Welfare issues in Italian heavy pig production

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Abstract

This thesis explores research published between 2013-2017 and focuses on welfare issues in Italian pig production. This sector is characterized by animals (heavy pigs) slaughtered for dry-cured products at a minimum of nine months and 160 kg (compared to 110-115 kg of other EU systems). The first set of studies investigated the feasibility of avoiding tail docking under different husbandry conditions (Trial A and Trial B), besides the benefit of providing straw enrichment by racks (Trial A). Surgical tail docking within the first week of life is the routine solution in several EU countries to prevent tail biting, an abnormal behaviour causing stress, pain and tail injuries. In Trial A, tail presence did not significantly increase the risk of tail lesions, the level of acute phase proteins (*i.e.* blood markers of inflammation), or impair the health status (mortality, lung and gastric lesions) over the 30 weeks of fattening. Small amounts of straw (70 g/day per pig) increased the motivation for pigs to explore the environment, reduced serum haptoglobin (*i.e.* the inflammatory state) and reduced the risk of tail biting (at weeks 3, 9, 18) and ear biting (at weeks 3, 9). At slaughter, the straw group revealed a gastric ulcer risk ~ 70% lower than the one without straw (OR: 0.27). Males were at higher ulcer risk than females (OR: 1.52). In Trial B, tail presence had no effect on blood markers, conflicts, or ear and tail biting behaviours, both at the weaner and fattening phase. At fattening, however, undocked animals showed a higher prevalence of mild tail lesions (P<0.01) and a lower frequency of belly nosing behaviour (P<0.05). Blood samples taken from the animals in trial A and trial B were found to have a variable degree of spurious haemolysis. The release of free haemoglobin can bias the quantification of several analytes. Therefore, a further study evaluated the effect of physical haemolysis in 3 aliquots (on a scale of 1+ to 3+) of 30 non-haemolytic sera, in order to assess the threshold of acceptability for a panel of 27 blood analytes at increasing levels of haemolysis. A further issue explored was the assessment of heavy pig welfare at the time of slaughtering, in a European project which enabled the gathering of data with other Countries (Spain, Portugal, Finland, Brazil). Welfare Quality Protocol® was applied in nine Italian abattoirs, providing information for reference values of several animal-based indicators. Finally, the impact of sexual maturity in heavy female pigs was investigated, on the basis of previous studies on light pigs. They reported mounting and agonistic behaviour to affect animal welfare in both males and females, due to sexual activity. In the study, a reduction was found (P<0.05) in immunocastrated *vs*. entire females for aggressive interactions, haptoglobin levels, serum cortisol levels and back lesions at given timepoints throughout fattening. There was no effect

on slaughter performances (back fat thickness and percentage of lean tissue). Taken together, the outcomes of all these studies highlighted the presence of specific welfare concerns in the Italian pig sector, due to the heavier final live weight (problematic during certain slaughtering phases) and the achievement of sexual maturity (leading to increased aggression of females). Similarly to other EU systems, tail biting was manageable with proper enrichment material; it occurred mostly at an early age and under poor health conditions. The studies have also brought practical implications in routine blood testing (by overcoming the unsuitability of blood measures due to haemolysis) and in investigating the role of gastric ulcers as an innovative stress marker.

Dedication

At present, this work represents the ultimate effort during my career of researcher. Research however is mainly a teamwork, so I would like to dedicate the thesis to all my peers whom I worked with: veterinarians, co-author scientists and reviewers.

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Outputs and Dissemination

Scientific congresses

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Di Martino, G, Capello, K, Scollo, A, Gottardo, F, Stefani, AL, Rampin, F, Schiavon, E, Marangon, S, & Bonfanti, L 2011a, 'Effect of tail presence on the welfare status of Italian heavy pigs', in proceedings of 3rd European Symposium of Porcine Health Management, 25-27 May 2011, Helsinki, Finland, pp. 195-196.

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Garbo, A, **Di Martino, G**, Capello, K, Scollo, A, Marchesan, M, Caucci, C, & Bonfanti, L 2017, 'Gilts *vs.* barrows: the effect of gender on skin wounds in finishers reared over 160 kg', in proceedings of 9th European Symposium of Porcine Health Management, 3-5 May 2017, Prague, Czech Republic, p. 164.

Scollo, A, **Di Martino, G**, Bonfanti, L, & Gottardo, F 2011a, 'Age and gender effect on the use of different types of environmental enrichments in Italian fattening heavy pigs', in proceedings of XXXVII SIPAS Annual Meeting, 24-25 March 2011, Piacenza, Italy,

Scollo, A, **Di Martino, G**, Bonfanti, L, Prevedello, P, & Gottardo, F 2011b, 'Tail and ear biting in undocked Italian heavy pigs during fattening period', in proceedings of 3rd European Symposium of Porcine Health Management, 25-27 May 2011, Helsinki, Finland, p. 197.

Scollo, A, **Di Martino, G**, Gottardo, F, Contiero, B, & Bonfanti, L 2014, 'Tail biting in pigs: is it possible to rear the heavy pig avoiding tail docking?', in proceedings of XL SIPAS Annual Meeting, 27-28 March 2014, Montichiari, Italy, pp. 303-313.

International scientific publications

1. Dalmau, A, Nande, A, Vieira-Pinto, M, Zamprogna, S, **Di Martino, G**, Ribas, JCR, Paranhos da Costa, M, Halinen-Elemo, K, & Velarde, A 2016, 'Application of the Welfare Quality® protocol in pig slaughterhouses of five countries', *Livestock Science*, vol. 193, pp. 78-87.

2. **Di Martino, G**, Capello, K, Scollo, A, Gottardo, F, Stefani, AL, Rampin, F, Schiavon, E, Marangon, S, & Bonfanti, L 2013, 'Continuous straw provision reduces prevalence of oesophago-gastric ulcer in pigs slaughtered at 170 kg (heavy pigs)', *Research in Veterinary Science*, vol. 95, pp.1271-1273.
3. **Di Martino, G**, Scollo, A, Gottardo, F, Stefani, AL, Schiavon, E, Capello, K, Marangon, S, & Bonfanti, L 2015a, 'The effect of tail docking on the welfare of pigs housed under challenging conditions', *Livestock Science*, vol. 173, pp. 78-86.
4. **Di Martino G**, Stefani AL, Lippi G, Gagliazzo L, McCormick W, Gabai, G, & Bonfanti, L 2015b, 'The degree of acceptability of swine blood values at increasing levels of hemolysis evaluated through visual inspection versus automated

quantification', *Journal of Veterinary Diagnostic Investigation*, vol. 27, pp. 306-312.

5. **Di Martino, G**, Scollo, A, Garbo, A, Lega, F, Stefani, AL, Vascellari, M, Natale, A, Zuliani, F, Zanardello, C, Tonon, F, & Bonfanti, L 2017a, 'Impact of sexual maturity on the welfare of immunocastrated v. entire heavy female pigs', *Animal*, vol. 12, pp. 1631-1637.
6. Scollo, A, **Di Martino, G**, Bonfanti, L, Stefani, AL, Schiavon, E, Marangon, S, & Gottardo, F 2013, 'Tail docking and the rearing of heavy pigs: the role played by gender and the presence of straw in the control of tail biting. Blood parameters, behaviour and skin lesions', *Research in Veterinary Science*, vol. 95, pp. 825-830.

National scientific publications (in Italian language with abstract in English)

Di Martino, G, Zamprogna, S, Garbo, A, Caucci, C, Marchesan, M, Bonfanti, L, & Dalmau, A 2017b, 'Welfare assessment in Italian pig slaughterhouses', *Large Animal Review*, vol. 23, pp. 149-153.

Statement of Authorship

Publication number:

1. Execution of the experimental trial, data collection, interpretation and publication writing.
2. Execution of the experimental trial, data collection, interpretation and publication writing.
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4. Experiment idea, collection of blood samples, data analysis, interpretation and publication.
5. Data collection at the abattoir, contribution to publication writing.
6. Execution of the experimental trial, data collection, interpretation and publication writing.

I carried out the research, but now I want to tell you the story…

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1. Background

1.1. EU legislation for the protection of pigs

The ‘big bang’ of welfare for farmed animals exploded in the United Kingdom around fifty years ago, both in terms of social awareness (Harrison, 1964) and, subsequently, scientific investigation (Brambell, 1965). A limit for animal suffering was incorporated into five basic freedoms or essential needs, with regards to feeding, comfort, distress, health and behaviour. Afterwards, the European Union enabled these general milestones to be addressed more specifically for the different animal species or production categories (*e.g.* laying hens *vs*. broiler chickens) using a set of crosscutting (*e.g.* EU Reg. 1/2005 on animal transportation) and species-specific laws. However, not all species are explicitly covered yet (*e.g.* meat rabbits, dairy cows, meat turkeys).

The welfare of farm pigs is assured by Council Directive 2008/120/EC (European Council, 2008). This piece of law applies to all categories of pig and provides minimum standards for their protection. Each member state is required to implement the directive through their national laws (*e.g.* in Italy through Legislative Decree 122/2011). Several specific requirements are listed in terms of quality of the flooring surfaces (*e.g.* maximum width of the openings), minimum living space available, minimum lighting, maximum noise, etc. This high level of detail represents one of the best examples within the EU, although some criticisms remain.

The first criticism of Directive 2008/120/EC is with regards to the table (Article 3) stating the minimum floor area available to each pig which indicates ‘*one square metre*’ ‘*for pigs heavier than 110 kg live weight*’ (European Commission, 2008). This statement has probably been written on the basis of the conventional ‘light pig’ production (*i.e.* animals are slaughtered at 115-120 kg), but does not consider specialised production systems such as Italian ‘heavy pigs’, where fatteners are slaughtered at around 170 kg live weight for PDO (Protected Designation of Origin) dry cured products (*e.g.* Parma and San Daniele ham).

The second criticism of Directive 2008/120/EC refers to the statement that ‘*pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities, such as straw, hay, wood, sawdust, mushroom compost, peat or a mixture of such, which does not compromise the health of the animals*’ (European Council, 2008). As it emerged during the recent EU audit, this requirement is mostly ignored in Italy (European Commission, 2017), as essentially it refers to the pig's need for a material that is 'edible, odorous, chewable and destructible' (EFSA, 2007). Currently the law is being addressed with a wooden stump, which in most cases is hardly chewable and provides limited interest for animals (European Commission, 2017).

Finally, the EU legislation requires that tail-docking must not be carried out as a routine intervention for preventing tail biting later in life (European Commission, 2008). Before carrying out tail docking, other measures have to be taken first, including improved housing conditions and reducing stocking density. Despite this provision, currently only a few northern EU countries with a minor contribution to pig production (*i.e.* Finland, Sweden, Norway) have banned this practice (D'Eath *et al*., 2016). The widespread and routine use of this mutilation in the EU seems to be driven mainly by the absence of clear actions outlined by the law (in terms of improving the environment and reducing stocking density) that producers are required to take before resorting to it (D'Eath *et al*., 2016). Significant changes are needed in pig management and housing, in order to make the ban of tail docking a benefit for pig welfare (D'Eath *et al*., 2016).

1.2. The importance of rooting material

Pigs have evolved to search various feeding resources hidden under a complex ground substrate (D'Eath and Turner, 2009). The motivation to explore, or properly to root is still present in domesticated crossbreeds and explains the frustration pigs may feel when housed in a barren environment on slatted floors at a high stocking density (D'Eath and Turner, 2009). Lack of suitable rooting/enrichment materials is currently the main issue in intensive pig production due to costs and the opinion that edible materials such as straw would block the manure outflow (EFSA, 2007).

According to several scientific publications (EFSA, 2007) straw represents the best material for investigation and oral manipulation, with a significant impact seen in the reduction of aggressive behaviour (Fraser *et al.*, 1991; Beattie *et al*., 2000), and frustration behaviour (*i.e.* tail biting) (Zonderland *et al*., 2008), with no negative impact on growth rate (Jordan *et al*., 2008).

1.3. Tail biting

Despite the numerous publications on tail biting risk factors, this abnormal behaviour still remains an enigma. Curiously, it is not thought to be due to aggression as this occurs through frontal fighting and typically happens after mixing of different groups (Zonderland *et al*., 2011). Tail biting is mainly due to frustration, and tends to be associated with other behaviours involving a repeated oral manipulation, such as bar biting and tail suckling (Brunberg *et al.,* 2011). Limited available space, lack of environmental stimuli, poor health and bad air quality are considered significant risk factors (as reviewed by the European Food Safety Authority (EFSA), 2007 and Sonoda *et al*., 2013). EFSA (2007) also comprehensively discussed the role of other possible factors of tail biting occurrence and spread, such as nutritional deficiencies (*e.g.* sodium), genetic heritability (Breuer *et al*., 2005), age (the end of the weaner phase is the most risky) and sex (castrated males seem at lower risk compared to entire females) (Zonderland *et al*., 2010).

1.4. Sexual maturity

Sexual behaviour in intensive farming systems can represent a welfare concern, due to increased aggression, excitement and mounting behaviour (Rydhmer *et al*., 2006). Mounting, in particular, may increase the risk of injuries and skin lesions (Rydhmer *et al*., 2006, 2010). The consequences of sexual behaviours, in terms of fear and harm, are not limited to the receiver, but to all pigs in the pen (Rydhmer *et al*., 2006, 2010). In Italian production, males are castrated while females are not. Sexual maturity occurs at around 5-6 months of age (Hemsworth, 1985), and heavy pigs spend around 3-4 months on the farm after the achievement of sexual maturity. Although no scientific reference are available for heavy pigs, it can be hypothesized that that aggression and excitement become more likely, due to females on heat which can cause mounting of pen-mates.

1.5. Pig welfare at slaughter

Loading and transportation to the slaughterhouse is one of the most challenging moments during a pig's life (Grandin, 2007). Several factors can affect animal welfare: mixing of groups, movement restriction, injuries associated with handling and loading operations, thermal discomfort, feed restriction, sudden acceleration and deceleration of the transport vehicle and noise (EFSA 2004, 2011). At the EU Level, Regulation 1/2005 and Regulation 1009/2009 have been outlined for the protection of farmed animals during transportation and at the time of killing, respectively. The high attention to these critical phases can be revealed by the choice of enacting two regulations, which are characterized by an immediate application in all member states with no possibility of change.

The legal approach limits welfare assessment to 'compliant or not compliant' criteria. Rather, more flexible and sensitive tools are needed to assess animal welfare more thoroughly (*e.g.* providing a detailed score system). To this end, Welfare Quality (WQ) was an EU project funded within the sixth framework programme to integrate the welfare of farmed animals into the food chain in an easy manner (Welfare Quality, 2009). Under the umbrella of the Five Freedoms, the project highlighted twelve distinct but complementary welfare criteria (Botreau *et al*., 2007). For each one of these criteria different measures were developed for their application on farms and at the abattoir (Dalmau *et al*., 2009).

In contrast to the WQ protocol on farms for fattening pigs, the aggregation scoring was not developed for the evaluation protocol at the abattoir, due to the lack of information on the assessment of pig welfare at the time of slaughter. In fact, a major issue when developing an aggregation score is to define what is acceptable within a variety of different management conditions, *e.g.* by means of 'expert opinion' methodology (Botreau *et al*., 2013). For these reasons, research is currently needed to assess the variability of the measures used in the WQ protocol for pigs among slaughterhouses from different countries and under variable management/environmental conditions, in order to propose thresholds for the calculation of scores.

2. Contextual significance of the publications presented in this thesis

My research on 'Welfare issues in Italian heavy pig production' is integrated by addressing a number of specific areas, which are interrelated, as shown in Figure 1. Moreover, a follow-up section has been included (Chapter 2.1.2) in order to discuss the impact of my work on the following scientific literature and EU policy (*i.e.* EU Audit on tail docking carried out in Italy in 2017).

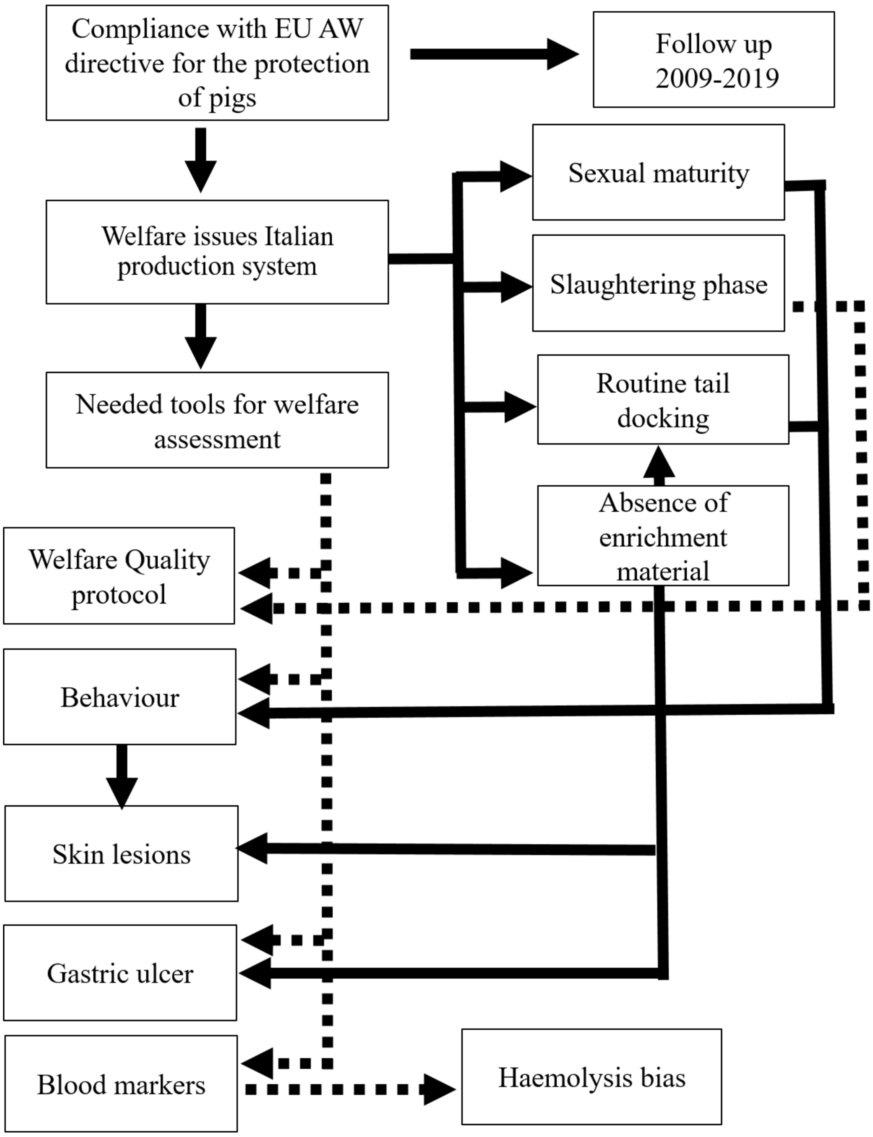

Figure 1. Interrelated areas discussed in the present thesis. Continuous arrows indicate a logical cause/effect connection; dotted arrows indicate a methodological connection.

2.1. Rearing undocked heavy pigs: is it really feasible?

2.1.1. A story of a balance: the strong connection between tail and straw

In 2009, I joined a scientific project on the feasibility of avoiding routine tail docking in Italian heavy pigs. At that time, numerous studies had already been carried out on tail biting, and a comprehensive EFSA report had been published two years before on 'The risks associated with tail biting in pigs and a possible means to reduce the need for tail docking by considering the different housing and husbandry systems' (EFSA, 2007). The novelty of the study proposal in Italy was the evaluation of the specific Italian heavy pig conditions, with a finishing cycle up to 9 months and 170 kg of live weight. The scientific question asked whether there was any possible specific risk for older and heavier pigs, as severe lesions seemed more likely to occur with pen-mates due to the higher muscular strength of the animals. Moreover, a possible sex effect was hypothesised, as observed in light pigs (Zonderland *et al*., 2008).

An experimental trial in the field was arranged in agreement with a major pig industry (Trial A), where 672 crossbred pigs (336 males and 336 females) were selected from the same piggery with weaner site (Figure 2). According to routine husbandry procedures, 168 males and 168 females had been tail-docked within their first week. Moreover, males had been surgically castrated (they are called 'barrows'). Surgical castration of males is considered necessary for the PDO production (Protected Designation of Origin). All technical details of Trial A are described in Di Martino *et al*. (2013) and Scollo *et al*. (2013). The object of discussion here is the evaluation of the scientific impact, explanation of the choices behind the rationale for the study, and finally some comments on the limitations of the study.

The first question that arose when developing Trial A focused on the husbandry conditions that needed to be applied (*e.g.* dry *vs.* liquid feed, slatted *vs.* solid floor, winter *vs.* summer period). The first option could consider the set of conditions more representative of the overall system (*e.g.* if slatted flooring is present in 80% of Italian pig farms, then a farm with slatted flooring should be chosen). The second option could consider the husbandry conditions actually available on a convenience basis. This is normally the least preferable solution. A third option is also possible, driven by the need to begin a set of trials with the most favourable conditions. This latter approach was chosen when designing the rationale of Trial A. Therefore, a farm with solid flooring was selected, as it is less stressful for animals compared to a slatted floor (Gillman *et al*., 2009). The farm also guaranteed a previous history of low mortality (*i.e*. lower than 2%). Good health status is another protective factor against tail biting. In fact, Moinard *et al*. (2003) found a 3.9 fold increase in the risk of tail biting when post-weaning mortality was above 2.5%. On this basis, the agreement with the pig industry was to repeat the experiment a second time under more challenging conditions (Trial B). All technical details of Trial B are described in Di Martino *et al*. (2015a).

In Italy, pigs are bred in 3 phases, while in other parts of Europe there are only two (Figure 2). Phase 1 refers to the piggery (*i.e.* piglets under the sow, until 21 to 28 days of life); phase 2 refers to the 'weaner phase' and lasts around 8 weeks in specific farms called 'site 2 farms'. Afterwards, pigs are moved to the fattening unit (site 3 farm) for around 30 weeks. In other European countries, due to a shorter fattening cycle, phase 2 and phase 3 can be merged, so no transfer and mixing of animals from weaner to finishing phase is needed. Indeed, moving and mixing animals is a welfare issue, due to transportation stress and stress due to creating new groups: *i.e.* hierarchies need to be determined by fighting, chasing and aggressions (Hessing *et al*., 1993; Erhard *et al*., 1997).

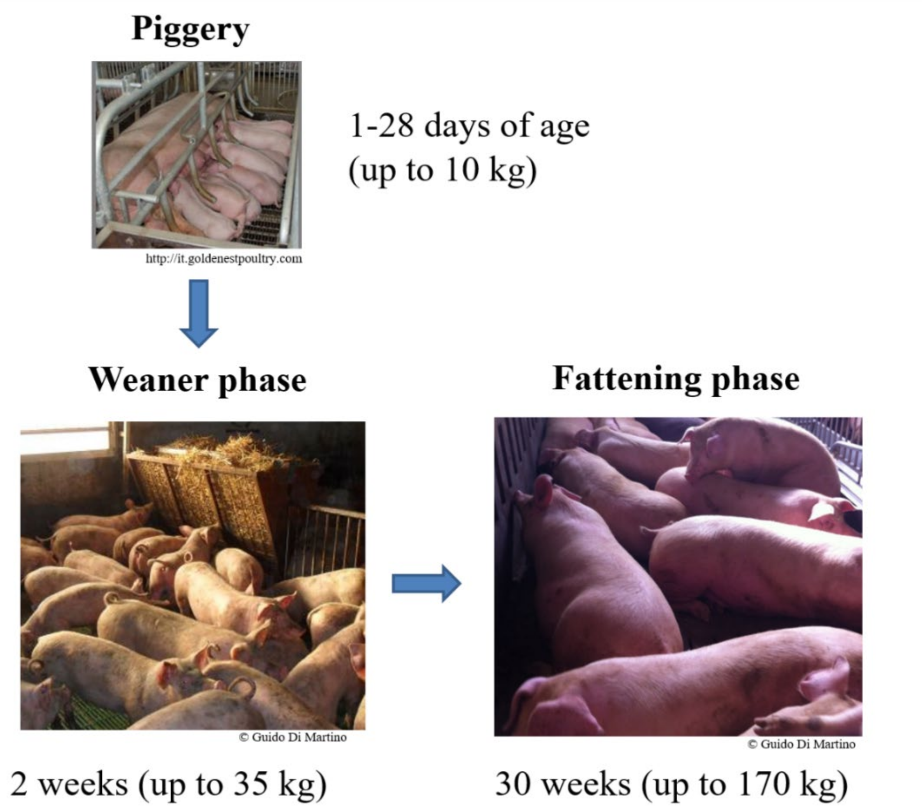

Figure 2. Production phases in Italian heavy pig production.

A major limitation of Trial A was not being able to properly monitor the weaner phase, as the pigs were made fully available at 12 weeks of age at the beginning of the finishing period. Weaner pigs for trial A were reared in a reproduction site with no possibility to make amendments to husbandry conditions and environmental enrichments, in order to be fully compliant with Dir. 2008/120/EC. Animals were housed in 20 pens of 37 heads each, according to sex and tail presence (*i.e.* 5 pens for each experimental group). The farm had slatted flooring and no environmental enrichment; stocking density was set at 0.38 metres squared per pig. It was possible to score skin lesions at week 1, 4 and 6 on 2 pens for each experimental group. In view of the limited possibility of investigation during this phase and the legal noncompliance in terms of environmental enrichment, it was decided not to include this data in a scientific publication. Rather, they were presented and discussed at a national congress (Di Martino *et al*., 2010).

The results of the weaner phase which preceded Trial A (Di Martino *et al*., 2010) highlighted an ear-damage risk two-fold higher in males than in females (OR: 2.56; CI 95%: 1.27-5.21) and a tail-damage risk 18 times higher in males compared to females (OR: 18.05; CI 95%: 4.19-78.63). The higher risk in males was inconsistent with the data published by Zonderland *et al*. (2008) which demonstrated a higher tail damage duration (20.2 days) following an outbreak of tail biting in all-female groups, compared to all male and mixed-sex groups, suggesting that females are more likely to tail bite compared to males. In our study, tail presence represented a protective factor against ear lesions (OR: 0.21; CI 95%: 0.09-0.49). Body and ear lesions were more frequent immediately after housing in the weaner unit, whereas tail lesions increased in frequency and severity at the end of the weaner age.

In Trial A, animals from the weaner unit were housed according to sex and tail presence in the same fattening shed (Figure 3), where all pens were provided with a metal chain and a chain with a rubber cover. The choice of two alternative types of chains was made in accordance with Manciocco *et al.* (2011), who found both these objects suitable for stimulating oral investigation. Twelve pens were provided with a rack on the wall where straw was always available. Such a 2 × 2 × 2 experimental design enabled the investigation of the main factors and their possible association (tail × sex × straw provision). On the other hand, there was a high risk of obtaining several interactions between factors, which are sometimes hard to explain. Moreover, in view of such a complex experimental design, it might be questioned as a further limitation of Trial A, whether the sample size was sufficiently large to reliably detect treatment differences.

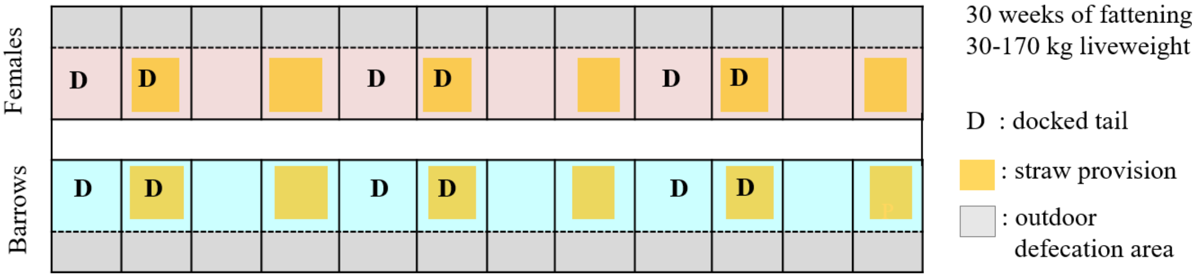

Figure 3. Distribution of the experimental groups of Trial A in 24 single-sex pens of 28 heads in a commercial fattening unit.

The finishing unit has an internal area with solid flooring (where the straw rack was set) and an outdoor defecation area with a slatted floor. The reported mortality was considered low compared to that typically expected in this production system (*i.e.* under 2%). Animals were fed with two liquid meals per day. Welfare was evaluated by skin lesion score, behaviour assessment and health/stress markers in the blood at time points throughout fattening. At the time of slaughter, lungs and stomachs were scored for the presence of lesions.

Tail presence did not increase either the risk of tail lesions, the level of acute phase proteins or the health status (mortality, lung and gastric lesions) over the 30 weeks of fattening. Straw increased the motivation for exploring, and reduced serum haptoglobin (a blood marker of inflammation, according to Kaneko, 2008) and the risk for tail biting behaviour (at weeks 3, 9 and 18 of fattening) and ear biting (at weeks 3 and 9 of fattening). At slaughter, the straw group revealed a reduced oesophago-gastric ulcer risk 70% lower than with no straw (OR: 0.27; CI: 0.17-0.41). The protective effect of straw was more evident in undocked pigs (OR: 0.16, 95% CI: 0.09-0.28), compared to docked ones (OR: 0.41, 95% CI: 0.25-0.69). Males were at higher risk than females (OR: 1.52; CI: 1.08-2.12).

According to the EFSA report (EFSA, 2007), several risk factors can predict a tail biting outbreak. Straw presence is not a complete solution but confers some advantage. This material has complementary properties able to raise pig motivation to explore, eat, smell, chew and modify a substrate. Indeed, the specific characteristics of the material and how it is provided may have a significant impact in determining the suitability: long straw *vs.* short straw, recently cut or not (*i.e.* tough when chewed or easily pulverized), easy or hard to extract from the rack on the basis of the grid size (Pedersen *et al*. 2014). According to our practical experience when fabricating the racks, a grid larger than 10 ×10 cm can produce a fast emptying of the rack and possible obstruction of the manure system, while when smaller than 2 × 2 cm it can dramatically limit pig interest (Di Martino, 2009; unpublished trials).

According to swine ethology (Spinka, 2017), pigs need to 'root' (*i.e.* to physically put the snout into the ground substrate), therefore the optimal straw provision should be as bedding material. This advice is present in the recent EC working document for the reduction of tail biting, where the straw rack is considered a suboptimal material, while straw bedding an optimal one (EU Commission, 2016a). On the other hand, straw bedding may produce a reduction in pig interest due to: 1) Dirtiness of the material, 2) The absence of the 'effort/reward' of reaching the substrate after having it extracted with difficulty from the rack (EFSA, 2007). Data from my research may support the second point, as the time spent looking for straw and attempting to extract the material was much longer than the time for eating some fibres. 18% of time was spent in straw investigation in Trial A and 36% in Trial B, compared with a very limited daily consumption per pig, *i.e.* 70g and 30g, respectively (Scollo *et al*., 2013; Di Martino *et al*., 2015a). At present, these results likely represent the lowest in the literature concerning the daily straw intake for finishing pigs. Recent publications suggest an intake of 200-300 g (Pedersen *et al*. 2014; Lahrmann *et al*., 2018). The reduced straw consumption in trial B compared to Trial A was probably due to the fact that in the former pigs were fed *ad libitum*, while in the latter they were fed restrictively. Similar outcomes were found by Zwicker *et al*. (2013).

Despite the small daily amount consumed, straw resulted in a protective factor against oesophago-gastric ulcers, which is a common pig health disorder (Di Martino *et al*., 2013). Ulcer of the pars oesophagea is a frequent cause of mortality in swine, while ulcer of the fundus is much more uncommon (Doster, 2000). The former has been studied in more depth and several predisposing factors have been found, such as fineness of feed particles, infections (porcine circovirus 2 and porcine reproductive and respiratory syndrome virus) and stress due to husbandry conditions (Wondra *et al*., 1995; Friendship, 2003; Amory *et al*., 2006). In my study, oesophago-gastric ulcer was diagnosed at the time of slaughter in 47% of the pig stomachs. All pigs had been subject to the same conditions (same shed with the same feed and health conditions), thus all of the differences are presumably attributable to the experimental treatment (*i.e.* the provision of the straw rack). Barrows were more prone than females, so a possible protective effect of oestrogens was supposed, in agreement with other studies in humans and mice (Shimozawa *et al*., 2006). No significant differences between docked and undocked pigs were detected. Nevertheless, because of a significant interaction between tail and straw (*P* = 0.007) was identified, the presence of straw acted as a protective factor particularly in undocked pigs, suggesting that in this group the absence of rooting material may have a stronger effect on welfare.

A recent publication (Holinger *et al*., 2018) has identified a surplus benefit of providing a rack with grass silage even in the presence of straw bedded pens. This research supports that rooting and looking for the manipulable material 'hidden elsewhere' may have a cumulative benefit with straw bedding. Moreover, it stresses the importance of increasing the type of enrichment stimuli to promote the sensitive experiences of animals, as suggested by the European Commission (2016a). This is the reason why the provision of metal chains should not be considered as unsuitable, but simply insufficient: pigs may spend a lot of time manipulating them, and this is a positive behavioural outcome (Bracke & Koene, 2019). However, their use needs to be combined with that of other complementary materials.

In order to overcome the limitations of Trial A and to repeat the experiment under more challenging conditions, the pig industry agreed to arrange Trial B. It is worth mentioning, however, that the distinction between Trial A and B in terms of favourable (Trial A) and challenging (Trial B) was based primarily on mortality rates and floor type (fully slatted in Trial B). Conversely, other aspects of the husbandry in Trial B were actually less challenging than those in Trial A (*i.e. ad libitum* diet *vs.* restricted feeding). 448 crossbred pigs (224 females and 224 barrows) aged 3 to 40 weeks were included in the study (Figure 4). In contrast to Trial A, the weaner phase was included, while the fattening site was characterised by the presence of a fully slatted floor, *ad libitum* pelleted diet, and a previous history of high mortality (*i.e.* over 5%). Moreover, in order to simplify the experimental design, the effect of straw was removed (*i.e.* all pens were provided with a straw rack). This decision unfortunately precluded confirmation of the beneficial effect of straw on oesophago-gastric ulcers.

Similarly to Trial A, tail presence had no effect on blood markers, conflicts, or ear and tail biting behaviours in Trial B, both at the weaner and fattening phase. However, an outbreak of tail biting was detected during the last week of the weaner phase in one pen of males. Moreover, at fattening undocked animals showed a higher prevalence of mild tail lesions ($P<0.01$) compared to docked ones, besides a lower frequency ($P<0.05$) of 'belly nosing' behaviour (*i.e.*, when an individual persistently thrusts their nose towards the belly of a pen-mate, nuzzling the teat and flank areas). The exact meaning of this behaviour is still an object of debate. Widowski *et al*. (2008) suggested it as a general indicator of stress, but a clear relationship could not be established. In general, abnormal behaviours tend to be a redirected response to frustration. In the case of undocked pigs, the long tail acts as an object of interest and consequently decreases the motivation to beat the others' flanks. Such an inverse correlation between tail biting and other abnormal behaviours not involving biting was also found by Brunberg *et al.* (2011). Interestingly, in Trial B ear lesions had a higher tendency to be found in docked pigs (*P*=0.076), similarly to what was found in the weaner (unpublished) phase of Trial A. These findings may represent an example of compensation between stimuli, where the presence of an extra one (the undocked tail) can protect from the motivation to investigate (in this case to bite) others (Goossens *et al*., 2008).

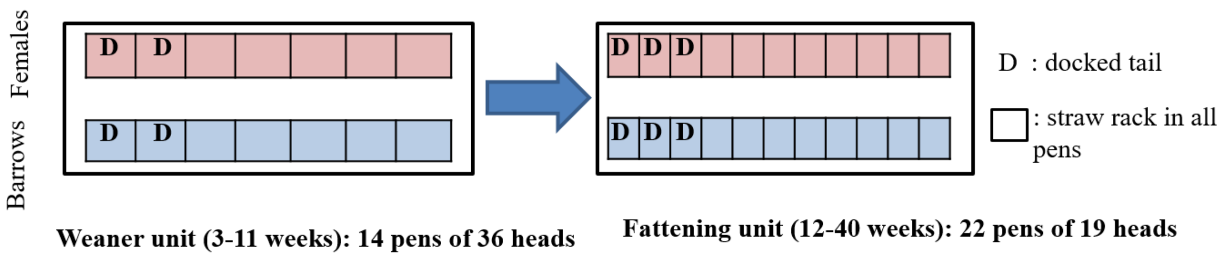

Figure 4. Distribution of the experimental groups of Trial B in a commercial weaner and fattening unit.

In both trial A and B the small amount of straw consumed from the rack did not create any management issues due to slurry outflow obstruction. A general limitation of these findings (raised also by the reviewers) is that we were unable to objectively measure and describe the outflow system (*e.g.* the size and length of the pipelines, presence of bendy routes or strictures). Moreover, a second reviewer argued that we should have quantified the amount of straw which was pulled out and lost in the manure and the amount pulled out but actually eaten. Future works will be needed to respond to these very hard questions.

In general, in view of the sporadic and unpredictable nature of tail biting in intensive pig farming, further studies with a rationalised design will be needed to confirm the outcomes of Trial A and Trial B, both in terms of more animals, more pens, but especially more farms involved. It is possible that several Italian farms will be available to be assessed in the near future, due to an action plan promoted by the Italian Ministry of Health for increasing the prevalence of undocked pigs. This initiative will be better described in the next chapter.

2.1.2. Ten years later

Chapter 2.1.1. has detailed the tail biting issue, and suggests the major remarks reported from mine and others' papers that might be employed when entering a pig farm and evaluating the risk of tail biting and the need for tail docking. The '3-S recipe' might be an easy approach: 1. Straw, 2. Space, 3. Skills (of the farmer). However, several further publications have followed my results in these ten years going deeper into this complex issue, each one focusing on further risk factors. In 2018, the motivation of pigs towards different wood species was tested (Chou *et al*., 2018) and interactions with spruce (*Picea sitchensis*) were higher compared with beech (*Fagus sylvatica*), larch (*Larix decidua*) and Scots pine (*Pinus sylvestris L.*). The effect of docking length (short *vs*. medium *vs*. long *vs*. undocked) was also evaluated: only the shortest docking length treatment reduced the tail biting risk (Thodberg *et al*., 2018).

The relationship between the tail posture (*i.e.* the positions such as hanging, lowered or upright) of a pig and tail damage was another promising focus of research, with the aims being to find an early prognostic marker of tail damage (Larsen *et al*., 2016; Wedin *et al*., 2018). Indeed, it was found that a tucked tail could be used as a detector of tail damage, but with the risk of false positives. Alternatively, according to Wedin *et al*. (2018), an increase in tucked tails and a reduction of curled tails is a reliable indicator of a tail biting outbreak, that gives prior warning at least seven days in advance of the outbreak.

Despite the large number of studies, very few papers have been able to outline easy and effective take-home messages for farmers (such as Figure 5, from Valros & Heinonen, 2015). To the best of my knowledge during these years of investigation 'in the real life' of pig farms, farmers' insufficient education is the major criticism when trying to both prevent and manage tail biting. They need to be fully aware of the higher risk of having intact tails in their farm, and be practically trained to look at tail posture, tail lesion, and tail sucking behaviour (also called 'tail-in-mouth' or TIM; EFSA, 2007). Moreover, they must be proactive in managing an outbreak as soon as possible and in an appropriate way: removing the biter, medicating the victims, eventually also removing the most injured pigs and calling the vet for systemic antimicrobial therapy.

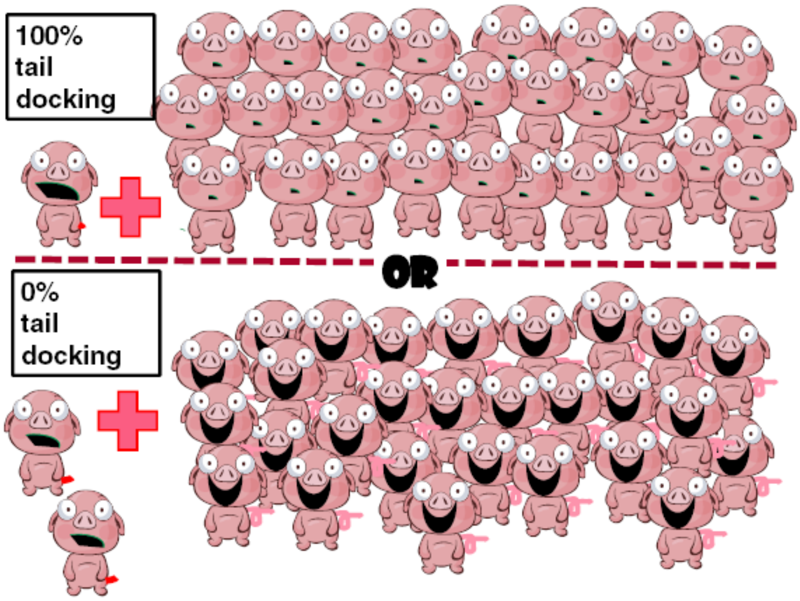

Figure 5. The tail docking balance: pro and cons, from the scientific paper of Valros & Heinonen (2015).

As explained with apparent simplicity by Figure 5 (Valros & Heinonen, 2015), the net benefit is made by the balance of avoiding a painful procedure to the whole group counteracted by an increased risk of injuries for some subjects. Moreover, we are also reminded that tail docking does not eliminate the issue, but can only decrease its frequency. Accordingly, the quantification of the risk is exactly what farmers should perform in order to understand if this balance is likely to be positive or negative in their farm. Where it is negative, further amendments in husbandry procedures should be carried out. This risk evaluation process has been formally requested by the EU Recommendation 2016/336 to all member states two years ago (European Commission, 2016b), but in Italy it was not put in practice.

In November 2017 the European Commission programmed an audit in Italy to evaluate the level of compliance with EU Directive 2008/120/EC and with EU Recommendation 2016/336. I was invited to the audit as a scientist to present my publications, and had the opportunity to follow the audit process. The report evidenced the lack of compliance of Italy, not only on tail docking, but also on the total absence of risk analysis as requested by Recommendation 2016/336. The report is publicly available at the European Commission website, in four languages (European Commission, 2017).

In response to this reprimand, the Italian government created a worktable of experts (which I was also invited to join) to create a checklist for farmers and to implement a three year action plan to improve pig welfare and decrease the need for tail docking. An education programme was established for farmers and for veterinarians, which taught them the theoretical basis behind the risk evaluation for tail biting. Trained veterinarians received the credentials to upload online in a private area of a website property of the Ministry of Health the results for each of their farms (with the agreement and the collaboration of the farmer). Afterwards, the system automatically creates dashboards for control authorities (Figure 6).

In 2019, farms at high risk are going to make amendments based on the specific risk factors identified by the system. Upon the decrement of the risk level, the introduction of a small number of undocked pigs will be requested in 2020, if farmers are thoroughly trained and supported by the local health authorities. With positive results the farmers will be asked to increase the percentage of undocked pigs, while in cases of unfavourable findings the farmers will be asked to further improve the quality of the environment (by switching within a three scale system on the checklist from 2 ‘acceptable’ to 3 ‘optimal’ for some parameters).

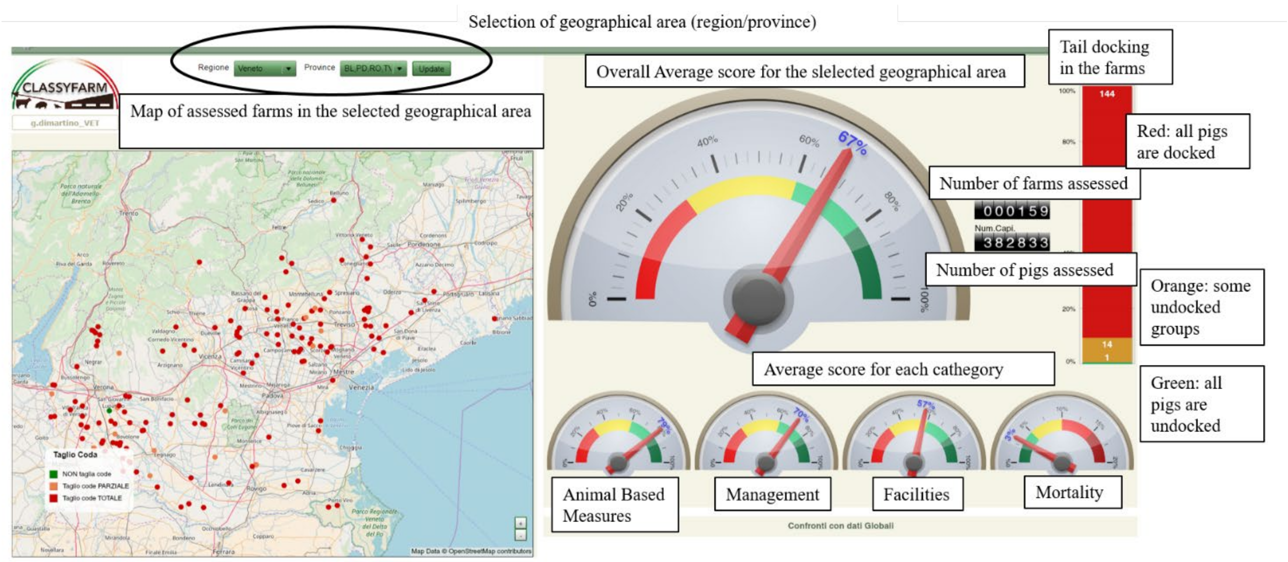

Figure. 6. Dashboard example with outputs of risk evaluation for tail biting in Italy (explanations in English language have been added).

2.2. Welfare assessment of Italian heavy pigs: a Rubik's cube

2.2.1. Behaviour, health, physiology: 'unity is strength'

In this section, I aim to make specific comments on the methods applied in my experimental studies, also thanks to the experience I have acquired. First, I would need a brief introduction by referring to two milestone papers on animal welfare assessment. In the former, Prof. Fraser explains how complex welfare assessment can be; as different indicators need to be associated, but can unexpectedly provide conflicting outcomes (Fraser, 2003). The key for interpretation is the 'value assumption,' *i.e.* to the weighted value a scientist decides to give to a single variable. Gestation stalls for sows is a suitable example, given that skin lesions and abortions are lower in individual stalls while behavioural indicators highlight poor welfare due to space confinement and animal frustration. In addition, the discussion of Korte *et al.* (2007) warns welfare scientists on the limitations of the five freedoms principles. In fact, animal welfare is not simply an absence of deprivations, but a fluid concept; properly a 'buffer zone' where some stress is needed, whether successfully sustained for a certain timeframe.

The stress fluctuation during time in the experimental groups is exactly the first issue for a researcher assessing animal welfare for a fattening period of 30 weeks. The time variable is significant for most of my measures and the trend is fluctuating in the different experimental groups. For example, in Trial A (Scollo *et al*., 2013) tail biting was higher in undocked pigs at 3, 9 and 18 weeks of fattening. Unexpectedly however, mild tail lesions were higher in docked pigs at week 14 and there was no clear explanation for this. We do not have a comparison between lesions and behaviour during the same week. This is due to two constraints: 1) The feasibility of making a sufficient number of evaluations without an excessive disturbance to animals 2) The need to separate the different evaluations during time, given that some (such as lesion scoring or blood sampling) could bias the behaviour assessment. More concordance between parameters can be found for the effects of straw provision, which significantly reduced both tail lesions (but only at week 3) and tail biting behaviour (at week 3, 9 and 18, but not at 29). Straw effect was also confirmed by a blood measure (serum haptoglobin, an indicator of inflammation), so in this case the interpretation seems clearer. I also found a fourth confirmation at the time of slaughtering in terms of oesophago-gastric ulcers. Indeed, oesophago-gastric ulcer revealed to be a promising welfare indicator, especially as pigs, like humans, suffer different grades of stomach erosion in response to stress (Doster, 2000).

Another issue to be faced is the proper interpretation of the results from other studies, in order to make appropriate comparisons. For example, comparisons can hardly be done between values of blood markers from different studies, because of different laboratory/sampling methodology (Kaneko, 2008). Moreover, the different study approaches can provide an apparent inconsistency. I can explain this occurrence with a practical example from my studies. Amory *et al*. (2006) evaluated several factors in the housing environment as possible risk factors for ulcers in pigs. The authors found an unexpected association between ulcers and floor type (slatted floor conferring an increased risk) and tail docking (tail-docked pigs had an increased risk). So it seems that unlike in my study where undocked tails had no effect on ulcer onset, according to Amory *et al*. (2006) the undocked tail is inexplicably a protective factor against oesophago-gastric ulcer (although the authors do not comment on this result).

The study of Amory *et al*. (2006), as with other ones on pig ulcer (Robertson *et al*., 2002; Gottardo *et al*. 2017) is an epidemiological investigation. Therefore, there is no proper comparison between two groups of animals which lived in the same environment and husbandry conditions apart from a single experimental treatment (as it happened in my experimental trials). Rather, there is a comparison between farms, with different husbandry and health conditions. It is very unlikely that surgical intervention within the first week of life can have an influence on a stress-related disease which typically occurs during the finishing period (Doster, 2000). Alternatively, a tail biting outbreak in the finishing period can induce a massive stress event, as well as impacting the amount of feed being consumed (*i.e.*, a pig may avoid the manger because in that moment it is more vulnerable to being tail bitten). Consequently, it could be speculated that undocked tails could lead to distress of the gastric mucosa, rather than docked ones. We are, however, looking at groups of pigs. If we look at farms, the results of Amory *et al*. (2006) may become more reasonable. In fact, as I explained previously, rearing pigs with intact tails is a challenge that improves farmers' skills: farmers able to rear undocked pigs are likely to employ better welfare to their animals for a variety of environmental conditions (air quality, quality of feeding, quality of manipulable materials, etc.). Therefore, it can be that these farms are characterised by a lower prevalence of oesophago-gastric ulcers.

Unfortunately, ulcer evaluation of different groups within the same farm is not easy, because it is hard to track individual animals and/or pen-groups up to the slaughterhouse (and after slaughtering). In fact, in the transport vehicle the different pen groups can be mixed, and during the conventional slaughter process the stomach is removed together with the intestine after the opening of the carcass and is directed to a specific cleaning room (*i.e.* it loses the association with the carcass, which instead is maintained for lungs and liver). Stomachs are then opened along the great curvature and cleaned before being put into a machine that washes the inner part and removes feed residuals (stomachs are used in the food industry to produce tripe). On the contrary, lungs and liver remain together with the carcass, so their traceability can be easily determined.

In my studies the pig industry provided major support in order to maintain the traceability of the experimental groups: 1) by keeping the pen groups separate during transportation and at lairage; 2) by providing a mark for recognition at the beginning of each new slaughtered group (a cut on the left ear of the first animal hung up on the slaughtering rail). However, the information regarding the pen of origin was still lost. For this reason, in the logistic regression for oesophago-gastric ulcer the animal was the statistical unit and the pen was not included as a cluster. A reviewer argued that this could be a limitation of the study, given that there could be a pen effect acting on ulcer occurrence and severity. In response to this criticism, which was not possible to refute, we have included a specific comment in the paper warning readers on the missing data.

A second reviewer argued that the results were very similar to the study of Scott *et al*. (2007). However, that study investigated the effect of flooring (slatted floor *vs.* straw bedding) and feeding (dry *vs.* liquid) by means of a 2 × 2 factorial design on gastric lesions of entire light pigs (104 kg l.w.) in mixed-sex pens. Therefore, our study was different because of several aspects: 1) we did not have mixed-sex groups but barrows *vs.* gilts, 2) we had fattening pigs of 169 ± 4 kg l.w., 3) we had all of the animals under the same husbandry condition (*i.e.*, the same shed, with the same liquid feed; 12 pens were provided with a straw rack and 12 without), 4) we did not use straw bedding, but 70g/day of straw, 5) we also investigated a group of undocked pigs (2 ×2 × 2 factorial design). Straw bedding is a well-known system to improve pig welfare and to prevent tail biting (EFSA, 2007). Nevertheless, the pig industry in most European countries (Italy, in particular) is promoting the adoption of slatted/partly slatted floors (EFSA, 2007), and the efficacy of 70 g/day of straw by a manger rack on the prevalence of gastric lesions had never been explored before. This aspect, within the traditional Italian production of heavy pigs, gives novelty to the study.

The rebuttal was accepted, but it is even greater to see after five years how the study has influenced the following research. The paper was cited 24 times, and several new studies included the investigation of oesophago-gastric ulcers in connection with the provision of a small amount of straw (*e.g.* Herskin *et al*., 2016) or other similar materials (Holinger *et al*., 2018). Finally, the EU commission has indicated the evaluation of oesophago-gastric ulcers as a reliable welfare indicator (EU commission, 2016a).

Despite the EU Commission's support, this interesting new welfare marker is probably still underused. At present no studies have investigated animal traceability, due to the practical constraints explained above. This possibility, although challenging, would potentially highlight interesting associations between some behavioural traits and individual ulcer susceptibility. As I was aware of this aspect, my study on immunocastration (Di Martino *et al*., 2017a; see Par. 2.3) was designed to maintain animal traceability up to the end of slaughter. To do this, I tattooed between the shoulders of each animal to recognise them during fattening. At each behavioural session, it was hard to read the tattoos from a distance, so I entered the pen and marked each pig with a spray coloured number (one to seven blue and one to seven red) that corresponded (by means of a legend) to the number in the tattoo (one to 56). In this way, I was able to attribute specific behaviour not to the pen but to the animals. Moreover, at the time of slaughtering one of my colleagues was in the stunning area and recorded the tattoo number in the order of the progressing chain. Another colleague was in the room where stomachs were delivered and they collected them in the same order. In the meantime, I was scoring the lesions on the skin. Afterwards, I inspected the lungs and livers and then went into the room to score the stomachs. Surprisingly, in that study the prevalence of ulcers was very low (*i.e.* 7% of ulcered stomachs) and did not allow a robust association with behaviours.

The sex effect on gastric ulcers merits further research as well. At present, only in our study (Di Martino *et al*., 2013), has it been found that females may be at reduced risk than males, and the possible role of female hormones has been hypothesised on the basis of that found in studies on humans and mice (Shimozawa *et al*., 2006). This evidence highlights the suitability of the pig as an important animal model for human medicine.

Two final remarks on oesophago-gastric ulcer refer to the current difficulty in determining the effective quantity of straw consumed *vs.* the amount which was removed and lost under the slatted floor and the high prevalence of this disease even when straw is provided. These aspects are also present in the recent study of Herskin *et al*. (2016), who provided 10, 500 or 1000g straw/pig/day from 30 kg live weight on partly slatted floor. Irrespective of the quantity of straw provided, 67% of the pigs showed signs of ulcers. Groups provided with either 500 or 1000g of straw/pig/day were pooled as 'permanent access'; the percentage of pigs with ulcers was reduced by permanent access to straw (7% *vs.* 33%; $P<0.05$). Most studies in the literature found a beneficial effect from around 100-200g/pig/day, but at present our study identifies the lowest effective dose. Nonetheless, in our study 32% of pigs provided with straw still exhibited oesophago-gastric ulcers. Comparisons across studies is challenging in view of the different ages and experimental conditions applied.

Behavioural investigation represents a fundamental element of welfare assessment (Martin & Bateson, 2007). Moreover, the sensitivity of behavioural indicators, which are able to detect a transient perturbation of equilibrium, that does not produce a lesion, a physiological change or a production loss. Unfortunately, behavioural studies in pigs also tend to refer to pens not to single animals. This is due both to the difficulty of getting individual recordings of behaviour and to the fact that most behaviours are strongly influenced by the pen unit, so it is more correct to evaluate them at the pen level (*i.e.* the proportion of pigs lying at each instant of observation, the number of aggressive interactions within the pen in a specific timeframe). This approach can be a constraint in studies where a limited number of pens are available (*e.g.* Di Martino *et al*., 2017a). In the study cited, the number of pens could not be increased due to the high cost of the immunovaccine, therefore an animal-centred approach was adopted.

Aside from the need for observing a sufficient number of animals and of pens, a sufficient quantity of time of observation is important as well. The required time is still the subject of discussion, as no gold standard has been determined. General rules on which scientific publications are based (Martin & Bateson, 2007) recommend a few uninterrupted hours during the period of maximum animal activity, if the intention is to detect some specific behaviour when it is more likely to be performed. The best choice to evaluate the 'time budget' (*i.e.* the distribution of the different behaviours during the 24 hour period) is a sample of recordings during a 24 hour period, but in this case you need infrared cameras to be installed in the farm to overcome the problem of darkness. Moreover, the analysis of video recording is very time consuming. For these reasons, new protocols for welfare assessments have proposed faster methods, focused on fewer priorities (*e.g.* aggressive behaviour, interaction with the enrichment material). Welfare Quality Protocol for pigs (on farm) suggests a period of only 20 minutes per pen for recording major pig behaviours (Welfare Quality, 2009), while the evaluation at the time of slaughter is faster, as it requests evaluation of the animals when being unloaded (to detect immobilisation, turning back and lameness) and briefly at lairage (focusing the evaluation to shivering, huddling and panting, *i.e.* all indicators of thermal distress).

A rapid behavioural method has been recently proposed by the European Commission (European Commission, 2016a) to help farmers to easily evaluate the pigs' interest in the enrichment materials. A pen has to be observed for 2 minutes (this time is needed for habituation). Afterwards, the observer has to count the number of pigs interacting with the enrichment/s (A) and the number of active pigs (B) (except for pigs drinking and eating), and determine the proportion: A divided by A + B. Where the subsequent percentage is lower than 18%, the enrichment provided is deemed insufficient.

It is likely in the future that innovative technology will be available for 'smart farmers'. Precision livestock technology is growing in importance not only in experimental contexts, but also in real world farm life, in order to increase productivity, ameliorate animal conditions and reduce farmers' workload using remote sensors (*e.g.*, Soundtalks® equipment for monitoring coughing in pigs, based on Ferrari *et al*., 2008) and smartphone applications (Bercksmans, 2018). In poultry, for example, a commercial company can provide cameras and software able to detect animal movement (each animal is extrapolated as a dot moving in the space at a certain speed) and calculate the descriptive statistics for the whole population during time. A significant decrease/increase in average speed/distance covered, or a detected increase in the variation (standard deviation) between individuals results in an alarm directly to the farmer' smartphone (Fancom, 2018). Recently, Bracke has described sensors that can be used to semi-automatically record pig manipulative activity by attaching a motion sensor to hanging objects (Bracke, 2018), to have a scale of weight to determine the 'enrichment value'.

The discussion on methods of welfare assessment should not forget to mention the important role of stress indicators, given that this section in welfare evaluation has received a high level of attention in past research, probably due to the appealing idea of finding a universal marker of stress. Conversely, as Korte *et al*. (2007) well remind us, it is not a simple direct correlation. In my studies, only a small subset of blood markers have provided evidence of a welfare impairment. Cortisol is highly variable and depends on age, sex and the time of day (Evans *et al*. 1988; Ruis *et al*., 1997). These aspects can be accounted for, but cortisol varies between individuals, and amongst a set of repeated samples from the same individual (Kaneko, 2008). If cortisol levels are evaluated from blood samples, ethical concerns may be raised surrounding its collection. Whether recorded from saliva, practical limitations may exist including the time taken for collection by swab with minimum interference with the animals. In both cases, criticisms can also be made regarding the need for a suitable baseline, which can hardly be established in field trials. It is generally accepted that a comparison between the same animals at different time points (under the same sampling conditions) is a suitable option. Again, this approach might be suboptimal during chronic stress, where stress markers may not behave as they do in an acute response, either remaining anomalously low or high (Kaneko, 2008). A similar comment can be made on acute phase proteins, as positive indicators of subclinical infection.

In my studies, acute phase proteins were more strongly linked to physical damage than cortisol (*e.g.* in severely tail bitten animals cortisol could be within the normal range, but haptoglobin and albumin-to-globulin ratios were high). Among the different molecules tested, haptoglobin provided the most reliable results, confirming evidence of welfare impairment obtained with other parameters (*e.g.* skin lesions, fever, pain). Haptoglobin is a serum glycoprotein whose primary function is to bind free haemoglobin in the blood (Kaneko, 2008). However, a role as a positive acute phase protein has been demonstrated due to bacteriostatic activity (limiting iron availability for bacterial growth), antioxidant property and ability to stimulate cells of the monocyte/macrophage lineage (Quaye, 2008). Previously, a 10- to 100-fold increase was reported in response to the noxious stimulus in ruminants and a 2- to 10-fold increase in swine (Murata et al., 2004). In particular, the increase of haptoglobin in pigs has been found in response to lameness, respiratory diseases, tail biting and ear necrosis (Petersen *et al*., 2002; Salamano *et al*., 2008; Heinonen *et al*., 2010).

In trial A, an unexpected sex effect was also found on haptoglobin, which had not been described before in the scientific literature. Females had higher values than males at the end of the finishing period (Scollo *et al*., 2013). This finding was confirmed in the second trial with undocked pigs (Di Martino *et al*., 2015a) and it was proposed to be a possible effect of stress hormones during oestrus. Further (and striking) confirmation arrived after my last field trial on immunocastration (Di Martino *et al*., 2017a), where I found that females had higher haptoglobin values after sexual maturity compared with immunocastrated females.

Two previous publications (Piñeiro *et al*., 2009a, 2009b) suggested Pig-MAP (Pig Major Acute Protein) is a sensitive acute phase protein in pigs, with an increase of more than 10-fold in response to infection, trauma and inflammation. For this reason, it was decided to include the quantification of this interesting parameter in the panel, using the same ELISA kit proposed by Piñeiro *et al*. (2009b) (PigMAP kit ELISA, PigCHAMP Pro Europa S.A., Segovia, Spain). Surprisingly, reproducibility (n = 10, CV > 20%) and repeatability (n = 20, CV > 20%) characteristics were unacceptable using the commercial kit. It is currently unclear why this occurred.

2.2.2. The unexpected interference of haemolysis

In Trial A, the decision to remove the quantification of PigMAP did not significantly compromise the rationale of the study, as other blood markers were included in the panel. However, another unexpected occurrence created an issue for the validity of the physiological measurements. Swine erythrocytes are physiologically characterised by a high level of fragility compared with other domestic animals (Matsuzawa *et al*., 1979). Such a natural characteristic is likely to produce a variable degree of haemolysis during blood sampling, and potentially compromise the quantification of several analytes. Therefore I sought to determine the extent to which haemolysis interfered with accurate analytical results.

Haemolysis may be explained by the specific blood sampling process which is particularly challenging for pigs, as they need to be restrained with a steel lace tightening the snout. The snout becomes reddish and congested, and the animal is distressed. The proper positioning of the pig is fundamental, with the head raised and the front legs to the rear, to expose the jugular groove and raise the veins. Using the right hand, the operator has to stand on the right side and to blindly stick (*i.e.* the jugular veins are not visible) the needle into the jugular furrow at the lowest point of the groove 5-13 cm in front of the point of the shoulders (Muirhead, 1981). The detailed description is needed to fully appreciate the difficulty of this procedure, and to understand why the expected percentage of haemolytic specimens can be as high as 7-8%, even though well trained personnel have applied best veterinary practice (Di Martino *et al*., 2011, preliminary results presented at SIPAS national congress).

Anecdotal reports between veterinarians correlate the level of haemolysis to the inexperience of the operator, but this hypothesis has not been tested by the evidence during our sampling session, where the same skilled operator gained both haemolytic and non-haemolytic samples. Moreover, the time needed for filling the vacutainer (from around one second when the vein is fully penetrated immediately, to more than 10 seconds when the vein is taken sketchily and limited blood drops drip into the vacutainer) was not clearly correlated to the degree of haemolysis (Di Martino *et al*., 2011, preliminary results presented at SIPAS national congress).

Haemolysis is a complex and only partly understood process associated with a number of potential interfering factors, including artefactual dilution due to release of intracellular compounds, release of proteolytic enzymes and analytical interference (Lippi *et al*., 2008). In routine blood testing, haemolysis is not a concern, because veterinarians mostly collect blood to detect serological positivity to swine diseases, and antigen-antibody reactions are not affected by the presence of free haemoglobin (Dimeski, 2008). Conversely, in the evaluation of biochemical parameters, this occurrence can skew results.

I collected 30 non-haemolytic sera samples at the abattoir (where at the time I was involved in other activities) to investigate this issue. I divided the samples into three aliquots to which progressive degrees of physical haemolysis (on a scale of 0 to 3+) were induced by rotary shaking in the laboratory. This method is the one that best mimics 'spurious' haemolysis (*i.e. in vitro* haemolysis due to erythrocyte traumatisation when passing through the needle into the vacutainer). The level of haemolysis was visually determined, using a colorimetric reference scale (commonly in use in haematobiochemistry labs). Moreover, the visual method (Lippi *et al*., 2011) was compared with an analytical method (haemolysis index).

The ROC (receiver operating characteristic) curve analysis was applied to identify the best cut-off analytical values for differentiating the visual levels of haemolysis (0 *vs.* 1+; 1+ *vs.* 2+; 2+ *vs.* 3+). For each comparison, the specificity (Sp), sensitivity (Se) and the area under the curve (AUC; 95% CI) were calculated. According to the ROC curve analysis, a cut-off of 36mg/dL provided the best specificity (1.00) and sensitivity (1.00) in discriminating levels 0 *vs.* 1+ (AUC: 1.00; 95% CI: 1.00–1.00). A cut-off of 72mg/dL gave the best sensitivity (1.00) and specificity (0.97) in discriminating levels 1+ vs 2+ (AUC: 0.99; 95% CI: 0.99–1.00). A modest overlap was found between the 2+ and 3+ levels, with discriminatory cut-off values of 127mg/dL (Se: 1.00; Sp: 0.70) and 177mg/dL (Se: 0.80; Sp: 1.00), respectively (AUC: 0.97; 95% CI: 0.93–1.00). A graphical presentation of these results is given in Figure 7.

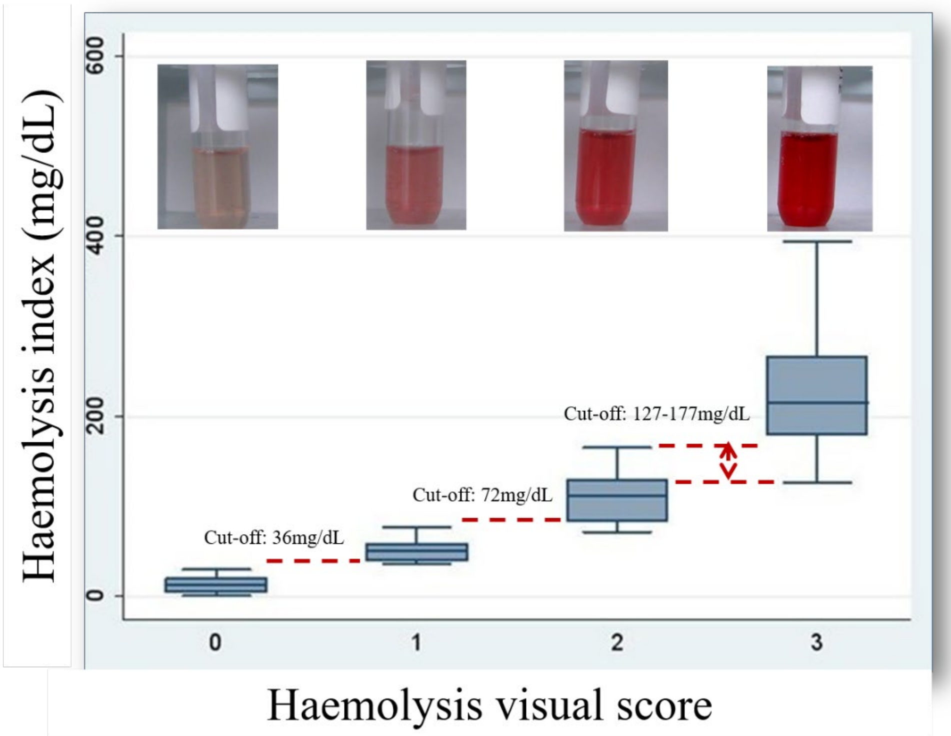

Figure 7. Identification of the best cut-off analytical values (haemolysis index) for differentiating the visual scores of haemolysis.

Twenty-seven blood analytes were tested; nonlinear trends of values obtained at progressive degrees of haemolysis confirmed the difficulty in establishing reliable coefficients of correction for adjusting the test results (Di Martino *et al*., 2015b). On the other hand, it was possible to determine the acceptability for each parameter according to the degree of haemolysis. I applied a non-parametric Wilcoxon signed rank test to determine whether there were significant differences compared with non-haemolytic samples.

At present, this work (Di Martino *et al*., 2015b) has been cited in seven studies, and read 577 times on Researchgate. Citations mostly came from studies on wildlife (tiger salamanders, yellow-bellied sliders). In fact, an unexpected group of readers had in common the same priority to determine the suitability of blood specimens avoiding as much as possible the need to recollect blood from the animals. Collection has to be limited either because the procedure is laborious or because a limited blood volume can only be taken without causing animal death. The paper was also discussed in a PhD dissertation evaluating the effect of oxidative stress in pigs on several blood markers, and suggesting interesting connections between the likelihood of haemolisation and the level of oxidative stress in pigs (Singhal, 2016). Previously, Adenkola *et al*. (2009) had used erythrocyte osmotic fragility as a welfare parameter to investigate the effect of antioxidant administration (ascorbic acid) to reduce stress in pigs transported by road for eight hours.

2.2.3. Like a paradox: measuring welfare at slaughter

When I was contacted by IRTA (*Instituto de Recerca y Tecnología Agroalimentaries*) to join a research initiative to be applied in a sample of Italian abattoirs, very little data was available on pig welfare at the time of slaughter. This lack of information is due to practical aspects, which are briefly discussed here. Firstly, the difficulty in accessing a slaughterhouse for specific reasons: 1) The staff responsible are understandably suspicious, as they face welfare issues as a potential legal non-compliance, but not (as for farmers) as a direct source of income (as it can be for farmers applying higher welfare standards, who can be safeguarded by specific logos such as Freedom Food or Slowfood). 2) For the public, the time of transport and killing is the worst moment during an animal's life. 3) For the public the moment of transportation is the only occasion where they can see farm animals, often overcrowded and in poor conditions. 4) The slaughterhouse is a much more dangerous place compared to a farm, and the staff responsible are focussed on HAPPC (Hazard Analysis and Critical Control Points). They have to devote much attention to both food and people safety: verifying that guests wear appropriate clothes and IPD (individual protection devices) to protect themselves against injuries and to avoid becoming a hazard for food safety.

The brief introduction is necessary to understand how difficult it was to recruit the abattoirs to join the Spanish project, which aimed at applying the WQ Protocol (Welfare Quality, 2009) in Spain, Portugal, Finland, Brazil and Italy. Each country should have provided data on 10 big abattoirs (*i.e.* that slaughter more than 1000 pigs per day). Unfortunately, it was not possible to reach this sample size in all countries, which was reduced to 5 in Finland, 8 in Portugal and 9 in Italy. From a methodological point of view, the major criticism is not only the limited number of units, but also the sampling scheme was biased based on convenience and practicality, *i.e.* only slaughterhouses available to participate were recruited in a limited timeframe (while the protocol should be repeated during different seasons). This limitation could have resulted in underestimated bad outcomes. On the other hand, the paper (Dalmau *et al*., 2016) did not aim to find a comparison between countries, but rather to explore the variability of the parameters (similarly to a pilot study) as a useful way to establish reliable thresholds for future use in the WQ protocol to define what is acceptable within a variety of different management conditions.

Even without the possibility of a random sampling scheme of the slaughterhouses, several criticisms and legal non-compliance issues were evident in the different countries, particularly with the effectiveness of stunning. Surprisingly, this research attributed some unexpected placements of Italy in terms of ranking countries from good to bad, likely due to the specific production system of heavy pigs.

The WQ sampling scheme is based on defined sample sizes and takes into account all slaughtering phases (unloading, lairage, stunning, jugulation). In the unloading area, the levels of fear, slipping, lameness, space, thermal comfort, sick and dead animals are assessed. At lairage, space allowance, drinking points and levels of thermal comfort are considered. Vocalisations are counted by means of two measurements (continuous and instantaneous) when pigs were moved from lairage to the stunning system. Stunning effectiveness is assessed by means of four parameters: corneal reflex, rhythmical breathing, righting reflex (*i.e.* any attempt to regain posture) and vocalisations. The level of skin lesions and any sign of diseases (pneumonia, pericarditis, liver parasites) are evaluated after slaughtering the animals. From a methodological point of view, the major limitation of the WQ protocol concerns the categorisation of continuously distributed variables (*i.e.* all assessed items are grouped into a 0-1-2 scoring system) that may reduce the sensitivity in detecting treatment differences. On the other hand, this choice has probably been made by the WQ developers in order to make this tool as easily manageable as possible, after a short time of training.

As the international publication (Dalmau *et al.*, 2016) proposes a summary of data in different countries, I will briefly expand here the results from the Italian dataset, to discuss in detail some important aspects and methodological issues, which are found in the Italian language in a further publication in a national journal (Di Martino *et al.*, 2017b). Evaluations were made from March to June 2014 by myself and another colleague, who attended a specific training course at IRTA together with the assessors of other countries. After the course my colleague repeated the training for me and shared the theoretical material she received from IRTA. The duration for each assessment was around 4 to 5 hours, mostly due to the need to wait for the arrival of the lorries (the protocol requires the evaluation of the unloading phase in six lorries). Plants slaughtered 51,000 to 860,000 pigs per year, on average from 650 to 3,600 per day and from 75 to 390 heads per hour. In seven plants, electric stunning was used (six were manual systems with prods applied by an operator, while one was an automated system), while in two, gas stunning with carbon dioxide was applied.

Results on animal reactions at unloading are given in Table 1 (pag. 41), while results on lairage and the pre-slaughter area are given in Table 2 (pag. 42). No animals were found dead in the lorry, while only one subject identified as unable to walk and was immediately euthanised by means of an emergency captive bolt device. Data on the post-jugulation phase (scores for lesions on carcasses and organs) are given in Table 3 (pag. 43).

With regards to ‘reluctant to move’ and ‘turning back’ animals, results from Italy ranged between 0% and 2.2% and between 0.4 and 4.6%, respectively. The situation appears much better than in Spain, Brazil, Portugal and Finland, where the former value reached 40% (Brazil) and the latter 20% (Finland). Similarly, incidences of slipping ranged between 0.6% and 13.2%, while in Brazil they reached 58%, 52% in Spain and 18% in Portugal. Lameness did not exceed 2%, while in Brazil it reached 58%, 52% in Spain and 18% in Portugal. Overall, the Italian outcomes were better also in terms of sick and dead animals and in terms of skin lesions of carcasses. In Italy, the mean value for sick animals at unloading was 0.01% ± 0.13%, while in other countries these percentages ranged from 0.11% ± 0.53% (Portugal) to 0.43% ± 0.72% (Brazil). In Italy, the mean value for dead animals was 0.00% ± 0.00%, while in other countries these percentages ranged from 0.05% ± 0.22% (Brazil) to 0.20 ± 0.73 (Spain). In Italy, carcasses with more than 10 lesions (‘score 2’ according to WQ method of assessment) ranged between 2-10% compared to a range of 8-22% in Portugal, of 2-34% in Finland, 2-48% in Brazil and 8-40% in Spain. Unfortunately, as only one visit was made per slaughterhouse and few slaughterhouses were available per country, no sufficient data were available for robust statistical inferences. Therefore, only descriptive statistics were presented in the paper (Dalmau *et al.*, 2016).

Taken together, the Italian results in Dalmau *et al.* (2016) are explainable by a higher economic value of heavy pigs compared to other rearing systems for fresh meat, which is strongly connected with the need to attain undamaged thighs. As a consequence, slaughter operators in Italy have developed a much higher sensitivity to avoid unnecessary stressors, likely perceived as ‘hazards’ impairing meat quality. In detail, several important protective actions were found to be applied in Italian plants: 1) In seven plants, a roof was set over the ramp to shelter animals when being unloaded. 2) In one plant (as an internal guideline at unloading), the truck and the trailer of each vehicle were separated in order to make the unloading procedure easier for animals (*i.e.* routinely the whole vehicle approaches the ramp, animals are first unloaded from the trailer and afterwards groups from the truck are unloaded by passing through the empty trailer). 3) In all plants, animal mixing was forbidden, so the pen groups were maintained both during transportation (by means of enclosures) and at lairage. 4) In five plants, CCTV was installed and a camera pointed towards the unloading ramp. The last one was a specific initiative of the abattoir responsible, so it was not clear whether staff would have watched the videos, or if the cameras were actually working. It was not part of our protocol to check CCTV, so I only reported the information as a point of interest. Some years later I realized that this is a pressing welfare concern in EU abattoirs, where different activist associations are pushing to make the installation of CCTV compulsory as a control tool for official veterinarians. In the UK, an official opinion by the Farm Animal Welfare Committee was published in recent years to evaluate this initiative (FAWC, 2015).

Within the Italian dataset, a high variability was observed in terms of materials, structures and type of flooring of the unloading ramp. With consent I photographed the sites (Figure 8), because it proves very useful to explore some details: the presence of fresh litter such as straw or sawdust; the presence of anti-slip surfaces (although which types are best is unknown); the difference in lighting; the inclination of the ramp (it cannot be steeper than an angle of 20 degrees, according to EU Regulation 1/2005). The most reliable ramp evaluation comes from animals (in terms of percentages of animals slipping, falling, reluctance to move), as it is difficult to isolate a single factor. In the Italian study, the plant with the worst scores at unloading (see Table 1) was H (Figure 8).

Figure. 8. Variability of unloading ramps observed during welfare evaluation in nine Italian pig abattoirs (from Di Martino *et al*., 2017b).

Space allowances (m^2/animal) per pen in Italian plants are hardly comparable with those from other countries, due to the different slaughter weight. Some attempts have been made in the paper to convert data to 110 kg of live weight. According to this approach, the stocking density in different countries ranged from 0.53m^2/110 kg in Spain to 0.75m^2/110 kg in Brazil.

Stocking density in lairage pens is not subject to any legal requirement, as EU Reg. 1099/2009 only states '*Each animal shall have enough space to stand up, lie down and, except for cattle kept individually, turn around'* (European Council, 2009). I realised when interviewing the slaughter operators that this concept is subject to different interpretations. Some operators were convinced that the same limits for stocking density established for pigs on the farm (European Council, 2008) should be applied. Others thought the same limits for stocking density established for pigs during transport applied (European Council, 2005). The most meticulous operators displayed advice on their walls (Figure 9) indicating the maximum allowable number of pigs not only on the basis of their live weight (heavy *vs.* light pigs), but also on the basis of the time of year (summer *vs.* winter). I was unable to find conclusive data on suitable stocking density for heavy pigs at lairage in the literature. Others have suggested long and narrow pens, with a minimum of 0.5m^2/pig (Grandin, 2007), but again this advice was targeted to farms with light pigs.

Figure 9. Advice present in one Italian abattoir, indicating a maximum number of pigs for a lairage pen in winter and summer (represented as ice and sun) on the basis of their weight: L: light ('leggeri', in Italian); P: heavy ('pesanti', in Italian). ©Guido Di Martino.

The number of vocalisations recorded near the stunning area was rather high in Italy compared to other countries. This may be because moving heavy pigs can be more problematic than moving lighter and younger animals: they tend to be more reluctant to move, exhibiting stubborn behaviour when they do not intend to go on (and the operator is authorized to use the electric prod to incite movement only when really needed). Moreover, in Italy electric stunning is more frequently used (Di Martino *et al.*, 2017b), but both types of stunning systems (gas or electric) require handlers to channel animals in a single row towards the stunning device. In fact, the Italian system for gas stunning (called 'gondola') is based on pushing animals (single file) into a moving carriage (swinging on a track like a hammock, or the traditional venetian boat) towards an underground room where carbon dioxide is present at a high percentage (*i.e.* >80% according to EU Regulation 1099/2009). In other countries, electric stunning is less frequently used in slaughter plants, and gas stunning devices for light pigs allow maintenance of a small group of animals together, which are gassed at the same time.

In general, stunning with carbon dioxide is not considered a welfare friendly method, as this gas has side-effects for pigs (Velarde *et al.*, 2007; Becerril-Herrera *et al.*, 2009). In particular, Velarde *et al.* (2007) found that the degree of aversion is positively correlated with the carbon dioxide concentration. Conversely, a decrease in the concentration increases the time to loss of consciousness. Therefore, if higher concentrations of carbon dioxide (*i.e.* 90% was tested) are recommended for rapid induction of anaesthesia, it needs to be assumed that this may be more aversive for pigs than lower concentrations (*i.e.* 70% was compared). Troeger & Woltersdorf (1991) suggested that pigs carrying the halothane gene (nn or Nn) are more likely to suffer for changes in the carbon dioxide concentration during the induction phase. However, Italian breeds are almost halothane-free due to the need to avoid PSE (pale soft essudative) meat, which is not compatible with ham production (Accomando, 2010). Therefore the effect of this variable can be excluded.

Regardless of the stunning method used, a fundamental welfare parameter is the short time from stunning to jugulation, to avoid as much as possible any recovery of consciousness. This time can be reduced to a few seconds in Italian systems, where the animal falls individually from the (gas or electrical) stunning chamber to a conveyor belt close to the jugulating personnel. This process might explain why in Italy after stunning, vocalisations and righting reflex were never observed, which is different from other countries where they had an overall prevalence of 0.2% and 1.5%, respectively.

During the consciousness assessment of gas stunned animals, a problem encountered was due to the high percentage of animals that had their eyes closed after stunning, which impaired the performance of the corneal reflex test (these animals were excluded from the evaluation). In this regard, the available literature offers alternative approaches but does not provide enough clarification. According to OIE: '*corneal reflex is difficult to verify and is often confounded with the palpebral reflex, which can be considered a false-positive. Thus, it must not be assessed in isolation*' (OIE, 2015). This interpretation is supported by Atkinson *et al*., (2012), who indicate the risk of recovering consciousness being characterized by the co-presence of four signs (corneal reflex, rhythmical breathing, righting reflex and vocalisations). On the other hand, according to EFSA (EFSA, 2013), effective electrical and gas stunning should lead to abolition of both palpebral and corneal reflex.

2.3. Is sexual maturity a welfare issue?

When reviewing the five freedoms for animal welfare (Brambell, 1965), one specifically requires the performance of 'normal behaviour'. Scientists are still debating what exactly 'normal' means within a significantly abnormal environmental setting such as indoor intensive conditions for pigs. An approach that might be embraced refers (with caution) to the natural conditions of the ancestral species. The importance of rooting material for pigs or nesting material for sows provides a good example, as they essentially exhibit the same behaviour as in wild boars (Spinka, 2017). Therefore, the EU legislation (Dir. 2008/120/EC) requires the provision of materials allowing pigs to satisfy these needs. It is still questionable however, whether all innate behaviours need to be promoted.

Sexual behaviour is innate, instinctually driven, and part of the normal repertoire of a species. When impossible to perform (*e.g.* due to lack of a suitable mate), it can result in severe frustration. In pig rearing, sexual behaviour has to be controlled (*e.g.* in order to avoid aggression between sows) and skilfully managed (*e.g.* in order to stimulate puberty). In fact, only 30 minutes of exposure to males (and to male pheromones) in gilts from 160 days of age can induce the first heat within just a few days (Houpt, 2011). Also, the gilt group housing is able to stimulate puberty, due to the effect of female pheromones (Hemsworth, 1985). The pro-oestrus phase (*i.e.* the phase before oestrus, characterised by ovulation) in gilts results in increased social activity, physical contact, suckling, licking, oral investigation of genitals, flank nosing, and mounting (Pedersen, 2007). In sow pens close to a boar, more than 40 mounting acts within 24h have been observed (Pedersen, 2007). In nature, such motivation seems connected to the need to attract the attention of the boar (Hemsworth, 1985; Pedersen, 2007).

Social stress due to hierarchy seems to also have an influence on sexual mounting in sows (Pedersen *et al*., 1993; Pedersen *et al*., 1998): dominant subjects tend to mount subordinate pen-mates but rarely *vice versa*; moreover, pluriparous pigs mount eight times more than primiparous pigs (Pedersen *et al*., 1998). Mounting may become an animal welfare issue if the risk of skin lesions, injuries and lameness is increased. A high frequency of mounting aggressiveness is a cause of stress, fear and harm, not only for the receiver, but to all pigs in the pen (Rydhmer *et al*., 2006, 2010). In particular, Rydhmer *et al*. (2006) found in light weight pigs (116 kg) that 15% of males and 6% of females suffered lameness or injured legs and feet. Indeed most studies showed a higher frequency of aggressive interactions in males compared with females (*e.g.* 2.6 *vs.* 1 events per pig per hour for Fredriksen & Hexeberg, 2008). In extensive organic production, the mounting frequency was 0.3 events per pig per hour in males and only 0.01 events per pig per hour in females (Thomsen *et al*., 2012). In conclusion, studies on light weight pigs support the notion that sexual maturity is a stress factor for both males and females, although the stress is higher for males.

In order to investigate the issue of mounting in Italian production we did a preliminary study (Garbo *et al*., 2017) of skin lesions in 6,486 gilts and 6,967 barrows from 27 farms. They were scored on three body regions (ears, front, and back) at four weeks of fattening and again before slaughter (24 weeks of fattening), according to the WQ Protocol scoring system for finishing pigs (Welfare Quality, 2009). In a further seven farms, wounds were scored at the abattoir, from the carcasses of 724 gilts and 781 barrows. At 4 weeks of fattening, 81% of animals of either sex had an overall score of 0 (*i.e.* less than five lesions in each body region). In particular, 96% scored 0 at ears and in hind-quarters, while 92% scored 0 in the front. At 24 weeks of fattening, 86% of animals of either sex had an overall score of 0. Ears and hind-quarters were assessed as 0 in 97% of pigs. At the front region, 95% of pigs scored 0. Unexpectedly, this study (which was unpublished and only presented at an international congress) found a small percentage of skin wounds in finishers reared over 160 kg, with no sex effect. No sex differences were detected at the slaughterhouse, although in agreement with previous findings on nine Italian abattoirs (Di Martino *et al*., 2017b), the majority of animals scored 1 (*i.e.* between two and 10 lesions in each body region), most likely due to transportation, lairage and slaughtering operations (Garbo *et al*., 2017).

From a methodological point of view, however, the preliminary study of Garbo *et al*. (2017) did not make the right welfare comparison. If the aim is to assess the effect of sexual maturity on animal welfare you need to be sure that animals have achieved this status (*e.g.* quantify the level of progesterone in the blood); you should also consider a wide variety of indicators (behaviour, stress markers, skin lesions); finally, you need to compare this group with another group of females at the same age with no follicular activity. It is rather inappropriate to compare the welfare outcomes of the same animals before and after the demonstrated sexual maturity. In fact, you cannot exclude the possibility that the variation you find with time is not due to sexual maturity, but is more generally linked with age. Therefore, you need an identical group of females, under the same husbandry conditions, which you are certain have had no follicular activity. This experimental condition is not practical, where it is normally obtained by surgical castration of part of the animals. Differently from males, surgical castration of females is a difficult and dangerous intervention, likely to produce health and welfare consequences (*e.g.* infections, pyaemia, and death) in the short and medium term.

In recent years, the possible use of immunocastration has been shown to be a feasible alternative to surgical castration, by blocking the hypothalamic-pituitary-gonadal axis by means of an autoimmune response against gonadotropin-releasing hormone (GnRH) (Albrecht, 2013). GnRH is a tropic peptide hormone secreted by the hypothalamus which induces the release of follicle-stimulating hormone (FSH) and luteinising hormone (LH) from the anterior pituitary (Kaneko, 2008). In females, FSH acts on granulosa cells to stimulate follicle maturation and to produce oestrogens. Oestrogens induce the release of a LH peak: LH before ovulation stimulates theca cells to ovulate, while after ovulation it stimulates the production of progesterone by the corpus luteum. Sexual hormones produce a negative feedback signal by inhibiting the release of GnRH (Kaneko, 2008).

Immunocastration is obtained by means of two subcutaneous injections of a synthetic antigen which mimics GnRH to induce the response, but has no hormonal activity: the first injection creates sensitisation, while the second guarantees the immune reaction (Albrecht, 2013). Immunity is reached 20 days after the second injection and is maintained for almost 10 weeks (Pinna *et al*., 2015). A third injection is needed in heavy pigs compared to 2 injections in light pigs: this aspect explains why in Italy this drug (Improvac, Zoetis, Spain) is not frequently used as a reliable alternative to surgical castration. Rather, it is used as an emergency procedure either in cryptorchids or in improperly castrated subjects. In Italy there is currently no commercial immune vaccine for females, as Improvac is contraindicated in females. The exact reasons behind this statement on the drug leaflet however are unknown. Nonetheless, the same active ingredient under a different commercial name (Vacsincel, Zoetis, Spain) is available in Spain to guarantee immunocastration in '*cerdo iberico'* females (*i.e.* the traditional Spanish swine breed).

In light male pigs, some studies have investigated the effect of immunocastration on welfare and behaviour (Albrecht, 2013). After the achievement of the immunisation, immunocastrated male pigs showed less aggressive, mounting and sexual behaviours, which occurred at a similar frequency to barrows and females, and was significantly lower than unvaccinated males (Rydhmer *et al*., 2010). Prior to our work, no studies had investigated the effect of sexual behaviour on the welfare of heavy female pigs, by creating a control group of immunocastrated females. At that time, however, particular interest was given to immunocastration in females, as it was thought that the treatment could have an effect on growth performance and fat composition. This hypothesis was supported by similar findings in males (Albrecht, 2013), given that more active and aggressive animals are likely to remain leaner and to grow less.

In my study (Di Martino *et al*., 2017a), I found a reduction in aggressive interactions, haptoglobin, serum cortisol and back lesions at given timepoints throughout the finishing 30 weeks period. All details regarding the trend of these measures are given in Di Martino *et al*. (2017a). Rather, it is reported here the trend of GnRH antibodies and progesterone, attesting the right process of immunocastration (Figure 10a and 10b, respectively).

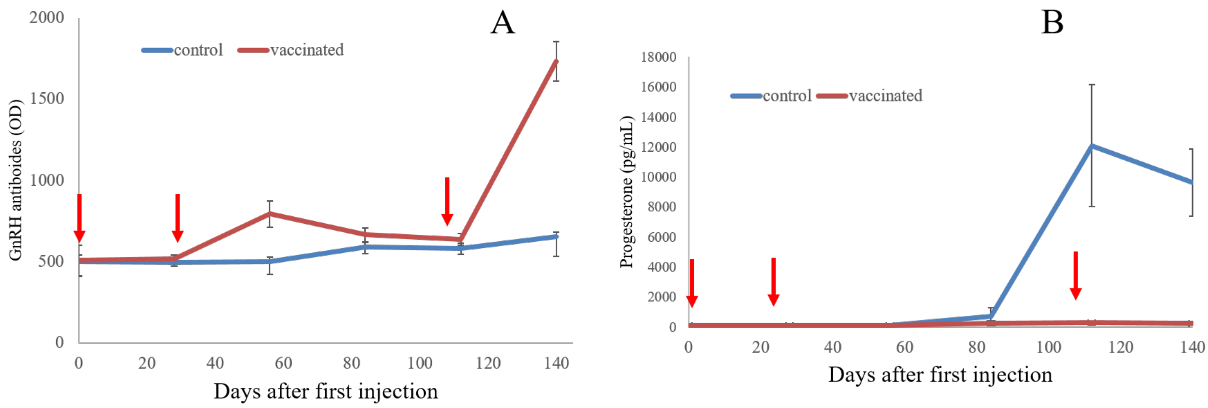

Figure 10. Trend (mean with error bars) of GnRH antibodies (A) and progesterone (B) in immunocastrated (vaccinated) and entire (control) heavy female pigs. Red arrows indicate the vaccine injections at 16, 20 and 32 weeks of age.

No significant effect of immunocastration was found on slaughter performances (growth rate, back fat thickness and feed efficiency). Taken together, these findings suggest a small, yet non-negligible, impact of sexual maturity on the welfare of heavy female pigs. Moreover, the results discourage the possible use of immunocastration as a tool to improve productivity, as proposed in studies on males.

As a final remark, I report here some unexpected and not clearly explainable results regarding ovary histology compared with macroscopic observation. As expected on the basis of progesterone and GnRH antibody titres, during fattening, macroscopic examination of the ovaries at the abattoir showed reduced size, smooth shape and no visible follicles (the typical shape of a pig ovary is similar to a blackberry) (Figure 11). Histologically, however, ovaries from immunocastrated pigs presented some evidence of follicular activity (Figure 12), and also the presence of *corpora lutea* in two subjects. In particular, the cortex of the ovaries showed follicles in different stages of development in both groups (primordial, primary, secondary, tertiary follicles and mature follicle). However, mature follicles in immunocastrated females appeared macroscopically not visible and histologically smaller (0.4-1.7 mm *vs.* 2.2-7.2 mm) compared with control females. To my knowledge, no other study has evaluated ovarian histology in immunocastrated gilts. Previous studies (Zeng *et al.*, 2002; Dalmau *et al*., 2015) reported the absence of macroscopically visible follicles and the presence of some non-respondents. Zeng *et al*., (2002) found two non-respondents characterised by follicular activity, *corpora lutea*, and ovaries not macroscopically different (in weight and shape) from uncastrated females. Dalmau *et al*. (2015) reported the presence of one immunised subject with visibly mature follicles (*i.e.* 8-11 mm of diameter) in Iberian pigs.

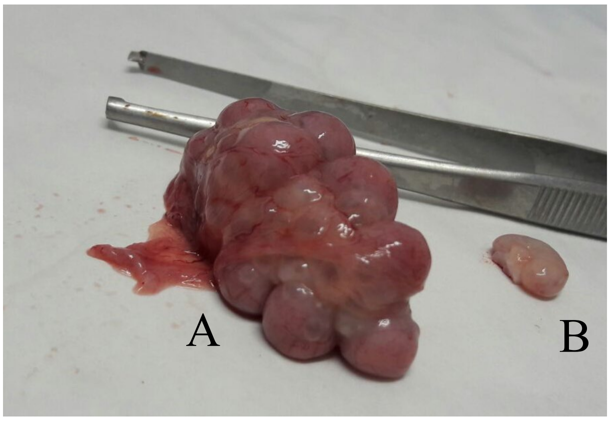

Figure 11. Macroscopic morphology of an ovary from an entire (A) and an immunocastrated (B) female pig. From Di Martino *et al*. (2017a).

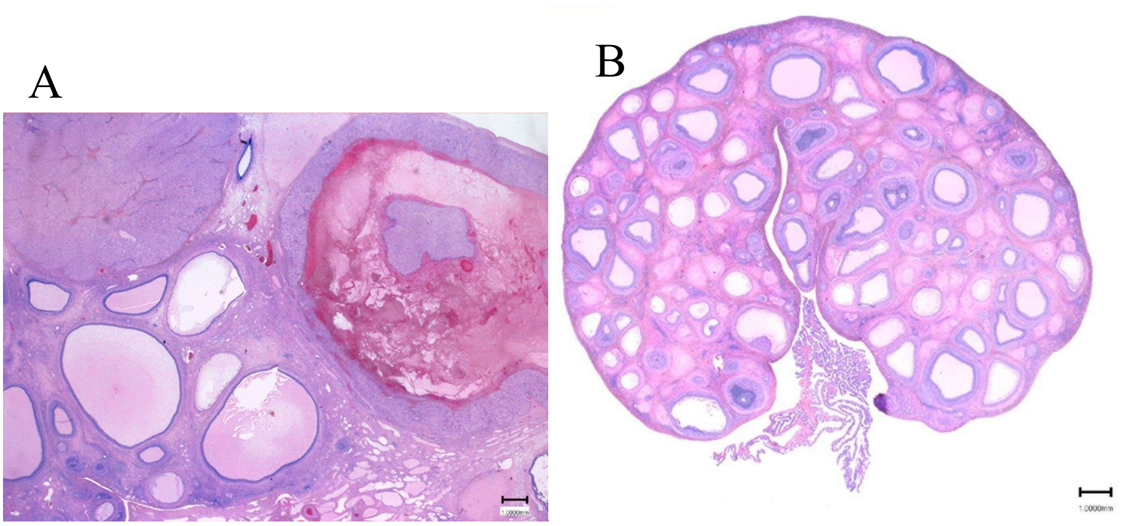

Figure 12. Histological section of an ovary of an entire (A) and an immunocastrated (B) female pig. The scale bar indicates 1 mm. From Di Martino *et al*. (2017a).

3. Conclusions and implications

This thesis has focused on different subject areas related to the welfare of Italian heavy pigs, both in terms of major welfare concerns (the consequences of tail biting and mounting behaviour) and of methodological issues (welfare assessment at slaughter; analytical interference in blood testing due to spurious haemolysis).

The studies discussed have explored the presence of specific criticisms in Italian pig production, with reference to the heavier slaughter weight (160-170 kg) and the prolonged fattening cycle (*i.e.* three months after the achievement of sexual maturity) compared to light pigs (110-115 kg) reared in other European countries. Despite this, when a trial was carried out with undocked heavy pigs, tail biting was found to be problematic mostly at an early age (weaner phase) and under challenging conditions (*i.e.* poor health status).

Tail biting represents, like in other European Countries, one of the major issues in the pig industry. However, the Italian Ministry of Health is currently implementing an action plan to improve farming conditions and reduce the need for tail docking. The published works discussed here had a direct impact on government initiatives and to solicit farmers' preparedness and education.

A low, yet not negligible, welfare impact of sexual maturity was found on heavy female pigs, due to mounting and agonistic behaviours (compared to an immunocastrated counterpart). This outcome may support the use of immunocastration as a tool to reduce aggressiveness in specific situations (*e.g.* in farms with high levels of aggression among pen-mates) or in outdoor production systems.

Small amounts of straw administered by racks were found to be beneficial in terms of reduction of aggressive behaviour and improvement of gastric health. The sensitivity of pigs to oesophago-gastric ulcers in response to environmental stressors has pushed the European Commission to consider gastric lesions a reliable welfare indicator.

WQ protocol for the slaughter phase was been shown to be a feasible and reliable method of assessment. It also highlighted useful margins of improvement for Italian abattoirs (*e.g.* the level of vocalisations in the pre-stunning area). Conversely, most parameters relating to unloading and lairage procedures got a better score compared to other countries, probably due to the higher economic values of heavy carcasses for ham production.

Blood markers were a useful support to welfare evaluation in the different experiments discussed, with particular reference to acute phase proteins. However, spurious haemolysis due to sampling procedures and high swine erythrocyte fragility needs to be taken into account as a potential bias in testing pig blood. The outcomes of Di Martino *et al.* (2015b) can be used to establish the acceptability of the laboratory results for several analytes in relationship with the level of haemolysis.

4. Tables

Table 1. Evaluation of pig reactions at the time of unloading in nine Italian abattoirs.

Abattoir	Fear			Slipping[3]	Falling[4]	Lameness		Density	Ramp	
	Reluctant[1]	Turning back[2]	Tot.			Moderate[5]	Severe[6]	(kg/m^2)	Length (m)	Steep (°)
1	0.0%	4.4%	4.4%	6.7%	0.0%	1.5%	0.8%	209	6	18°
2	0.0%	1.8%	1.8%	3.8%	0.8%	1.9%	0.0%	216	6	18°
3	0.4%	0.4%	0.7%	3.4%	0.4%	1.4%	0.0%	221	2	10°
4	1.8%	0.4%	2.2%	3.5%	2.0%	0.4%	0.0%	232	3	15°
5	1.1%	3.0%	4.1%	0.6%	0.0%	0.0%	0.4%	204	6	15°
6	0.0%	1.5%	1.5%	3.1%	0.5%	0.8%	0.0%	211	3	15°
7	0.8%	0.8%	1.5%	13.2%	1.9%	1.1%	0.0%	215	2	15°
8	1.1%	3.6%	4.7%	6.0%	0.4%	2.1%	0.0%	212	2	10°
9	2.2%	4.6%	6.8%	6.7%	0.7%	1.8%	0.0%	217	2	15°
Mean	**0.8%**	**2.3%**	**3.1%**	**4.9%**	**0.7%**	**1.2%**	**0.1%**	**215**		

[1] n. of subjects that stop and remain immobile for at least 2 seconds when unloading.
[2] n. of subjects that turn back inside the vehicle when unloading.
[3] n. of subjects with loss of balance in one limb without floor contact.
[4] n. of subjects with loss of balance in one limb with floor contact.
[5] movement disharmony with reduction of load on one limb.
[6] movement disharmony with no load on one limb.

Table 2. Evaluation of pig reactions at lairage in nine Italian abattoirs.

Abattoir	Panting	Shivering	Huddling	m^2/pig[1]	n. pigs[1] per drinker[2]	Vocalisations		
						Total[3]	Istantaneous[4]	
							Single	Multiple
1	0.0%	0.0%	1.1%	1.0±0.1	9±4	20	4	0
2	0.0%	0.0%	3.7%	0.8±0.1	18±5	30	1	0
3	0.0%	0.0%	6.4%	1.2±0.3	11±3	33	8	3
4	0.0%	0.0%	4.2%	0.8±0.4	19±6	31	7	1
5	0.0%	0.6%	10.4%	0.8±0.1	28[5]	33	8	0
6	0.0%	0.0%	6.9%	0.7±0.1	20±4	31	4	1
7	0.0%	0.0%	0.0%	0.9±0.1	10[5]	31	4	2
8	0.0%	0.0%	5.5%	0.9±0.1	7±3	32	9	1
9	0.0%	0.0%	0.0%	1.6±0.5	7±2	20	1	0
Mean	**0.0%**	**0.1%**	**4.3%**	**1.0±0.3**	**13±7**	**29**	**5**	**1**

[1] Results as mean ± standard deviation.
[2] All plants except n. 5 and n.7 were provided with nipples.
[3] Sum of vocalisations collected in 3 sections of 4 minutes each.
[4] At second 1-20-40-60 single or multiple vocalisation were recorded. Data given as sum of instants with presence of vocalisations (on a total possible of 48).
[5] n. of pigs per meter of drinking trough

Table 3. Evaluation of pig reactions at jugulation and health conditions post jugulation in nine Italian abattoirs.

Abattoir	Post jugulation				Health status				Skin lesions[1]	
	Corneal reflex	Righting	Breathing[2]	Vocalisations	Pleurisy	Pneumonia	Liver parasites	Pericarditis	1	2
1	2%	0%	17%	0%	20%	13%	5%	0%	82%	3%
2	0%	0%	20%	0%	15%	23%	37%	8%	73%	10%
3	0%	0%	2%	0%	10%	8%	0%	0%	88%	7%
4	0%	0%	3%	0%	30%	3%	17%	7%	77%	5%
5	n.d.	0%	18%	0%	2%	3%	10%	0%	85%	5%
6	n.d.	0%	37%	0%	3%	7%	0%	0%	82%	2%
7	0%	0%	3%	0%	15%	5%	20%	2%	85%	3%
8	0%	0%	5%	0%	18%	3%	2%	5%	82%	10%
9	0%	0%	22%	0%	15%	2%	0%	3%	73%	2%
Mean	**0%**	**0%**	**19%**	**0%**	**14%**	**8%**	**10%**	**3%**	**81%**	**5%**

[1] score 0 was attributed when each body area presented up to one lesion. Score 1 was attributed when each body area presented between 2 and 10 lesions. Score 2 was attributed when each body area presented more than 10 lesions or a lesion with a diameter larger than 2 cm.
[2] two acts or more within 36 seconds.

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6. International publications