeneity (points)	6.21	6.32	0.24	7.29	6.86	0.22
Colour bitonality (points)	3.32	3.04	0.31	1.86	2.07	0.2
Marbling (points)	3.96	4.29	0.35	1.29	1.5	0.12
<u>Fat</u>						
Ham fatness (points)	3	3.21	0.19	3.43	3.07	0.27
Fat firmness (points)	5.68	5.95	0.14	5.5	5.79	0.16
Overall evaluation (points)	6.36	6.93	0.23	6.86	6.93	0.15

[a] SEM = Standard Error of the Mean
Note: Evaluation was expressed on a scale ranging from 1 to 10 where 1 is attributed to the absence of the trait and 10 to its maximum presence. Overall evaluation was assessed as the total impression the panelist got evaluating an ham, expressed on a scale ranging from 1 to 10, where 10 is attributed to hams with optimal characteristics, whereas 1 is attributed to very bad hams.

3.5 Discussion

The trial conditions did not appear to influence the pigs' health and no occurrence of disease was recorded during the trial periods. It is worth noting that since pigs seem to dislike excessive light intensities (>400 lux) and they prefer darkness for sleep (Baldwin and Start, 1985; Taylor et al., 2006), in all the trials light intensity was kept within a moderate range (i.e. not exceeding 80 lux), and the artificial photoperiod always allowed for an 8-h period of darkness for sleep.

Despite a tendential higher slaughtering weight, pigs subjected to a longer photoperiod (Trial A) did not show any differences in lean (i.e. fine) cut yield as was demonstrated by similar F-o-M values and single cut percentages for the whole carcass. Several hypotheses can be formulated to explain this result. According to our previous results (Martelli et al., 2005), pigs exposed to a longer photoperiod demonstrated a higher degree of calmness leading to a possible reduction of energy waste through the expression of behaviours, such as pseudo-rooting, which are typically observable under stressful and/or frustrating conditions. Furthermore, taking into account the fact that pigs were fed-restricted (no differences in feed intake) we cannot rule out an increase of Growth Hormone (GH), even though we did not carry out any specific analyses. Dubreuil et al. (1988) have, in fact, demonstrated that darkness produces a decrease in the baseline GH level in pigs of both sexes. Furthermore Laurentie et al. (1989) observed an increase in GH secretion in lambs during resting. The joint effects of a shorter dark period and a longer time spent resting may have improved GH secretion and hence overall body development, which would explain the tendential higher body weights at slaughter given an identical carcass composition (no increase was observed in the ratio of fat to muscle). Considering that we did not observe any effect either on growth parameters or on body weight in pigs exposed to a higher light intensity (Martelli et al., 2010), it may be supposed that light duration has a greater impact on body growth than light intensity.

As concerns fatty acid profiles of raw thighs, the increase in the saturation degree of the subcutaneous fat of the animals exposed to the longer illumination regime could be associable, according to Lo Fiego et al. (2005) and Virgili et al. (2003), to the higher (albeit only tendential) body weight of these animals. Covariance analysis applied to the fatty acid composition using the animals' body weight as a covariate confirmed this relationship. In the case of heavy pigs intended for Parma Ham production, such a modification can be positively considered as it makes possible to obtain a fat whose characteristics are suitable for the dry-curing process, being less subjected to lipid oxidation.

On the whole the results from the present experiments fall within the ranges reported by other Italian authors (Scipioni and Martelli, 2001; Virgili et al., 2003; Lo Fiego et al., 2005; Pugliese et al., 2006). The Iodine number was below 70 and proteolysis index was between 24 and 31% for both trials, according to the limits indicated by Parma Ham production rules (Consortium for Parma Ham, 1992).

Ham yields and their weight losses during the curing process reflect the different initial weights of the raw thighs and all the data are consistent with the standard values reported by Mordenti et. al. (1994) for weight losses of Parma hams after a 12-month curing period (26–28%). With respect to the present experiments it is worth noting that the seasoning period was even more prolonged (18 months).

As concerns dry-cured hams, their quality depends, as is well known, on multiple elements, such as animal breed, animal age, feeding, environmental conditions prior to slaughtering (*ante mortem* factors), product handling at the slaughterhouse and ripening conditions, the raw material quality and the ripening conditions being the most important factors (Gonzalez and Ockerman, 2000; Virgili and Schivazappa, 2002). Consequently, marked differences are detectable among Mediterranean dry-cured hams with respect to quality and sensory properties, which in turn are mainly tied to the type and extent of proteolitic, lipolitic and oxidative processes occurring in muscular and fatty tissues during ripening. With respect to the chemical composition of the cured hams in the present trials, the only significant difference found was a lower sodium chloride content found in hams from group A2 versus group A1. Once again, this difference might be due to the higher weight of the tights from the group A2, which is likely to have slowed down salt penetration. For all groups the sodium chloride content fell within the limits for Parma Ham production (4.5–6.7%; Consortium for Parma Ham, 1992). Overall results concerning the fatty acid composition of fat from cured hams agree with those reported by several authors for Italian heavy pigs intended for Parma ham production (Mordenti et al. 1994; Lo Fiego et al., 2000; Scipioni and Martelli, 2001; Musella et al., 2009). Besides its well known benefits for human health (Katan et al., 1994), the higher percentage of polyunsaturated fatty acids found in group B2 did not negatively impact on the organoleptic properties of hams. The sensory analysis of cured hams, in fact, did not reveal any significant differences among groups in terms of colour and consistency of the lean and the fat components.

3.6 Conclusions

The specific illumination requirements of pigs are linked to their need to receive an appropriate sensory input and to express their behavioural repertoire. Our previous studies suggested that an increase in light duration or intensity can positively affect Italian heavy pig welfare and, in the case of light duration, also growth parameters (Martelli et al., 2005; 2010). Present results on post slaughtering outcomes demonstrate that, within a moderate range of light intensity (i.e. not exceeding 80 lux) and given a minimum of 8 hours of darkness per day to allow optimal conditions for animal rest, increased light duration or intensity above the minimum mandatory levels has no negative impact on carcass composition or the quality of the meat and cured ham derived from Italian heavy pigs. Rearing pigs in a semi-darkness environment in order to avoid competitions between the animals is once again confirmed to be a baseless practice contrary to animal welfare.

PREFACE TO CHAPTER 4

Based on EU legislation, all pigs over 2 weeks of age must have permanent access to fresh water. Nevertheless, rearing techniques based on water restriction are still commonly used in Italy, particularly while rearing heavy pigs intended for dry-cured ham production (*e.g.:* Parma Ham). The aim of this research has been to investigate the effects of water restriction on animal welfare and meat quality, taking into account not only fresh meat, but also dry-cured hams characteristics.

It is hoped that the data obtained from this trial will contribute to a better understanding of water requirements of liquid-fed heavy pigs, with the purpose to develop a strategy to reduce water spillage (and therefore the total volume of manure produced) without affecting animal welfare and production traits.

Research paper based on the chapter:

- Nannoni E., Martelli G., Cecchini M. , Vignola G.; Giammarco M.; Zaghini G., Sardi, L. (2013). Water requirements of liquid-fed heavy pigs: effect of water restriction on growth traits, animal welfare and meat and ham quality. *Livestock Science* 151, 21-28.

CHAPTER 4:

WATER REQUIREMENTS OF LIQUID-FED HEAVY PIGS: EFFECT OF WATER RESTRICTION ON GROWTH TRAITS, ANIMAL WELFARE AND MEAT AND HAM QUALITY

4.1 Abstract

Reducing water waste, and therefore the total volume of manure produced, is one of the ways to lower the environmental impact of intensive pig farming. The aim of this trial was to verify whether the absence of additional fresh drinking water could compromise the production traits or behaviour of liquid-fed heavy pigs. 60 animals (initial BW 78 kg) were divided into two experimental groups, both fed a liquid diet (water-to-feed ratio 3:1 w/w). All pens were equipped with nipple drinkers; one of the groups had permanent access to fresh water thanks to nipple drinkers installed in the pen (working drinkers—WD), whereas the other group had no water supply except that delivered with food (dry drinkers—DD). The pigs were housed in temperature- and humidity-controlled rooms. They were brought to a weight of 160 kg and then slaughtered. Hams were dry-cured according to the directives for Parma ham production. No significant differences ($P > 0.05$) were observed between the experimental groups with respect to growth parameters (ADG and FCR), behavioural traits, blood parameters or the qualitative traits of carcasses (dressing out, lean meat yield, backfat thickness), meat (pH, colour, WHC, fatty acid composition of subcutaneous fat and tenderness) and cured hams (weight losses, sensory properties, chemical composition and oxidative status). With respect to drinking behaviour, a low number of visits to the drinker was recorded for both groups and data seem to indicate a high amount of water wasted by pigs provided with additional water delivery by nipple drinkers. Liquid feeding did not suppress drinkers use or drinker manipulation in both groups.

4.2 Introduction

Water is often referred to as “the forgotten nutrient”, and it has received less attention than any other nutrient (Brooks and Carpenter, 1990). This is the reason why the water requirements of pigs are not as well understood as those for other nutrients. There are two main reasons why it is difficult to establish water requirements: first of all, water needs can vary considerably depending on the animal's physiological state, rearing environment and diet. Secondly, a consumption-based approach to the water requirements of pigs may not be accurate due to inevitable waste. The amount of water that flows through the drinkers should not be assumed as the pigs’ intake, since much of it is not ingested by the animals but wasted. Brooks (1994) reported 60% waste in growing pigs and Fraser and Phillips (1989) reported 23 to 80% waste in sows, although it is generally accepted that wastage from the drinkers will depend on flow rate as well as mounting method and position.

The EFSA opinion (2007) on the welfare of fattening pig states that the availability of fresh drinking water is important, particularly for dry-fed pigs. It has been extensively confirmed that water intake influences dry matter intake and therefore pig growth performance, and that the welfare of pigs is compromised if water is unavailable (Kyriazakis and Savory, 1997). According to EC legislation (Council Directive 2008/120), all pigs over 2 weeks of age must have permanent access to a sufficient quantity of fresh water. However, the legislation provides no indication as to how much water should be supplied to the animals or how.

It is difficult to obtain representative water consumption data from the literature, as the only available data are based either on old studies (for instance Gill et al., 1987) or on practical guidelines (*e.g.*: National Pork Board, 2002). According to the cited references, water consumption varies from 4.2 to 20 L/pig/day. Gill et al. (1987) recorded voluntary water consumption of 0.44 L in growing-finishing pigs fed at a 3:1 water-to feed ratio. More recently Brumm et al. (2000) confirmed the wide range in water use and dependence of water use and manure volume upon feeder and drinker type. Furthermore, to our knowledge, the only research dealing with water consumption of animals over 100 kg BW was carried out by Faeti et al. (1998), who found in pigs between 42 and 170Kg BW an average water consumption ranging from 5.0 to 7.5 L/pig/day; besides, the available literature dealing with water requirements of heavy pigs never considered behavioural traits. Since heavy pigs are intensively reared in a relatively barren environment up to at least 9 months of age, stereotypies and redirected exploratory behaviour can represent a serious welfare problem, probably because of oral dissatisfaction due to restricted feeding and/or to the lack of rooting materials in animals kept on slatted floors (Scipioni et al., 2009). Therefore, considering that heavy pigs are one of the categories with the most limited amount of behavioural information, a broader approach to water needs would be needed, taking into account not only water consumption, but also behavioural traits.

The Integrated Pollution Prevention and Control (IPPC) Directive reference document (EU, 2003) clearly states that water should be efficiently used in order to reduce waste water and manure production. Even if a reduction in animals' water consumption is not among the IPPC recommendations, they acknowledge the existence of some production strategies that include restricted water access.

As in other countries, liquid feeding is a common technique in Italian heavy pig production. Feed is mixed with water or with by-products from the human food industry. In Northern and North-Eastern regions of Italy, the historical availability of whey as a by-product of cheese production (*e.g.* Parmigiano Reggiano and Grana Padano) has encouraged liquid feeding as a widespread practice because of its relatively low costs. Liquid feeding generally has a beneficial effect on pig performance, mainly due to improved nutrient digestibility. The amount of water in the feed influences the outcomes (Della Casa et al., 1991; Hurst et al., 2008). A water-to-feed ratio of 3:1 or 3.5:1 improves growth and feed efficiency (Barber et al., 1991); a ratio of 4:1 might be necessary if salt rich feed are fed, *e.g.* whey (Mordenti and Scipioni, 1993), whereas at higher ratios (>4:1) dry matter intake and ADG are depressed (Choct et al., 2004).

Theoretically, liquid-fed pigs should not require an additional source of water given that their water requirements are satisfied through the daily allotment of liquid feed, and this is assumed to be the case with the traditional 3:1 water-to-feed ratio (Mavromichalis, 2006). However, there are many unpredictable circumstances under which water requirements increase, and in these situations pigs can benefit from additional drinking water. Furthermore, it has been shown that even though voluntary water intake decreases in pigs given liquid feed, liquid-fed pigs are motivated to work for additional water depending on the feeding system used (Vermeer et al., 2009).

The present study had a double aim. The first was to gain knowledge about the water requirements of liquid-fed pigs by investigating how water waste and therefore manure output could be quantitatively reduced. The second aim was to verify, under controlled experimental conditions, whether animal welfare and performance could be affected by a lack of fresh drinking water. The quality of meat and dry-cured hams was taken into account as well.

4.3 Material and methods

The experiment was carried out in the facilities of the Faculty of Veterinary Medicine of the University of Bologna, Italy and it was conducted in observance of current Italian legislation implementing EU legislation on pig protection. The experimental protocol was approved by the Ethical Committee of the University of Bologna. During the trial no sanitary problem occurred.

4.3.1 Animals, housing and feeding

Sixty hybrid pigs with an initial average body weight (BW) of 78 kg were used. Pigs were kept in collective pens (5 animals per pen) on a totally slatted floor with a floor space of 1.20m^2/pig (Figure 4.1). Each pen was equipped with a nipple drinker and a collective feeder, which was not provided with individual separations. Hanging chains were provided as environment enrichment. Pens were located in temperature- and humidity-controlled rooms (22°C, 70-80% RH) equipped with a forced-air ventilation system. Pigs received 10 hours of artificial light every day (8 AM—6 PM), supplied by neon tubes. According to the guidelines for Parma Ham production (Consortium for Parma Ham, 1992), they were slaughtered at an average BW of 160 kg, after a 15-h fast.

In order to meet the pigs' requirements, two commercial feed formulations (espressed on as-fed basis) were used:

- <u>from 78 kg to 110 kg BW</u>: 3195 Kcal DE/kg, 15.83% CP, 0.80% lysine;
- <u>from 110 kg BW to slaughtering</u>: 3199 Kcal DE/kg, 14.39% CP, 0.73% lysine.

Pigs were liquid-fed at 9% of their metabolic BW ($BW^{0,75}$) up to a maximum of 3.1 kg of feed per pig, divided into two meals (8:30AM and 3:30PM). The water-to feed ratio was 3:1, corresponding to a 22.5%DM content of the liquid feed. The fattening phase took place between October and February.

Animals were homogeneously allotted (on the basis of their sex and BW) to two experimental groups:

a) <u>WD (Working Drinkers)</u>: six collective pens each having a nipple drinker which allowed the animals to drink water *ad libitum*;

b) DD (Dry Drinkers): six collective pens each having a nipple drinker, but which was not working: water supply to these pens had previously been discontinued. Consequently, these pigs had no fresh water available, and the only source of water for them was liquid feed.

Nipples were installed at 75cm above the floor and the flow rate was adjusted at 1 l/min. in the WD group.

Figure 4.1: On the left, inside view of one of the pens. On the right, the portable system for feed preparation and distribution which was used during the experimental trial.

4.3.2 Growth and blood parameters

Pigs were individually weighted at the beginning of the trial (day 0), on day 49, and at the end of the trial (day 124) to calculate average daily weight gain (ADG). Feed intake of every pen was recorded, in order to calculate feed conversion rate (FCR). Data collection of growth parameters stopped on day 124, when half of the pigs reached the required slaughtering BW of 160 kg. The remaining pigs were kept under the experimental conditions up to the day in which these pigs in turn attained the final body weight of about 160 kg.

Blood samples were collected at the end of the trial from a total of 30 randomly-chosen animals (15 from each group). Samples were analysed for Hematoctrit (HCT), Haemoglobin (Hb), Red Blood Cells (RBC) and White Blood Cells (WBC) counts. Smears of whole blood were made, air-dried and stained with May-Grünwald-Giemsa. Differential cell count was performed by two independent operators under light microscope in order to obtain Neutrophil-to-Lymphocyte ratio (N:L).

4.3.3 Water disappearance, visits to the drinkers and behaviour

In the WD group, water-meters (Superdry, Eur-8, Idrotech, Udine, Italy) were installed along the water distribution system (Figure 4.2). Every water-meter recorded water disappearance from the drinker of a single pen. Water disappearance was recorded every 2 weeks and average daily water disappearance per pig was calculated.

Figure 4.2: One of the water-meters installed along the water distribution system.

Daily behaviour (from 8 AM to 6 PM) of 40 pigs (eight pens of five animals; four pens per group) was videotaped once a week by means of a digital closed circuit system (Figure 4.3). No recording of vocalization was made. A total of 15 videotaping sessions were recorded. Videos were examined by a single trained operator and behaviours were assessed by scan sampling at 10 minutes intervals according to a predetermined ethogram for heavy pigs (Martelli et al., 2010). Videos were then watched continuously and drinking behaviour (number and duration of visits to the drinkers) was recorded. A visit was defined as a contact with the drinker lasting more than 1 second and, when visible, followed by deglutition. If two consecutive contacts were less than 3 s apart, they were considered as a single visit.

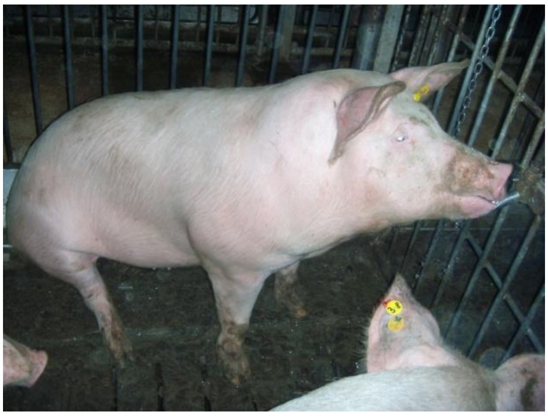


Figure 4.3: Behavioural observations. On the left, screenshot during feed distribution. On the right, one of the drinking bouts.

4.3.4 Carcass, meat and ham quality

In order to comply with the required BW for Parma Ham production (160 kg), pigs were slaughtered in two sessions. At the slaughter plant, carcass weight and the weight of the main carcass cuts (thigh, loin and shoulder) were recorded; lean meat percentage and back-fat thickness were measured by Fat-o-Meater (FOM-SFK, Copenhagen, DK). Dressing out percentage and the yield of the main cuts were subsequently calculated on the basis of carcass weight. pH measurements were taken in the *Longissimus dorsi* (LD) muscle pH at 45 min post mortem (pH 45') and 24h post mortem (pH 24h) by a portable pH meter (model 250A, Orion Research, Boston, MA). At 24 h post mortem, instrumental colour (Minolta CR-200 Chromameter Minolta Camera, Osaka, Japan, D65 illuminant, colour space L*a*b*) was measured in the LD muscle, and samples of the muscle were taken in order to determine drip loss and cooking loss according to Honikel (1998). Shear Force was measured on six cores from the cooked samples using an Instron Universal Testing Machine, model 1011 (Instron Ltd., England) fitted with a Warner-Bratzler (WB) device at a cross-head speed of 200 mm/min (Figure 4.4).

Figure 4.4: Determination of WBSF in the cooked meat. On the left, Instron Universal Testing Machine. On the right, detail of the blade and the cilindical core

Subcutaneous fat was sampled in the area overhanging *Biceps Femoris* (BF) muscle. Total lipids were isolated (Folch et al, 1957) and, after methylation, fatty acid composition was determined by gas chromatography (HRGC8560 Series Mega 2 gas chromatograph; Fisions Instruments, Milan, Italy). Fatty acids were esterified using 5% metanolic hydrogen chloride. The fatty acid methyl esters were separated by gas chromatography using a Supelco SP-2330 capillary column(length: 30 m; internal diameter: 0.25 mm; film thickness: 0.2 mm; Supelco, Bellefonte, PA, USA). Injector and detector temperatures were kept at 220 °C and 280 °C, respectively. The column was programmed as follows: 140 °C for 1 min; the temperature was then raised to 220 °C (3 °C/min) and held constant for 15 min. Fatty acids were identified by comparing the retention times of the peaks with those of known standards. Results are expressed as percentages of total fatty acids. The iodine number was determined according to the AOAC method (2000).

Hams were followed during the whole dry-curing process. They were weighted after dissection from the carcass, after trimming, after salting, after 12 months and at the end of the dry-curing period (18 months). Weight losses were calculated for each productive step.

At the end of the dry-curing process, 32 hams (16/group) were randomly selected and deboned. A sample-slice (including BF and SM muscles) was taken transversally from the caudal portion of the ham to the middle of the femoral bone impression. The slice was evaluated by a panel of trained experts. Evaluation was expressed according to Sardi et al. (2012) on a scale ranging from 1 to 10 (1=absence of the trait; 10=maximum presence) for the following parameters: wet surface, texture, colour inhomogeneity and marbling for the lean portion; texture, thickness and oily surface for the fat. An overall score was attributed as a global evaluation of the ham, expressed on a scale ranging from 1 to 10 (1=very bad quality; 10=optimal characteristics). With the same techniques described before (Minolta colorimeter), colour of the SM muscle and of the subcutaneous fat was measured. Samples were taken from the BF muscle. Moisture and crude protein were analysed according to AOAC methods (AOAC, 2000), sodium chloride content and proteolysis index (non-protein nitrogen/protein nitrogen) were determined (Baldini et al., 1992, Careri et al., 1993). Purified lipids were analysed for peroxides (AOAC, 2000). TBARS (Tiobarbituric Acid Reactive Substances) were assessed according to the method proposed by Wang et al. (2002), specifically adapted: during the analysis, with the only exception of the incubation phase, samples were kept on ice in order to reduce lipid oxidation.

4.3.5 Statistical Analysis

Data were analysed using the STATISTICA 10 package (StatSoft Inc., 2011). Normality of data was assessed by the Kolmogorov–Smirnov test and the data obtained were submitted to one-way analysis of variance using the presence/absence of fresh water as the main effect. The statistical unit was the pen for the growing, behavioural and water consumption data; the individual (pig or ham) for carcass, meat and ham quality data. The effect of time on water disappearance from the drinkers (WD group) was submitted to one-way analysis of variance. The Bonferroni *t* test (α=0.01; 0.05) was used for pair-wise comparisons of variables differing by $P<0.05$. For nonparametric data (behavioural traits and sensory evaluation of hams), the Mann-Whitney test was used. The significance level for all statistical tests was set at $P < 0.05$.

4.4 Results

Table 4.1 shows the animals' growth and blood parameters. The experimental conditions (presence or absence of *ad libitum* fresh water) did not significantly affect any of these parameters.

The behavioural pattern of pigs, expressed as a percentage of total observed behaviours, is reported in Table 4.2. No statistically significant differences were observed between the experimental groups. Animals spent most of the observation period (approximately 72%) lying down (either lateraly or sternally), whereas exploring activities occupied 16% of the observation period. The time spent inactive (either sitting or standing) was between 5% and 8% of the observation period. The frequency of occurrence of other behaviours such as eating, drinking, walking or fighting was low (between 4% and 7% overall).

Table 4.1: Growth and blood parameters

Group		**WD**[a]	**DD**[b]	**RMSE**
Pigs	n.	30	30	-
Initial BW (day 0)	Kg	78.8	78.3	12.12
Intermediate BW (day 49)	Kg	117.1	118.2	11.71
Final Weight (day 124)[c]	Kg	159.5	161.2	13.93
ADG (days 0-49)	g/d	784	814	186.5
ADG (days 49-124)	g/d	571	596	85.99
ADG (days 0-124)	g/d	656	683	105.79
Total duration of the trial	D	138	135	17.20
FCR (days 0-49)	kg DM/kg BW	3.36	3.24	0.30
FCR (days 49-124)	kg DM/kg BW	5.57	5.33	0.45
FCR (days 0-124)	kg DM/kg BW	4.49	4.32	0.28
HCT	%	42.3	41.9	3.86
Hb	g/dL	15.2	14.9	2.27
RBC	$*10^6/\mu L$	7.8	7.6	0.71
WBC	$*10^3/\mu L$	14.1	14.7	3.26
N:L		0.61	0.59	0.20

Data analysis evidenced no statistically significant difference(P>0.05) between the experimental groups.
[a] Working Drinkers [b] Dry Drinkers
[c] Data collection of growth parameters stopped on day 124, when half of the pigs attained the required BW for Parma Ham production and were slaughtered.

Table 4.2: Daily behavioural pattern of heavy pigs (percentage of total observed behaviours)

Groups		**WD**		**DD**	
Pigs	**n.**	**20**		**20**	
Replicates	**n.**	**4**		**4**	
		Average	**SD**	**Average**	**SD**
Standing inactive	%	2.84	2.54	1.99	2.34
Sitting inactive	%	4.68	4.31	3.36	2.28
Lateral recumbency	%	43.69	11.77	44.37	13.21
Sternal recumbency	%	27.25	8.11	27.82	9.29
Eating	%	1.80	1.78	1.45	1.66
Drinking	%	0.02	0.15	0.04	0.29
Walking	%	1.43	1.58	1.22	1.66
Exploring the floor	%	15.28	6.43	16.48	6.36
Fighting	%	0.59	0.95	0.29	0.61
Other[a]	%	2.36	1.77	2.98	1.59

Data analysis evidenced no statistically significant difference(P>0.05) between the experimental groups.
[a] "Other" includes all behaviours that are not listed, *e.g.*, changing position and social interaction.

Water disappearance data are reported in Figure 4.5. Average daily water disappearance from the drinkers was 0.76 ± 0.41 L/pig and no time effect was detectable. If we consider that each animal received an average of 8.9 L of water per day mixed with feed, the total average daily water consumption of WD group (i.e., water delivered with food + water disappearance from the drinkers) was 9.7 L/pig. Recorded water disappearance from the drinkers varied greatly from one pen to another though, with values ranging from 0.48 to 1.35 L/pig/day (Figure 4.6)

Figure 4.5: Average water disappearance from the drinkers (WD group)

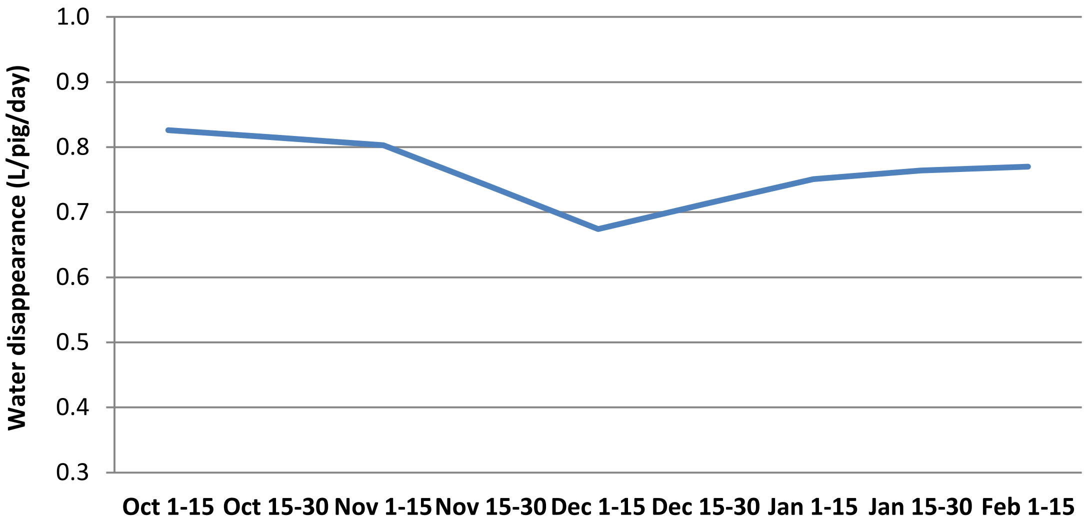


Figure 4.6: As an exemplification of the water consumption differences observed between the pens, this graph shows water consumption data from the two pens where the highest (water meter 1) and the lowest (water meter 2) water consumptions were recorded.

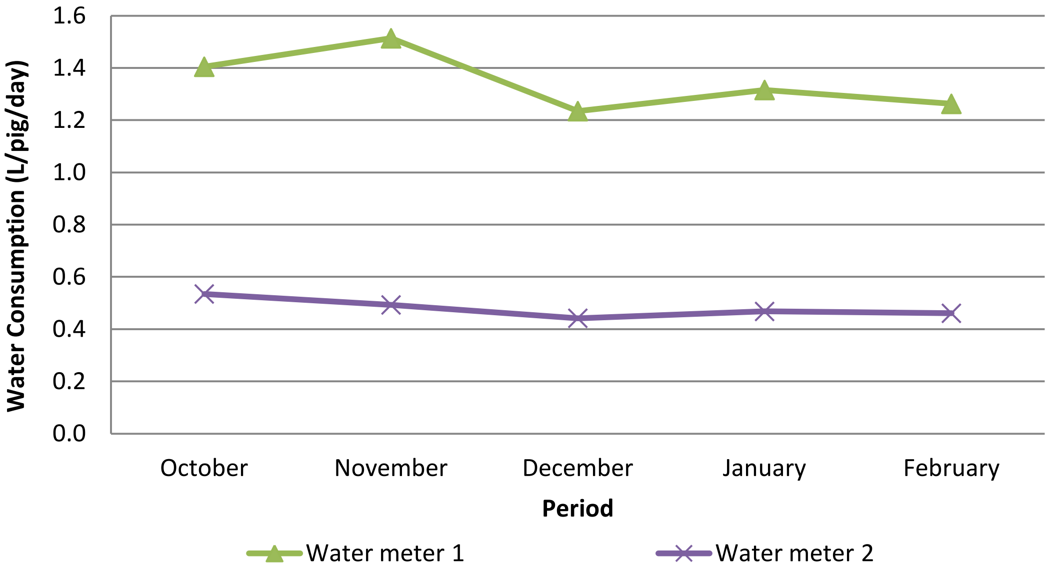


Figure 4.7 shows the number of visits to the drinkers (*i.e.* drinking bouts or drinker manipulations) per pig per day. The average value was significantly lower in the DD than in the WD group (0.6 ± 0.4 vs. 1.3 ± 0.4, P<0.01). Drinking behaviour/drinker manipulation was not significantly different between the two groups (total daily time spent at the drinker: 7.61 vs. 6.28 seconds/pig in the DD and WD groups, respectively), with most visits to the drinkers (74.9%) occurring between 9:30 AM and 3:30PM (Figure 4.8).

Figure 4.7: Daily visits to the drinkers (number of visits per pig, per day). WD=Working drinkers, DD=Dry drinkers. Average number of visits (WD: 1.3±0.44; DD: 0.6± 0.4) was significantly different between the experimental groups (p<0.01)

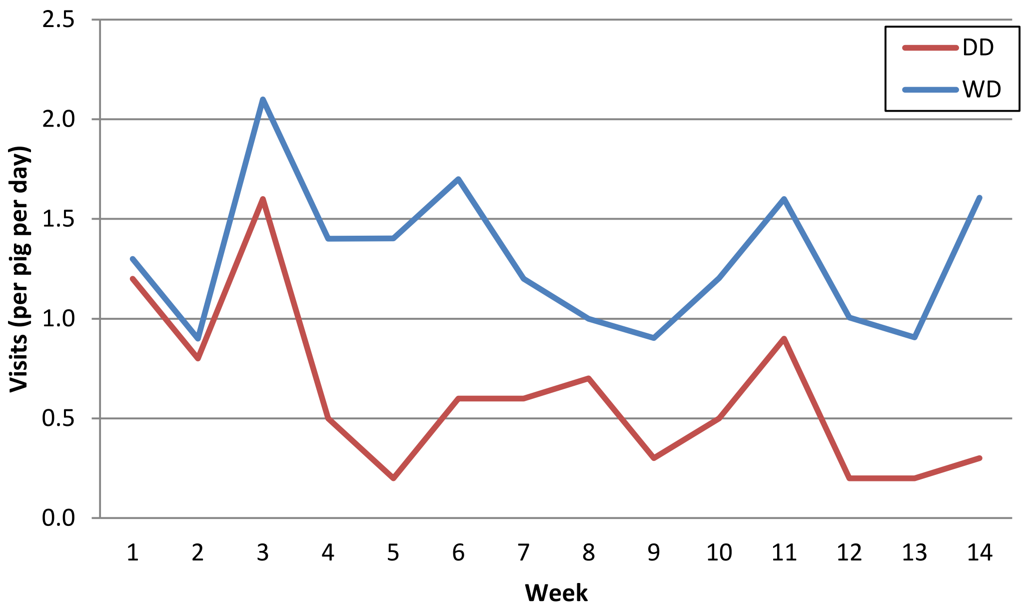
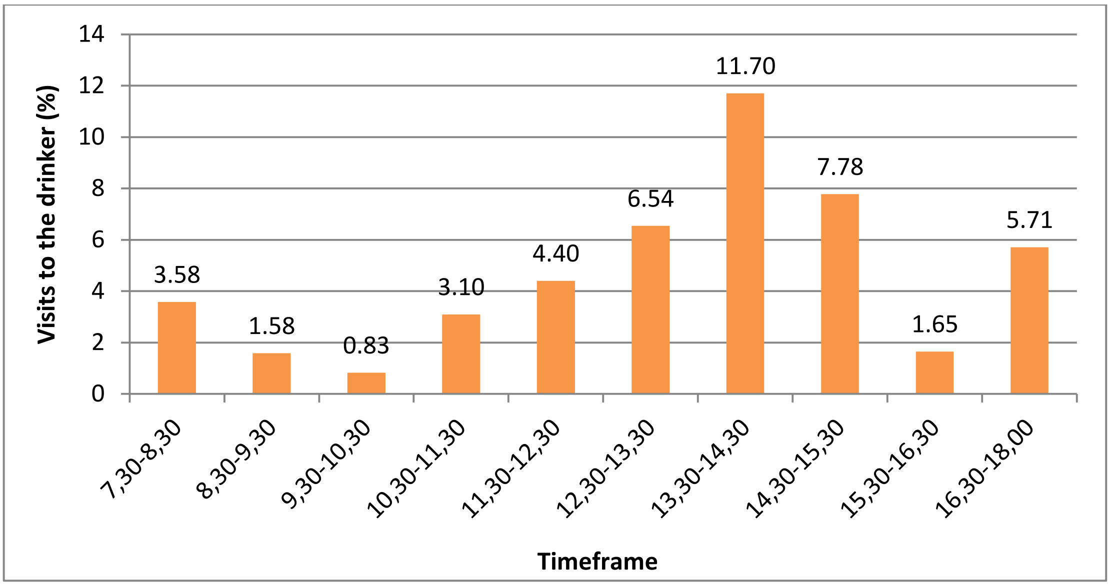


Figure 4.8: Distribution of visits to the drinkers during the observation period (average between the experimental groups)



As concerns carcass and meat quality, no significant differences were detected (Table 4.3): also fatty acid composition and iodine number of the raw thighs subcutaneous fat did not show any significant differences between treatments (Table4.4).

Table 4.3: Carcass and meat quality

Group		WD	DD	RMSE
Pigs	n.	30	30	-
Dressing Out	%	84.51	84.21	1.0
Lean Meat (F-o-M)	%	52.20	52.19	2.53
Back Fat thickness	mm	22.14	22.06	4.19
Loin	%CW	24.11	24.16	1.17
Shoulder	%CW	14.82	14.67	0.81
Thigh	%CW	25.06	25.18	1.08
pH45'LD		6.51	6.49	0.21
pH24hLD		5.65	5.62	0.14
L*		44.23	43.79	2.92
Hue[a]		0.77	0.75	0.11
Chroma[b]		9.50	9.64	1.71
Drip Loss	%	1.08	1.07	0.23
Cooking Loss	%	22.02	21.75	2.36
WB Shear Force	kg/cm^2	4.64	4.37	1.25

Data analysis evidenced no statistically significant difference(P>0.05) between the experimental groups.
[a] Hue= arctg(b/a)
[b] Chroma=sqrt(a^2+b^2).

Table 4.4: Acidic composition and iodine number of raw hams

Group		WD	DD	RMSE
Hams	n.	16	16	-
C 14:0	%	1.56	1.53	0.17
C 16:0	%	23.38	23.42	1.18
C 16:1	%	2.47	2.49	0.20
C 18:0	%	10.91	11.11	1.14
C 18:1	%	44.98	44.87	1.88
C 18:2	%	13.27	13.00	1.42
C 18:3	%	0.93	0.97	0.17
C 20:1	%	0.76	0.80	0.14
C 20:2	%	0.70	0.65	0.14
C 20:4	%	0.33	0.35	0.04
SFA	%	36.09	36.56	2.19
MUFA	%	48.47	48.46	1.85
PUFA	%	15.22	14.97	1.53
Iodine Number	gI/100g	67.10	67.76	2.72

Data analysis evidenced no statistically significant difference($P>0.05$) between the experimental groups.

Table 4.5 shows the main qualitative parameters (weight losses, sensory analysis, chemical composition and oxidative status) of the cured hams. With the exception of a higher weight loss after trimming and a lower water content observed in the DD group ($P<0.05$ for both parameters), no significant differences were detected between the groups during the whole process. As concerns the fatty acid composition of the subcutaneous fat from the dry-cured hams (Table 4.6), no statistically significant difference emerged between the experimental groups.

Table 4.5: Ham qualitative traits

Group		WD	DD	RMSE
Thighs	n.	16	16	-
Weight losses during the dry-curing process				
Weight loss after trimming	%	14.46^{b}	15.16^{a}	1.42
Weight loss after salting	% trimmed	6.30	6.26	0.58
Weight loss after 12 months	% trimmed	27.95	27.93	1.96
Weight loss of cured hams	% trimmed	30.58	30.76	2.03
Sensory analysis				
Lean Wet Surface	Points	1.5	1.5	0.52
Lean Texture	Points	5.83	5.92	0.77
Lean Colour dishomogenity	Points	1.83	2.08	1.01
Lean Marbling	Points	2.00	2.83	1.21
Fat Texture	Points	6.00	6.08	0.71
Fat Thickness	Points	3.50	4.08	1.22
Fat Oily surface	Points	2.17	2.33	0.45
Overall score	Points	6.42	6.25	0.76
Lean Colour (SM muscle)				
L*		35.29	35.64	1.62
Hue		0.61	0.64	0.04
Chroma		13.81	13.53	1.05
Fat colour				
L*		75.90	75.76	2.24
Hue		-0.96	-1.22	1.02
Chroma		9.00	8.79	0.54
Chemical analysis				
Humidity ^{c}	%	61.05^{a}	59.97^{b}	3.74
Crude Protein ^{c}	%	26.41	26.97	1.47
Proteolysis Index ^{c}	%	27.33	27.34	2.19
Sodium Chloride^{c}	%	6.04	5.79	0.41

^{a},^{b} Values with different superscripts within the same row are significantly different ($P < 0.05$)
^{c} Values assessed on the lean portion

Table 4.6: Acidic composition, peroxide value and TBARS value of subcutaneous fat from dry-cured hams

Group		WD	DD	RMSE
Hams	n.	12	12	-
C 14:0	%	1.36	1.32	0.10
C 16:0	%	22.33	21.90	0.65
C 16:1	%	2.60	2.35	0.32
C 18:0	%	10.63	10.68	0.79
C 18:1	%	48.48	49.14	1.34
C 18:2	%	11.18	11.01	1.18
C 18:3	%	1.48	1.23	0.14
C 20:1	%	0.41	0.42	0.05
C 20:2	%	0.58	0.63	0.07
SFA	%	34.88	34.45	1.03
MUFA	%	51.50	51.92	1.27
PUFA	%	12.62	12.52	1.13
Peroxide Value[d]	$mEqO_2$ /kg	38.39	35.69	7.59
TBARS[d]	MDA mg/kg	2.03	1.96	0.44

Data analysis evidenced no statistically significant difference between the experimental groups.

4.5 Discussion

The results of the present trial indicate that under our experimental conditions the absence of *ad libitum* fresh water had no effect on the growth and slaughtering parameters of liquid-fed heavy pigs. Blood parameters (Hb, HCT, RBC, WBC) fall within the physiological range for swine (Thorn, 2000). Productive parameters are consistent with data reported for Italian heavy pig production in the InterPIG (2011) report. Our findings are also consistent with data reported by Faeti et al. (1998), who analysed the effect of different water-to-feed-ratios (2:1; 2.5:1; 3:1) in the presence/absence of fresh drinking water. In their two trials, one run in the hot and one in the cold season, they did not find any significant difference with respect to growth and slaughtering parameters.

It is worth noting that our results refer to specific experimental conditions: during this trial, the pigs were not exposed to any factor which could increase their water requirements: they were housed in temperature-controlled rooms, received a low-protein diet, had no health problems (*e.g.* fever, diarrhoea) and they received liquid feed at a 3:1 water-to-feed ratio, which is considered adequate to fulfil water requirements and improve feed digestibility (Hurst et al., 2008).

Nor behavioural traits differed between the experimental groups. The pigs spent most of the day lying (sternal recumbency + lateral recumbency), regardless of treatment. The recorded behavioural pattern was similar to previously observed ones (Guy et al., 2002; Martelli et al., 2010; Morrison et al., 2003; Scipioni et al., 2009; Stolba and Wood-Gush, 1989) and showed very low frequencies of abnormal behaviours and stereotypies (*e.g.:* bar biting, aimless exploring, dog-sitting posture). This is particularly interesting because the animals were fed-restricted and environmental enrichment was provided only by hanging chains, which offer a limited advantage in terms of pig welfare (Brake, 2006; Studnitz et al., 2007). On the other hand, Scott et al (2007) observed that liquid feeding can also have some behavioural effects as compared to dry-fed pigs, liquid-fed pigs spent less time standing and investigating. In our study, the unaltered behavioural pattern, as well as the N:L ratios recorded — which were similar between groups and consistent with those obtained by other authors before stressing events (McGlone et al., 1993; Morrow-Tesch et al., 1994; Puppe et al., 1997) — indicate that under our experimental conditions the absence of fresh water did not act as a stressor.

Total water consumption (9.7 L/day/pig) was comparable to that recommended by most Authors (*e.g.* Thacker, 2001). Average water disappearance from the drinkers is in agreement with what recorded by Vermeer et al. (2009), who observed in liquid-fed finishing pigs (liquid feed DM: 23.6%) an additional water intake of 0.7 L/pig/die. Under our experimental conditions, considering the flow rate (1 L/min) and the average time spent at the drinker (about 7 s over a 10-hour period), each pig drunk approximately 0.12 L from the drinkers. We can consequently suppose that more than 80% of the water obtained from the drinkers (0.64 L, expressly the difference between the 0.76 L of water delivered from the drinkers and the 0.12 L of water drunk) was actually not ingested by the animals, but wasted due to either accidental triggering of the drinkers or drinker manipulations lasting less than 1 s. Therefore each animal consumed 9.7 L/day (including water ingested with liquid feed) with a water spillage equivalent to 6.2% of total daily water delivery.

Such a water waste is low if compared with the literature dealing with dry-fed pigs (Li et al., 2005; Mroz et al., 1995). Also the number and duration of drinking bouts/drinker manipulations we recorded were extremely low compared with the findings of other authors (Li et al., 2005; Turner et al., 1999, 2000;), who recorded in dry-fed pigs 25-30 drinking bouts per pig per day and a total drinking time of 10-14 min over the 24-h period. To our knowledge, none of the available literature deals with pigs fed restricted liquid feed in a trough. The low number of visits and small amount of time spent at the drinkers is indicative of a low motivation for extra water use, suggesting that, under our experimental conditions, water requirements were basically met by the daily allotment of liquid feed. Furthermore, according to Patience (2012), luxury intake (*i.e.*, water consumption beyond what would be considered physiological need) can be due to stress, boredom or hunger. Under our experimental condition most drinking bouts occurred between the two meals, *i.e.*, when the animals were more active, therefore it cannot be ruled out that the animals could access to the drinkers because of boredom and/or hunger.

Even if water intake from the drinkers was low, it's worth noting how it was not completely suppressed by liquid feeding. Besides, the DD group visited the drinkers significantly less times than the WD group, but time spent at the drinkers was not different between the experimental groups, implying a longer mean duration of each visit in the DD group. This behaviour can probably be interpreted as a redirected explorative behaviour. Nevertheless, considering that before the start of the trial all pigs received water also by means of drinkers, a motivation to obtain additional water by DD pigs cannot be completely ruled out.

The difference in water consumption from working drinkers between pens can be ascribed to the individual attitudes of pigs towards drinkers: large individual variations in drinking patterns and total daily drinking time have been observed both in dry- (Turner et al., 2000) and in liquid-fed pigs (Faeti et al, 1998). Considering that only 40 pigs out of 60 were videotaped, it cannot be ruled out that the higher water consumption in some pens may have been due to some animals spending more time than their pen-mates at the drinker, either in order to play or as a stereotyped behaviour. In these pens, social facilitation can play a role as well, by increasing the probability of other animals engaging in the explorative or stereotyped behaviour directed towards the drinker.

With respect to fresh meat quality and acidic composition of the subcutaneous fat of the uncured tights, no significant differences were detected between the experimental groups.

As concerns the quality of hams (raw and cured), the iodine number of raw fat fell within the limit of 70 imposed by the Consortium for Parma Ham (1992). With respect to the oxidation products in cured hams, the TBARS values agree with those reported by Vestergaard and Parolari (1999), though our peroxide values were higher; however, they were similar to those reported by Antequera et al. (1992) and Martín et al. (2000) for lipids extracted from the BF and SM muscles of Iberian dry-cured hams. The slight differences observed with respect to the weight loss after trimming and the humidity content of DD hams might be due to the fact that hams were fatter (higher fat thickness and higher marbling degree of muscle) according to the sensorial score. Although statistically significant, these differences have no practical implications considering that no other differences were detected at any time during the curing process, either for the total weight loss or for the remaining chemical parameters of the cured hams. As expected, also acidic composition of the subcutaneous fat from the cured hams didn’t reveal any significant difference between the experimental groups. On the whole, it can be concluded that the absence of fresh drinking water did not affect the quality of the end products as regards both fresh meat and dry-cured hams.

4.6 Conclusions

Under practical conditions there are many physiological, pathological and environmental variables, in some cases unpredictable, which can increase water requirements. Our data indicate that, under strictly controlled environmental conditions, rationing the water of liquid-fed heavy pigs may be an acceptable method to reduce water spillage (and therefore the total volume of manure produced) without affecting some animal welfare or production traits. Even if the experiment was carried out during the winter season, water intake from the drinkers was not completely suppressed by liquid feeding as well as non-functioning drinkers manipulation. Therefore, in order to attain a high level of animal welfare, water waste should primarily be controlled through proper selection, installation and maintenance of drinkers, together with the provision of appropriate environmental enrichment materials in order to reduce explorative and stereotytped behaviours directed towards the drinkers.

PREFACE TO CHAPTER 5

During road transport, livestock is exposed to a large number of stressful conditions. If animal welfare and meat quality concerns are similar across countries, conditions of transportation may largely vary. Differences can include transportation times and distances, rest, feed and water intervals (which are regulated by legislation), road conditions, trailer design, animal genetics and extreme (cold and hot) weather conditions.

Large three decks trailers (often referred as Pot-Belly trailers -PB) are the most common vehicles for swine transportation in Canada, but their use has been associated with increased dead or fatigued animals and reduced pork quality, depending on animal location within the truck (deck and/or compartment position in the truck).

The research trial presented in the next chapter has been conducted in Canada during the hot season with the aim to assess the effectiveness of water sprinkling pigs in a stationary PB trailer in terms of pig response to heat stress and pork quality variation, and possibly identify a cut-off ambient temperature to ensure the greatest effectiveness of this practice.

Research paper based on the chapter:

- Nannoni E., Widowski T, Torrey S., Fox J., Rocha L.M., Gonyou H., Vanelli Weschenfelder A., Crowe T., Martelli G., Faucitano L., The effects of water sprinkling on exsanguination blood parameters and carcass and meat quality variation in pigs transported during summer. Under review for publication in the *Meat Science* journal (revised version submitted on February 13th, 2013).

CHAPTER 5:

THE EFFECTS OF WATER SPRINKLING PIGS IN A STATIONARY TRAILER DURING SUMMER ON SELECTED EXSANGUINATION BLOOD PARAMETERS AND CARCASS AND MEAT QUALITY VARIATION

5.1 Abstract

In each of 12 weeks between May and September, 2011, two identical pot-belly trailers were loaded with 208 pigs each and transported to the slaughter plant (2 h trip). One of the two trailers was equipped with a water sprinkling system (WS) installed inside the truck compartments whereas the other one transported pigs under standard commercial conditions (control, CONT). The water sprinkling system was activated for 5 min in the stationary truck, both at the farm (at the end of loading) and at the plant (immediately before unloading). Blood lactate levels at exsanguinations, carcass and meat quality traits were assessed on a sub-sample of randomly selected pigs (n=384/576). Exsanguination lactate levels decreased (P=0.02) in WS pigs compared to CONT, regardless of temperature. Concurrently, the pH value of the *Longissimus dorsi* (LD) muscle at 1h post-mortem (pH1) was greater (P=0.009) in WS pigs compared to CONT, regardless of ambient temperature. The effects of water sprinkling recorded differed according to pigs location inside the truck: water sprinkling reduced exsanguination lactate levels in pigs transported in compartments 5 and 8 (which are located at the front and at the rear of the middle deck, respectively): such a reduction was observed in compartment 5 at 15°C (P=0.03) and 18°C (P=0.009), and in compartment 8 at 22°C (P=0.03) and 25°C (P=0.04). In compartment 5, the pH1 value in the LD muscle of WS pigs was higher than in the CONT group at 18°C (P=0.002), 22°C (P<0.0005) and 25°C (P=0.005); pH1 in the SM muscle of WS pigs was lower at

18°C (P=0.01) and 22°C (P=0.02); and drip loss in the WS group was lower than in the CONT group at 22°C (P=0.01), and at 25°C (P=0.02). No significant effect was detected in compartment 4 (which is located at the rear of the top floor), or in compartment 9 (which is located at the front to the bottom deck). The results of this study showed that the sprinkling protocol applied was effective, particularly in some trailer compartments, in reducing stress response and improve pork quality at ambient temperatures greater than 20°C.

5.2 Introduction

In Canada, animal losses during transport increase during the summer months (Haley et al., 2008) as a result of the limited capability of pigs to cope with hot temperatures (Warriss, 1998a). The highest deaths recorded in the above-mentioned Canadian transport survey were during the month of August (0.40%) when the maximum ambient temperature was 33.6 °C. Truck design usually ensures adequate natural ventilation to prevent the internal temperature from reaching the upper threshold of thermal tolerance when the truck is moving, but when it is stationary, the internal temperature can rapidly increase (Marchant-Forde & Marchant-Forde, 2009).

In North America truck designs vary widely, from small single deck trucks to large three-deck punch-hole trailers (often referred as PB trailers). PB trailers are often dual-purpose (transporting either pigs or cattle) and allow the transportation of large loads of pigs (more than 200) on three decks (10 compartments) in one journey. However, these vehicles incorporate multiple (up to 5) and steep (up to 40° slope) internal ramps and 180° turns, which result in the reduction of handling ease during loading and unloading, increasing the use of electric prods and extending the load and unload times. As reviewed by Schwartzkopf-Genswein et al. (2012), these observations have been associated with a higher proportion of dead-on-arrival (DOA) and fatigued pigs in the PB trailer when compared to other vehicle types that are equipped with hydraulic decks, such as a double-decked truck or a flat-deck trailer.

Animal location (deck and/or compartment position within the truck) during transportation has an impact on welfare and meat quality (Bench et al., 2008). According to Weschenfelder et al. (2012), the compartments in the middle and bottom front of a stationary pot-belly (PB) trailer, were up to 6 °C warmer than the external ambient temperature during Canadian commercial transports. These environmental conditions may have contributed to a higher incidence of poor quality pork from pigs located in these compartments as reported in previous transport studies (Correa et al., 2009, 2013). Within the PB trailer, higher temperatures have been recorded in the front compartments of the middle and bottom deck (or "belly"), while the upper compartments presented lower temperatures (Brown et al., 2011b). In their study, the higher and lower temperatures have been explained by reduced ventilation and poor insulation (increased thermal radiation), respectively. According to Huynh et al. (2007), when high temperature is associated with high humidity, the importance of skin evaporation increases relative to respiratory evaporation, therefore in these conditions pigs should be able to wet their skins. As suggested by Brown et al. (2011b), in the summer, bottom and front compartments of a stationary PB trailer can be cooled by increasing the ventilation rate using fans in combination or not with water sprinkling to increase evaporative cooling.

Recently, the European Food Safety Authority (EFSA) on the welfare of animals during transport recommended the development of water misting devices to ensure pig comfort during transport (EFSA Panel on Animal Health and Welfare, 2011). Despite the interest in water misting as a mean to reduce thermal stress, studies demonstrating its effects on animal welfare and meat quality and science-based recommendations on its application are very few (Nanni Costa, 2009). For this reason, controlled studies are needed to assess the efficacy of water sprinkling on pig thermal comfort (Ritter, 2009b) and validate the existing, and contradictory, recommendations on the cut-off ambient temperature for the application of this procedure in a stationary truck.

Christensen and Barton-Gade (1999) recommend to sprinkle pigs using an intermittent misting system during transport when temperature is over 25°C. With environmental temperatures above 10-15°C, Colleu and Chevillon (1999) recommended to water sprinkle pigs for 5 minutes immediately after loading in order to reduce their body temperature by 10 % (3-4°C) and mortality rate (-25%). However, the increased comfort during transport did not result in any meat quality improvement in their study. Grandin (2002) recommended to use wet bedding (sand or wood-shavings) between 15 and 27°C, and, when the temperature is higher than 27°C, to sprinkle pigs if the truck has to remain stationary. Ritter (2009a) recommends, once a trailer deck has been loaded, to shower the pigs just long enough to get the pigs and the absorbent material wet. Keeping pigs in a stationary vehicle prior to unloading has been shown to increase animal losses and the incidence of PSE (pale, soft, exudative) pork, especially when the temperature is over 20°C (Driessen & Geers, 2001; Ritter et al., 2006), but sprinkling pigs prior to unloading at the slaughter plant is not a common practice and its impact on animal welfare and meat quality has not been assessed.

Pre- slaughter handling (such as transport and lairage) has been identified as one of the most stressful periods in the pigs' life (García-Celdrán et al., 2012), and any of the stress factors during pre-slaughter handling can result in changes in the metabolites of muscle ultimately having a detrimental effect on carcass and meat quality (Aziz, 2004). Blood lactate was proven to be an early indicator of physical stress and exhaustion (Benjamin et al., 2001), and to have a good correlation with the rate of early post-mortem metabolism and muscle drip loss (Edwards et al., 2010a), therefore it was adopted as a stress indicator in this study, together with the haematocrit value, which rises both as an initial response to stress, and during dehydration (Hall and Brashaw, 1998).

The overall objective of this study was to evaluate the effectiveness of water sprinkling in a stationary truck (both after loading and before unloading) in warm climate conditions, in terms of blood stress indicators and variation in carcass and meat quality parameters.

5.3 Materials and methods

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

5.3.1 Transport and water sprinkling protocol

A total of 4,992 Duroc x (Yorkshire x Landrace) crossbred pigs (115±10 kg) of mixed genders, originating from a single commercial finishing farm located near Thedford (Ontario, Canada) were transported to a slaughter plat located in Breslau, Ontario (2 h trip - 120±13min) using 2 tri-axle, dual purpose pot-belly (PB) trailers. A trip (or replicate) per trailer was carried out each of 12 weeks between May and mid-September 2011. Both PB trailers transported 208 pigs distributed across three decks and comprising of 10 compartments (4 in the upper and middle decks and 2 in the bottom deck) at a density of 0.40 m^2/100 kg (245 kg/m^2).

Of the two trailers, one was equipped with a custom-made water sprinkling (WS) system (Weeden Environments, Woodstock, Canada), while the other had no sprinkling system installed (control, CONT). The sprinkling system was operated in the stationary PB trailer. Two 5–min sprinkling sessions were performed:

- after the end of loading (i.e. immediately prior to departure from the farm);
- at the end of the 30—min wait at the plant (i.e. immediately before unloading).

Each 5 min sprinkling session delivered approximately 125 L of water evenly throughout the trailer through twenty-two 180^{o} spreader nozzles spraying in from each side of the trailer. According to the manufacturer, droplet size of the water was 900-1000 microns. Although this droplet size would apply more to the official definition of water misting, i.e. “a water spray for which 99% of the total volume of water is in droplets with a diameter less than 1000 microns” (NFPA, 2010), in this paper the word “sprinkling” will be used. This decision was made given the technical impossibility to verify the droplet size in field conditions and based on the observation of the water jet pattern through the sprinklers and the effects observed inside the trailer. Indeed, from a practical standpoint, misting can be defined as very fine droplets that evaporate while in the air thereby reducing air temperature, whereas sprinkling refers to coarse droplets that fall and wet the objects and the environment.

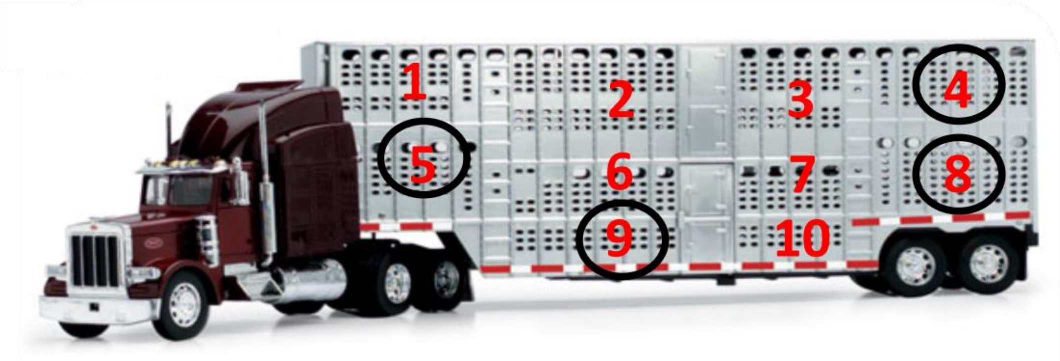
Figure 5.1: Pictures showing one of the nozzles and the inside of the WS trailer during the sprinkling session.

In each replicate (or journey), 48 “sentinel” (or test) pigs were randomly chosen at the farm and distributed at loading into 4 separate test compartments on each trailer (6 test pigs/test compartment). As shown in Figure 5.2, the following test compartments were chosen due to previous results showing compartmental variations in trailer microclimate (Brown et al., 2011b; Weschenfelder et al., 2012):

- compartment 4 (C4) located at the rear of the top deck;
- compartment 5 (C5) located at the front of the centre deck);
- compartment 8 (C8) located at the rear of the centre deck);
- compartment 9 (C9) located at the front of the bottom deck (“belly”).

Loading and unloading order between trailers and decks were randomized each week (by alternating the loading order through decks, i.e. loading the top or the bottom deck first) in order to avoid the confounding effect of the outside temperature variation and wait time in each deck (or compartment). Environmental temperature during transport was recorded according to the hourly data from a nearby Environment Canada Weather station. The average ambient temperature registered between loading and unloading for each journey was 19.5°C, ranging from 14.1 to 25.8°C.

Figure 5.2 Compartment position inside the Pot-Belly trailer. Test compartments are circled.



The driver of each trailer and the handler at the farm were the same through the 12 weeks. Pigs were raised on slatted floors in a growing-finishing unit and fed a liquid corn and soy based diet, including a balanced premix. On the day before transport, they were moved to two shipping rooms consisting of 8 pens each. Each group was kept in separate shipping rooms by treatment (sprinkling *vs.* no sprinkling) at a stocking density of 0.86 m^2/pig. The size of each pen corresponded to half the size of the truck compartment, so that mixing of unfamiliar pigs in the truck was reduced. Pigs were withdrawn of feed for approximately 18 h before transport (22 h before slaughter). During loading at the farm, pigs were loaded in small groups (6-8 pigs/group) using paddles. Electric prods were only used in a few occasions on a limited number of pigs, and only when it was absolutely needed, to prevent their negative effects on stress response and meat quality (Correa, et al., 2010). On arrival at the plant, pigs were unloaded using a paddle only and driven to separate lairage pens based on the transport compartment (no mixing was allowed). No water sprinkling was applied on pigs during lairage. After a period of lairage (122±13 min), pigs were driven using whips and paddles to a CO_2 stunner (Combi 77, Butina, Denmark) and exsanguinated.

5.3.2 Blood parameters

The day before transport, 6 “sentinel” pigs per test compartment were individually identified and weighed (Live Weight, LW) at the farm for the meat quality evaluation (n=576). Attempts were made to balance genders with a final representation of 57% barrows and 43% gilts). Within each group of 6 “sentinel” pigs, 4 pigs were randomly selected for the blood lactate study (n=384). These animals were restrained in a weighing scale and a small blood sample was obtained by pricking one of the animal's distal ear veins with a retractable 22 gauge needle. Lactate values were immediately assessed in duplicate by means of a hand-held lactate analyzer (Lactate Scout Analyzer, EKF Diagnostic GmbH, Magdeburg, Germany) (see Figure 5.3). This technique was successfully used in recent on-farm and pre-slaughter studies (Buzzard et al., 2010; Edwards et al., 2010a,b). Since blood lactate is a quick handling stress indicator, reaching its peak in 4 minutes after the stressor imposition (Anderson, 2010), pig handling was as gentle as possible and the blood sample was obtained within 2 minutes after the animal entered the scale. A second blood sample was obtained from these animals at exsanguination and lactate levels were immediately measured with the same technique. A third blood sample was collected at exsanguination (in K_2EDTA tubes), refrigerated (4°C) and subsequently analysed for haematocrit (HCT) determination with the microhematocrit technique according to a procedure described by Matte et al. (1986).

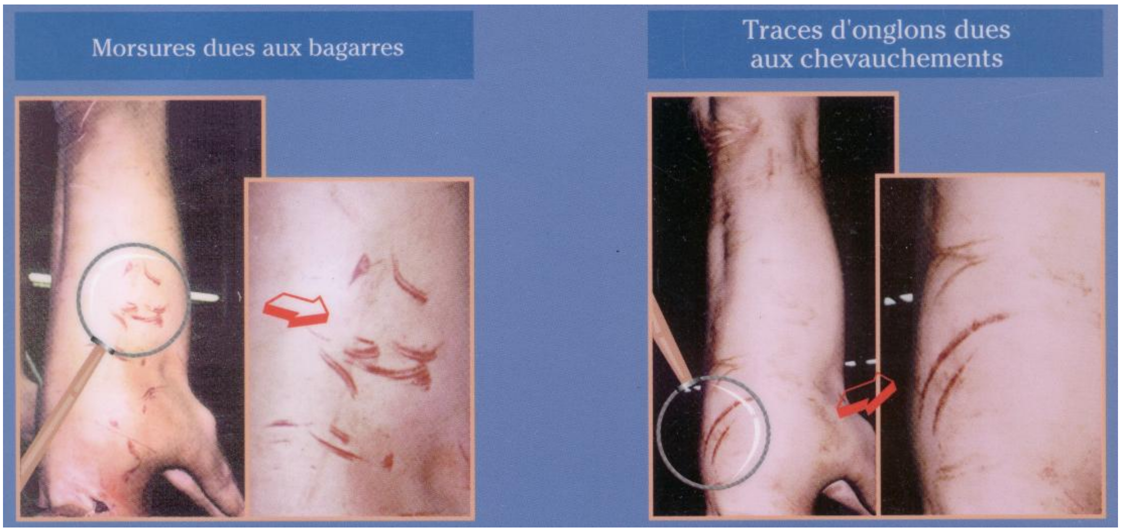
Figure 5.3: Basal lactate at the farm: sampling technique. On the left, vein pricking; on the right, collection of a drop of blood on a test strip with the hand-held lactate analyzer.

5.3.3 Carcass and meat quality parameters

Following slaughter, carcasses were eviscerated, split, and chilled according to standard Canadian commercial practices. Hot carcass weight (HCW) and carcass lean percentage (by Destron probe) were recorded, and HCW was used to calculate dressing percentage according to the following formula: dressing%=HCW/LW*100.

Skin damage was assessed on the day of slaughter in the cooler using the 5-point, photographic scale (1 = none to 5 = severe; MLC, 1985), whereas bruises were classified as fighting type bruises (1 = less than 10 bruises; 2 = 11 to 20 bruises; and 3 = greater than 20 bruises) or mounting (score 1 = less than 5 bruises; 2 = 6 to 10 bruises; and 3 = greater than 10 bruises) by visual assessment of shape and size according to the photographic standards of the Institut Technique du Porc (ITP, 1996) as described by Faucitano (2001). According to the ITP scale (Figure 5.4), bruises due to biting during fighting are 5 to 10 cm in length, comma shaped, and concentrated in high number in the anterior (head and shoulders) and posterior (ham) regions of the carcass. Long (10 to 15 cm), thin (0.5- to 1-cm-wide), comma shaped bruises densely concentrated on the back of pigs caused by the fore claws were classified as mounting type bruises.

Figure 5.4: Chart showing the difference between biting (on the left) and mounting type (on the right) bruises (ITP, 1992)



Muscle pH was measured at 1 h *post-mortem* (pH1) in the *Longissimus dorsi* (LD) and in the *Semimembranosus* (SM) muscles, and at 24 h *post-mortem* (pH24) in the LD, SM and *Adductor* (AD) muscles by means of a portable pH meter (Oakton Instruments Model pH 100 Series, Vernon Hills, IL) fitted with a spear tip electrode and an automatic temperature compensation probe (Cole Palmer, Vernon Hills, IL). At 24 h after slaughter, colour data were collected on the LD and SM after a 30 min bloom period. Visual colour of the LD muscle was evaluated using the Japanese Colour Standards (JCS - Nakai et al., 1975, Figure 5.5); marbling (NPPC, 1999) was also assessed in the LD muscle by the same trained operator. Instrumental colour (L*, a*, and b* values) was measured in the same muscle with a Minolta Chromameter (CR-300; Minolta Canada Inc., Mississauga, Canada) equipped with a 25-mm aperture, 0º viewing angle and D65 illuminant. Two-toning and blood splashes were visually assessed and their presence/absence was recorded both in LD and in SM muscle.

Drip loss was measured in the LD muscle using the modified EZ-driploss method of Correa et al. (2007). Briefly, three 25-mm-diameter cores were removed from the center of 2.5-cm-thick LD cross-section (removed at $3^{rd}/4^{th}$ last rib level), weighed, and placed into plastic drip loss containers (Christensen Aps Industrivaengetand, Hilleroed, Denmark), before being stored for 48 h at 4°C. At the end of the 48-h storage period, LD muscle cores were removed from their containers, surface moisture was carefully dabbed, cores were re-weighed, and drip loss percentage was calculated by dividing the difference between initial and final core weights by the initial core weight (Figure 5.6).

Figure 5.5: Subjective evaluation of loin colour by means of the Japanese colour scale.

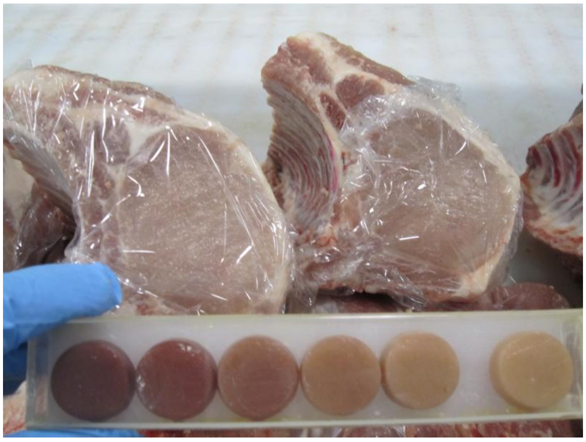


Figure 5.6: The EZ Drip Loss method: on the left, collection of samples from a loin steak; on the right, the plastic container

5.3.4 Statistical analysis

Continuous data were analyzed using the MIXED procedure (SAS, 2002). Environmental temperature (average value between loading and unloading) was used as a covariate in an analysis of covariance (ANCOVA). Week was used as a random block effect and sprinkling as a fixed effect. Since temperature was measured on the statistical block (week) instead of on the experimental unit (pig), a special approach was used (Milliken & Johnson, 2002). First, an exploratory model was tested to determine if the relation between the dependent variable and the temperature was similar for both treatments. If homogeneity of slopes was determined, the model was simplified to a one-way ANCOVA. Furthermore, if no linear relationship was found between the covariate and the dependent variable, a model without covariate was used and sprinkling was the only fixed effect tested (equivalent to a Student-T test). Binomial and ordinal data were analyzed according to a similar approach using the GLIMMIX procedure.

In the analysis per compartment, temperature was again used as a covariate in an ANCOVA analysis, with compartment, sprinkling and their interactions used as fixed effects. When presenting the results of the analysis per compartment, four values of the covariate (temperatures of 15, 18, 22 and 25°C) were chosen in order to represent high, medium and low temperatures and the adjusted means of the dependent variables for each of the selected temperatures were shown by compartment.

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

5.4. Results

The distribution of journeys (or replicates) within each ambient temperature interval was: 3 journeys below 15°C, 4 between 18 and 22°C, 4 between 22 and 25°C and 1 above 25°C.

5.4.1 Ambient temperatures – Losses during transport

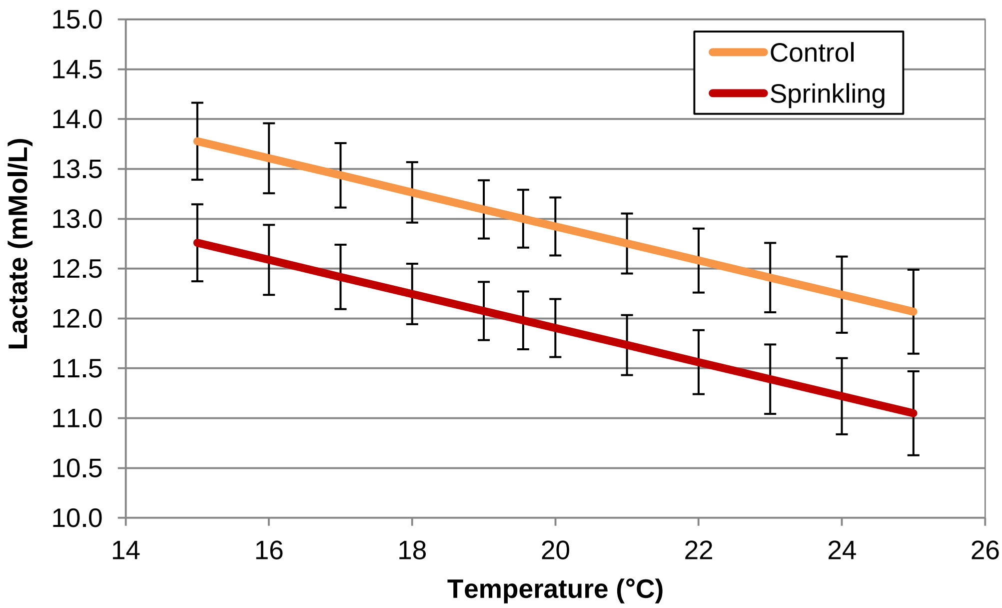
Temperatures recorded during the experimental trial ranged from a minimum of 12,8°C to a maximum of 29,0°C. The average between loading and unloading temperatures for each journey ranged between a minimum of 14,1°C and a maximum of 25,8 (average 19,5°C). During the whole trial, 1 pig was found dead on arrival (Compartment 6, CONT) and 2 were euthanized on the truck, the first one because of a broken leg (C7, CONT), and the second because it was non ambulatory-non injured (C4, WS).

5.4.2 Blood measurements

Average lactate (±SD) value at the farm was 2.7±1.15 mMol/L. There was neither statistical difference in lactate levels at rest (basal levels) at the farm between experimental groups nor in the gradient between sampling events. Furthermore, no effect of environmental temperature was detected on the basal lactate levels either.

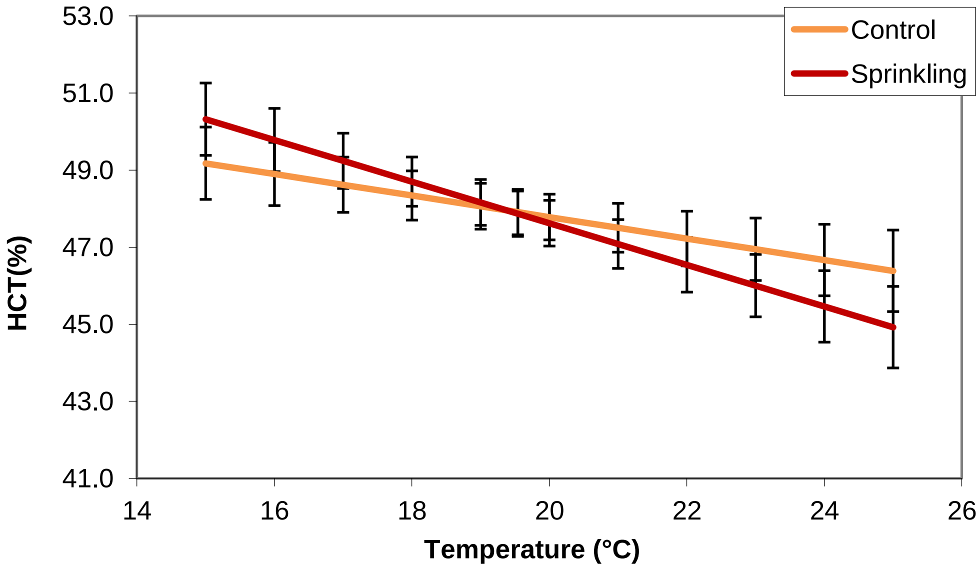
No interaction between sprinkling treatment and ambient temperature for blood parameters was found in this study. The application of WS in the stationary PB trailer resulted in lower blood lactate values at exsanguination (on average: 13.00 vs. 11.98 ± 0.29 mMol/L, *P* = 0.02). Furthermore, pigs transported during warmer ambient temperatures showed lower (*P* = 0.006) exsanguination lactate concentrations than those transported at lower ambient temperatures, as shown in Figure 5.7.

Figure 5.7: Homogeneous slopes model (LSMEANS ± SEM) for the effect of ambient temperature and water sprinkling on exsanguination lactate level (mMol/L) (n=192 per treatment). P-values: sprinkling 0.02; temperature 0.006.



In this study, HCT values at slaughter were only influenced by ambient temperature variation, with HCT values being lower (*P* = 0.02) in pigs transported at higher ambient temperatures (Figure 5.8). WS did not have any effect on HCT values at exsanguination, but a trend for an interaction between environmental temperature and sprinkling was detected (*P* = 0.10): at temperatures below 19.5°C, the WS group showed higher HCT values than the CONT group, whereas at temperatures above 19.5°C HCT values were lower in the WS than in the CONT group.

Figure 5.8: Heterogeneous slopes model (LSMEANS ± SEM) for the effect of ambient temperature and water sprinkling on HCT (%) at exsanguination. P-values: sprinkling 0.11; temperature 0.02; sprinkling*temperature 0.1.



5.4.3 Carcass and meat quality measurements

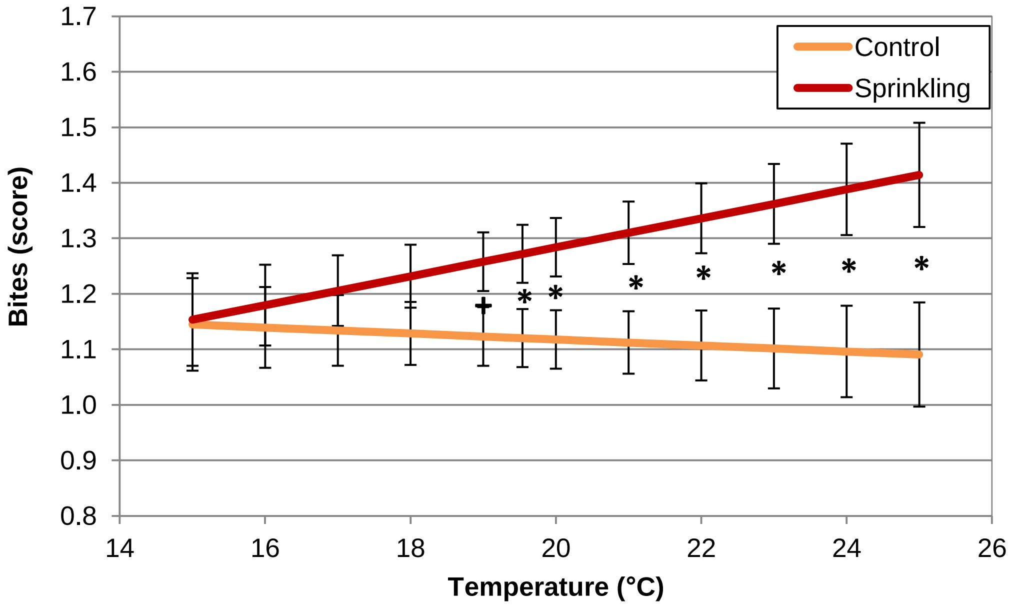
Except for fighting-type bruise score, the interaction WS x ambient temperature did not affect any carcass or meat quality traits in this study. Carcass weight tended to be lighter (*P* = 0.06) and carcass yield percentage was lower (*P* = 0.04) in WS compared to CONT pigs (Table 5.1). As concerns carcass damage, the overall skin damage score and the mounting-type bruises were not significantly different between treatments. However, an effect both of treatment (*P*=0.04 at 19.54°C and *P*=0.02 at 25°C) and of the interaction between sprinkling and temperature (p=0.10) was observed on bite marks, with WS pigs showing significantly higher scores than the CONT group, starting at 19°C (Figure 5.9).

Table 5.1. Effect of water sprinkling on carcass quality traits

	Treatment[1]			
	CONT	WS	SEM	*P*-value
n	288	288		
Carcass weight (kg)	94.4	93.6	0.74	0.06
Carcass dressing (%)	80.1	79.5	0.23	0.04
Lean yield (%)	61.7	62.1	0.21	0.26
Skin damage score[2]	1.19	1.22	0.0028	0.67
Mounting-type bruise score[3]	1.02	1.01	0.005	0.29

[1]CONT: Control, WS: water sprinkling
[2] 1 = none to 5 = severe (MLC, 1985)
[3] 1 = less than 5 bruises to 3 = greater than 11 bruises (ITP, 1996)

Figure 5.9: Heterogeneous slopes model (LSMEANS ± SEM) for the effect of ambient temperature and water sprinkling on fighting-type bruise score (*$P < 0.05$; $^+P \leq 0.10$). Carcass scores ranged from 1 = less than 10 bite marks to 3 = greater than 21 bite marks (ITP, 1996).



Meat quality data are shown in Table 5.2. Except for pH1, which was higher in the LD and SM muscles of WS pigs (*P* = 0.009 and *P* = 0.02, respectively), WS had no effect on any meat quality trait in this study. However, ambient temperature influenced mean drip loss in the LD muscle, with loins from animals transported during the warmest days having higher (*P* = 0.004) drip loss values than those transported at lower temperatures (4.5 ±0.19 % at 15°C; 4.9 ±0.15 % at 19.5°C; 5.4±0.21 % at 25°C; data not presented). No significant differences were observed as far as concerns subjective meat colour (JCS evaluation), bloods splashes, two-tones or marbling (data not shown).

Table 5.2. Effect of water sprinkling on meat quality traits as measured in the *Longissimus dorsi* (LD), *Semimembranosus* (SM) and *Adductor* (AD) muscles

	Treatment[1]			
	CONT	**WS**	**SEM**	***P*-value**
n	288	288		
LD muscle				
pH1	6.14	6.23	0.025	0.009
pH24	5.58	5.57	0.020	0.16
L*	53.32	53.61	0.418	0.46
a*	8.09	8.11	0.208	0.81
b*	5.13	5.30	0.161	0.18
Drip loss (%)	4.98	4.77	0.146	0.31
SM muscle				
pH1	5.95	6.00	0.029	0.02
pH24	5.64	5.62	0.018	0.28
L*	52.81	52.67	0.346	0.72
a*	7.91	7.79	0.193	0.42
b*	4.42	4.42	0.168	0.99
AD muscle				
pH24	5.75	5.73	0.018	0.30

[1]CONT: Control; WS: water sprinkling

5.4.3 Effect of the truck compartment on blood lactate and meat quality traits

Figures from 5.10 to 5.13 show the effects per compartment of the sprinkling treatment on exsanguination lactate, pH1h LD, pH1h SM and Drip Loss at four selected temperatures.

A significant WS x ambient temperature x truck compartment interaction was found for some compartments, with WS pigs located in C5 showing lower blood lactate values at exsanguination at 15 and 18°C than CONT pigs (P = 0.03 and P = 0.009, respectively; Figure 5.10). In this compartment, blood lactate values also tended to be lower (P = 0.08) in WS pigs than in CONT pigs at 22°C. Water sprinkled pigs transported in C8 showed lower blood lactate levels than the CONT group at 22 and 25°C than CONT pigs (P = 0.03 and P= 0.04, respectively).

As is shown in Figure 5.11, the LD muscle of WS pigs transported in C5 and C8 showed a reduced *post-mortem* muscle acidification rate as pH1 tended to be higher at 15°C (P = 0.10) and was higher at 18°C (P = 0.002), 22°C (P = 0.0005), and 25°C

(P=0.005) in WS pigs when compared with the CONT group. The value of pH1 also tended to be higher (P = 0.09) in the LD muscle of WS pigs than in the CONT group in pigs transported in C8 at 22°C.

Figure 5.10: Least squares means (± SEM) of the effect of sprinkling on exsanguination lactate level (mMol/L) by compartment, at four selected temperatures. ^{a,b} $P < 0.05$; ^{A,B} $P < 0.10$

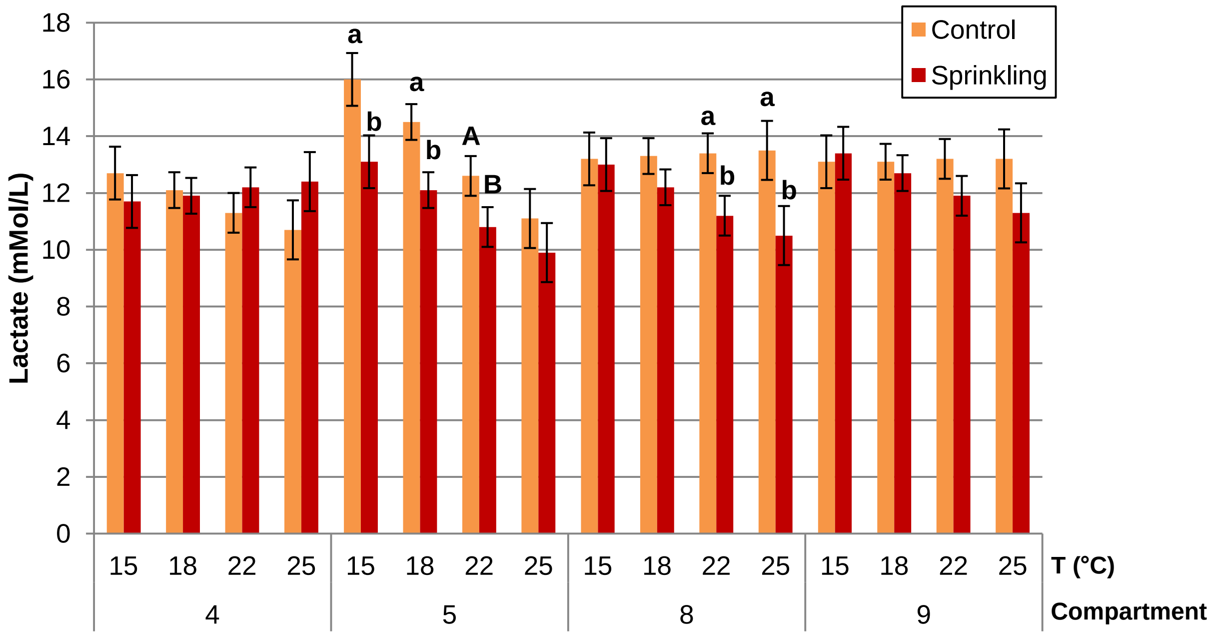
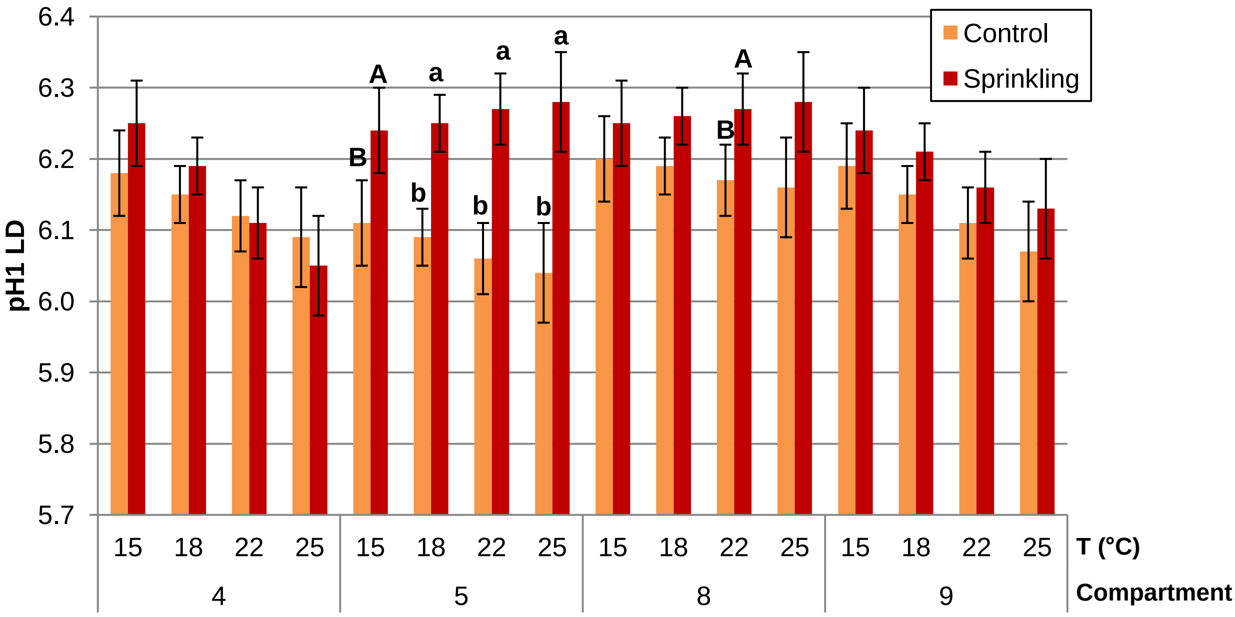


Figure 5.11: Least squares means (± SEM) of the effect of sprinkling on pH1 variation in the LD muscle by compartment , at four selected temperatures. ^{a,b} $P < 0.05$; ^{A,B} $P < 0.10$



A similar pattern of variation was found in the SM muscle, with higher pH1 in the WS group than in the CONT group in C5 at 18 (P = 0.01) and 22°C (P = 0.02) and a trend for higher (P = 0.10) pH1 at 25°C (Fig. 6). Water sprinkled pigs from C9 also tended to have higher pH1 in this muscle at 22°C (P = 0.06) and 25°C (P = 0.09) than the CONT group (Figure 5.12).

As shown in Figure 5.13, drip loss was significantly lower in the WS groups than in the CONT groups located in C5 at 22°C (P = 0.01) and at 25°C (P = 0.02).

The increased lactate values are related to decreased pH1 in the LD and SM muscles (r = - 0.35 and r = - 0.30, respectively; $P < 0.0001$) and increased drip loss in the LD muscle (r = 0.29; $P < 0.0001$)

Figure 5.12: Least squares means (± SEM) of the effect of sprinkling on pH1 variation in the SM muscle by compartment at four selected temperatures. ^{a,b} $P < 0.05$; ^{A,B} $P < 0.10$

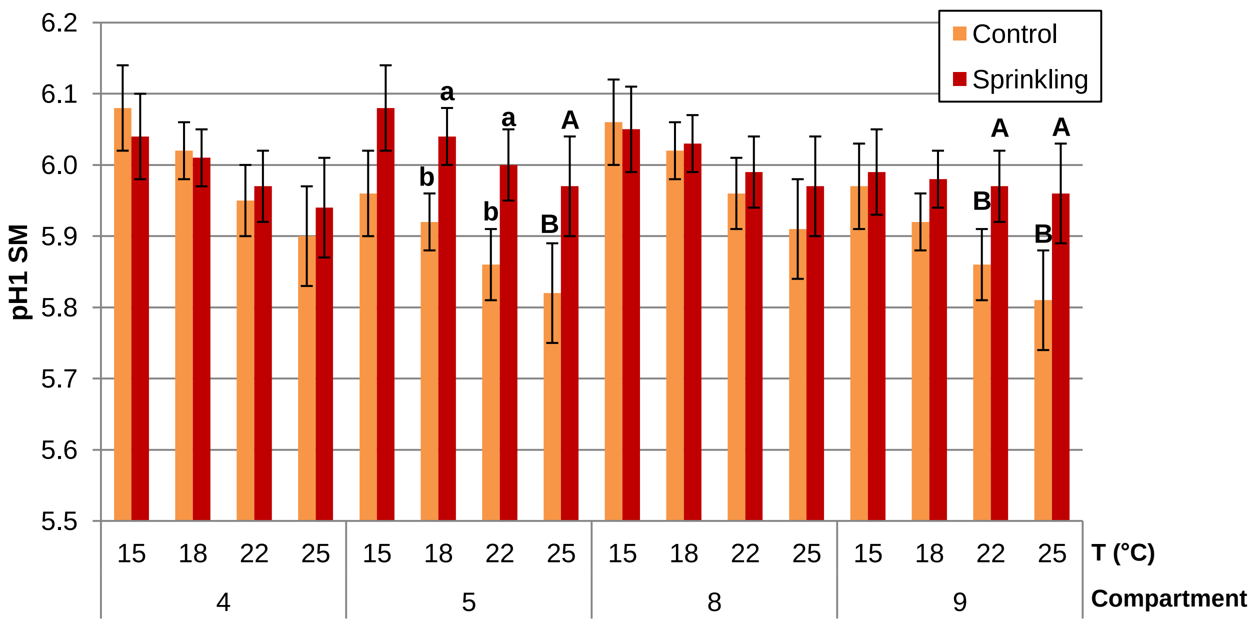
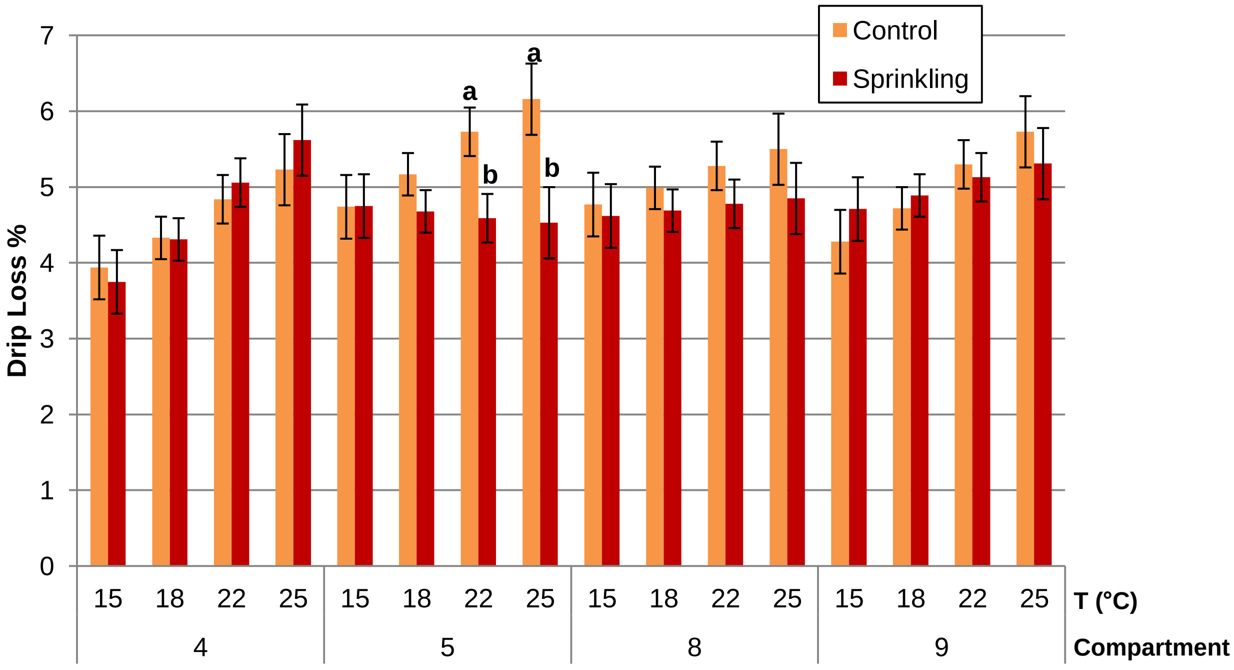


Figure 5.13: Least squares means (± SEM) of the effect of sprinkling on drip loss (%) variation in the LD muscle by compartment at four selected temperatures. [a,b] $P < 0.05$; [A,B] $P < 0.10$



5.5 Discussion

5.5.1 Blood measurements

Baseline lactate levels recorded in this study are lower than those observed by Edwards et al. (2010b). However in this study the absence of a relationship between basal and exsanguination lacatate levels, and between temperature and baseline lactate levels led us to focus on lactate levels at exsanguination.

The reduced blood lactate levels observed at exsanguination in the WS group could indicate the beneficial effect of WS on thermal comfort of pigs during transport. Besides, the reduction in blood lactate concentration at warmer ambient temperatures may be explained by the lower physical activity in the truck and in lairage (shorter latency to rest in the pen) which was observed in these pigs compared to colder temperatures in this study (Fox et al., 2012). These results agree with those reported by Brown et al. (2011a) who recorded lower blood lactate levels and increased lying behaviour in pigs transported during summer than in those transported during winter. The increased resting behaviour under these ambient conditions may be either due to fatigue or to the pig's attempt to minimize muscular heat production by reducing standing and walking behaviour under warm ambient conditions (Brown et al., 2011a; Huynh et al., 2005a). Furthermore, the lying behaviour may also be explained by the pigs' attempt to increase surface contact with the aluminium trailer structure, *i.e.* to remove heat via conduction.

In this study, HCT values were lower in pigs transported at higher ambient temperatures. Although no literature is currently available on pigs, lower HCT values were also reported during summer than during winter in calves and in humans and this variation was explained to be partly due to haemodilution occurring under warm weather conditions (Thirup, 2003; Borgna-Pignatti et al., 2006; Litwińczuk et al., 2009). Water sprinkling did not have any effect on HCT values at exsanguination, although a lower number of drinking bouts per pig were observed in sprinkled compartments during lairage in this study (Fox et al., 2012). This lack of association between drinking behaviour and exsanguination blood HCT may indicate that the lower drinking behaviour observed in WS pigs during lairage may not have been sufficiently large to change their hydration status.

5.5.2 Carcass and meat quality measurements

The higher level of activity in the truck observed in WS pigs in this study (Fox et al., 2012) may explain their larger body weight losses. Previous research already showed higher exploratory behavior and general activity in pigs being water sprinkled in the lairage pen (Weeding et al., 1993). The greater activity of WS pigs in the truck may also help explain the trend for the increased fighting-type bruise score observed starting from 19°C.

Except for the reduced acidification rate early *post-mortem* (higher pH1 in the LD and SM muscles), WS didn't significantly affect any meat quality trait in this study. Ac concerns the effect of ambient temperature on drip loss in the LD muscle, the increase in the production of exudative pork under warmer ambient conditions is frequently reported in the literature (Santos et al., 1997; Gispert et al., 2000; Guàrdia et al., 2004; van de Perre et al., 2010). It appears that the positive effects of water sprinkling on meat quality observed early *post-mortem* were not maintained at 24 h *post-mortem* or later. In particular, as for drip loss, the effects of sprinkling could not overcome the overall effects of high temperatures.

5.5.3 Effect of the truck compartment on blood lactate and meat quality traits

A number of studies (review by Bench et al., 2008) evidenced that the deck and transport compartment environment have an impact on welfare and meat quality, with pigs transported either in the front or in the rear compartments producing poorer meat quality (PSE or DFD), having higher body weight losses, carcass bruises and lactate levels compared with pigs travelling in central pens. According to our findings, compartments 5 and 8, located in the front and in the rear of the middle deck, were those where water sprinkling had the greatest effect, both on lactate values and on meat quality. It appears that in these compartments the applied sprinkling protocol was adequate to reduce the discomfort experienced by the animals during transport and to subsequently improve meat quality.

In previous summer transport trials using a similar PB trailer model (Brown et al., 2011b), C5 was reported as being the warmest location inside the PB trailer due to the poor ventilation flow caused by its design (solid front wall) and position. This compartment is, in fact, located immediately behind the tractor and above the tractor drive wheels and drive train, which radiate heat to the exterior of this compartment (Brown et al., 2011b). Thus, the WS protocol applied in this study appears to have improved the comfort of pigs in this critical trailer location.

The effects of sprinkling on blood lactate levels of pigs from C8 at slaughter is harder to explain as, differently from other studies where pigs presented higher body temperature during transport at this location (Faucitano et al., 2009), in this study this compartment was the coldest and pigs transported in it presented the lowest increase in gastrointestinal tract temperature (as measured by means of orally-administered temperature data-loggers) (Fox, 2013). Furthermore, a greater proportion of pigs from C8 were observed standing (less rest) during lairage (Fox, 2013), which may have contributed to more fatigue at slaughter and consequently higher exsanguination lactate levels (Edwards et al., 2010; Rocha et al., 2012).

The reduced physiological response to heat and transport stress (lower exsanguination blood lactate values) in pigs located in C5 and C8 due to the application of WS in a stationary truck resulted in improved pork quality in this study. Indeed, the LD muscle of WS pigs transported in these compartments showed a reduced *post-mortem* muscle acidification as pH1 tended to be higher both in LD and in SM muscles. The correlation between exsanguination blood lactate levels and early *post-mortem* acidification rate and drip loss found in this study confirms what was already reported in previous studies (Hambrecht et al., 2005; Edwards et al., 2010; Rocha et al., 2012).

5.6 Conclusions

Overall, the results of this study suggest that the application of water sprinkling in a stationary truck (after loading and before unloading) can help reduce the discomfort experienced by the animals and can improve some meat quality parameters resulting from transport during warm ambient conditions (>20^{o}C). These results provide the evidence that thermal environment and its effects on signs of heat stress and meat quality vary considerably in pigs in different compartments within the same trailer. However, as in this study only 3 repetitions were possible with ambient temperatures of ≥25^{o}C upon arrival at the plant, further validation of water sprinkling in the truck would be needed under hotter ambient conditions where temperature control becomes more critical and physiological heat dissipation in pigs becomes less effective. In these conditions, pigs should be given the possibility to wet themselves during transport, in order to lose heat more efficiently by means of skin evaporation.

5.7 Acknowledgements

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CHAPTER 6:

GENERAL CONCLUSIONS

The work presented in this dissertation leads to several considerations. On the whole, it can be concluded that many opportunities exist to effectively improve the welfare of growing-finishing pigs in different scenarios. The results from the first experimental trial (presented in Chapter 3) showed that prolonging the light phase and increasing light intensity can improve animal welfare without negatively affecting meat and ham quality. The second trial (Chapter 4) studied the effects of water restriction in liquid-fed pigs and showed how this practice didn't affect meat or ham quality. However, even though no modification in animal behaviour and blood parameters was observed, such a practice doesn't appear to be respectful of the animals' needs. Lastly, the third trial (Chapter 5) proved that an appropriate protocol of water sprinkling in the stationary truck can reduce the heat stress experienced by pigs transported during the hot season.

In the three scenarios considered, only cost-effective interventions were proposed to improve animal welfare. The cost of these interventions may have considerable variation in the required investment.

It's worth noting how, at farm level, an increase in light intensity could be achieved at a minimum cost, simply by regularly replacing the broken lamps and cleaning the illumination system. Duration of the photo-phase can be incremented at an increased energetic cost, but its positive effects include, according to previous findings, reduction in aggressive behaviour and improvement of productive parameters. As concerns water restriction, a reduction of water waste is achievable by alternative means: proper installation and maintenance of the drinkers, together with the provision of environmental enrichment material. Lastly, even though at an increased initial cost due to the installation of a water sprinkling system, heat stress during transport could be reduced, particularly in critical truck compartments, simply by applying short sprinkling sessions in the stationary truck.

As concerns the quality of the animal-derived products, meat quality was positively influenced by water sprinkling (through a reduction of the early *post-mortem* acidification rate), but the modification was not maintained at 24h *post-mortem*. Neither fresh meat nor dry-cured ham quality of Italian heavy pigs were affected by the different illumination regimes or by the availability/absence of fresh drinking water in the pens.

As a further element of originality, this dissertation focuses on the relationships between animal welfare and the quality of PDO products. In the context of PDO production methods, research often neglects the aspects related to the final product quality and focuses instead on the raw matter characteristics. However, considering the complex relationships between raw materials and processing techniques, targeting the desired end product quality is an aspect that should be adequately stressed, since both producers and consumers should be guaranteed that no alteration in the final product is associated with welfare-improved rearing systems.

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Note: All the websites in the reference list were last accessed in March 2013.