LAMENESS IN SOWS: VISUAL ASSESSMENT AND EFFECTS ON MECHANICAL NOCICEPTIVE THRESHOLDS

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List of symbols and abbreviations

CV: coefficient of variation
EFS: Electronic Feeding Station
ESF: Electronic Sow Feeder
e.g.: exempli gratia (for example)
Fig.: figure
g: standard acceleration
i.e.: id est (that is)
Intra-OR: intra-observer repeatability
Inter-OR: inter-observer repeatability
IOR: inter-observer repeatability
MNT: Mechanical Nociceptive Threshold
N: Newtons
SE (SEM): standard error of the mean
SD : standard deviation
VAS: Visual Analogue Scale
tVAS: “tagged” VAS

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Preface

Farm animal welfare is very important for European citizens, who expect that farm animals, as sentient beings, be spared any unnecessary pain and suffering and be better protected than is currently the case (Special Eurobarometer 442, 2015). This concept is reflected in several European laws, not only those that regulate in detail how livestock should be treated, but, most notably, in the Treaty on the Functioning of the European Union (TFEU). Animal welfare is perceived in the European Union as a societal, legislative and moral issue. Article 13 of Title II of the TFEU states that:

"In formulating and implementing the Union's agriculture, fisheries, transport, internal market, research and technological development and space policies, the Union and the Member States shall, since animals are sentient beings, pay full regard to the welfare requirements of animals, while respecting the legislative or administrative provisions and customs of the Member States relating in particular to religious rites, cultural traditions and regional heritage."

All health conditions that can cause acute or chronic pain and that can affect a high proportion of animals in a herd are problematic from an animal welfare point of view. Lameness in sows is one such example. Lameness is the clinical manifestation of a series of locomotion disorders characterised by alterations in the normal gait and posture and by a reduced mobility due to pain or discomfort (Bourne, 2011). It is estimated to affect up to 41.7% of sows in the European Union (range: 8-41.7%; Heinonen *et al.,* 2013; Quinn, 2014). The situation is cause for concern: all the available evidence suggests that in most cases lameness causes pain and discomfort, and that it affects the overall productivity of the sow herd by decreasing average longevity (Anil *et al.,* 2009; Pluym *et al.,* 2013). For these reasons, research efforts are increasingly being concentrated on sow lameness, in an attempt to unravel the underlying pathogenetic mechanisms, the risk factors, the remedial actions, and the welfare consequences for the animals.

Acknowledging that there is a problem is the first step. The next steps will be to develop sensitive and reliable instruments that allow to correctly identify lame animals and to assign a degree of severity to the condition. We also need to better understand the degree of welfare impairment that sows experience when lame, how different degrees of lameness affect their ability to satisfy their basic needs, and beyond that, to live “a life worth living”. The ultimate goal is to develop strategies to prevent and treat lameness on farm, by means of improved breeding, management, and provision of resources such as adapted flooring, rubber mats, bedding, hospital pens, detection technologies, etc. From a scientific perspective these are all important issues that justify the increasing body of literature on this topic. It remains to be seen to what extent the evidence that is accumulating on the overall negative effects of lameness on sow welfare and productivity will influence the way farmers manage their sow herds. Ultimately, on a day-to-day basis, the wellbeing of sows depends on farmers and stockpersons. The example of dairy cattle is quite daunting. The economic losses linked to each individual case of lameness in dairy cattle are widely known; visual scoring methods are readily available; farm veterinarians are trained and willing to provide advice on lameness prevention and treatment. Yet, according to a scientific opinion of the European Food Safety Authority (EFSA, 2009), despite all the research and the increasing awareness on dairy cattle lameness in relation to lost welfare and productivity, no significant improvement in the incidence of lameness has been seen in the past 20 years. The limited success in changing the attitudes and decision-making processes of dairy farmers is partially attributed to poor communication between farm veterinarians and their clients. Veterinarians are often too “directive” in style and should try to show more empathy to farmers, and to establish a relationship of cooperation and engagement (Bard, 2016). More careful and adapted communicative strategies can possibly promote a sense of “being understood” and a more proactive attitude of farmers towards prevention and treatment of dairy cattle lameness (Bard, 2016). Maybe these new communication strategies will help make the case for sows as well. However, the economic aspect of prevention and treatment must not be forgotten. The losses due to lameness and claw lesions in sows are not always directly measurable as is the case for dairy cows. Detection and scoring methods for sow lameness are not nearly as developed and well understood as are those for lameness in dairy cattle. Additionally, the European pig sector is facing a deep and prolonged financial crisis, and global competitiveness issues created a mentality whereby any further investment in welfare is generally viewed as an additional cost that will never be recuperated. Considering all this, some questions arise: will all this research finally have a practical impact on the welfare of sows? Will it be possible to engage farmers in this process? The answers will largely depend on the strength of the societal drive for change in Europe, and on the future global economic landscape for pig meat, which will determine the cost-benefit tradeoff for preventive and remedial actions against lameness.

Chapter 1 - General introduction

Adapted from: E. Nalon, S. Conte, D. Maes, F.A.M. Tuyttens, N. Devillers (2013). Assessment of Lameness and Claw Lesions in Sows. Livestock Science, 156, 10-23.

1.1. Prevalence and consequences of lameness in sows

Lameness is a symptom of pain or discomfort during locomotion associated primarily with a range of underlying conditions affecting the musculoskeletal apparatus of pigs. Housing, flooring, nutrition, group and feeding management, claw health, limb conformation, traumas and infectious diseases can play a role in the development of lameness. The main pathologies that are identified in direct relation with lameness in sows are summarised in **Table 1.1**. As can be seen from the table, claw and foot lesions, one cause of lameness, are also extremely common, with a prevalence varying from 50% to 100% (Anil *et al.,* 2007; Knauer *et al.,* 2007; Enokida *et al.,* 2011; Pluym *et al.,* 2011).

Lameness has several negative direct and indirect consequences on sow performance (Anil *et al.,* 2005; Anil *et al.,* 2009), particularly on longevity (Stalder *et al.,* 2004; Anil *et al.,* 2009; Pluym *et al.,* 2013a), and is now considered as one of the main reasons for the involuntary culling of breeding animals (Anil *et al.,* 2005; Engblom *et al.,* 2008; Kirk *et al.,* 2005; Jensen *et al.,* 2010; Knage-Rasmussen *et al.,* 2014). Additionally, claw lesions on the wall and the sole are associated with lower reproductive performance (Lisgara *et al.,* 2015; Pluym *et al.,* 2012, 2013a; Wilson and Ward, 2012).

Table 1.1. The most common disorders of the locomotor system in adult pigs.

Organ	*Disorder*	*Description*	*Prevalence*
Joints & Cartilage	Infectious arthritis	Inflammation of the joint caused by infection. Most common infections are caused by: *Erysipelothrix rhusiopathiae*, streptococci or *Acranobacterium pyogenes*[1,7]	18% of sows < 18-months old[1] 64% of sows > 18-months old[1] 44.8%†[2]; 8.8%**[3]; 14.3%†[5]
	Osteochondrosis 'Leg weakness'	Non-infectious, degenerative, focal disturbance of endochondral ossification[1,8]	21.9%†[2]; 49.1%**[3]; 27.8%†[4]
	Arthrosis Osteoarthrosis Degenerative joint disease	Nonspecific, degenerative conditions of the cartilage that develops in chronic joint disease. Arthrosis is usually secondary to osteochondrosis[1]	7% of sows < 18-months old[1] 82% of sows > 18-months old[1] 49.1%*[3]; 89.4%†[5]
Bones	Osteomalacia, Osteoporosis, 'Downer sow syndrome'	<u>Osteomalacia</u>: adult form of rickets due to phosphorus or vit. D3 deficiency, causing softening of the bones, which can bend or fracture. <u>Osteoporosis</u>: thinning of the bones caused by excess decalcification or inadequate levels of calcium in the diet, or lack of exercise. Typically occurring during lactation or post-weaning period and can lead to paraplegia or fracture.[1,7]	Up to 30% of first parity sows[7]
	Fracture	Fractures usually occur when an animal struggles to free a leg, falls or fights. It can be secondary to bone disease[1,7]	10.4%†[2]; 10.2%†[5]
Foot & Claws	Foot lesions	Cracks, overgrowth or tear of the different parts of the main claws or the dew claws (side wall, white line, sole, heel, heel-sole junction)[4]	8.3%†[2]; 17.5%**[3]; up to 99%†[4]; 47.2-76.2%†[5]; 86.4%†[6]
	Infectious pododermatis ('foot rot') Septic laminitis ('bush foot')	Infections that can begin within the soft tissues between claws (<u>foot rot</u>) or that is secondary to foot lesion, and can lead to deep necrotic ulcer of the laminae, the coronary band (<u>bush foot</u>) and reach the tendon, the phalangeal bone or the joint. Most common infections are caused by: *Fusobacterium necrophorum*, *Acranobacterium pyogenes*, or spirochetes[1,7]	50-64%*[1]; 21.1%**[3]
	Foot and mouth disease	Highly infectious vesicular viral disease. Clinical signs are lameness, fever, small vesicles on snout, coronary band, teats and between claws[1,7]	North America, majority of South America, Western Europe, Australia, New-Zealand are free of the disease[9] High morbidity, 5% mortality[1]
Muscles	Back muscle necrosis	Exudative necrosis and hemorrhage of back muscles. Part of the porcine stress syndrom[1,7]	Rare*[1]
	Muscle dystrophy	Vitamin E/Se deficiency[1]	Less common*[1]

* prevalence in adult pigs; ** prevalence in lame sows; † prevalence in euthanized and culled sows

References: [1]Dewey, 2006; [2]Engblom *et al.*, 2008; [3]Heinonen *et al.*, 2006; [4]Jørgensen, 2000; [5]Kirk *et al.*, 2005; [6]Knauer *et al.*, 2007; [7]Muirhead and Alexander, 2002; [8]Ytrehus *et al.*, 2007; [9]OIE (www.oie.int).

Besides the negative effects in terms of farm profitability, lameness has several potential adverse consequences on animal welfare. Depending on the origin of the condition, acute or prolonged pain will constitute a first challenge. The physiology of animal pain is a complex field of research, but there is increasing interest in investigating the short and longer-term effects of pain on animal welfare. Livestock species in particular often suffer from conditions (e.g., lameness, mastitis) that can have a long duration and that may remain undetected. For instance, sheep and cattle affected by lameness can develop hyperalgesia, a heightened sensitivity (or, in other words, a lower threshold) to pain that can persist after the cause of the original injury has subsided (Whay *et al.,* 1995; Laven *et al.,* 2008). Another potential consequence of the presence or persistence of pain can be a limitation in the ability of an animal to meet its basic needs. Lameness does not only affect pain perception, but is also capable of modifying sow behaviour. Parsons *et al.* (2015) measured several behaviours before and after chemically inducing lameness (synovitis) in a group of multiparous, non-pregnant sows. Lame sows decreased the frequency of drinking, standing, lying sternal and being in the drinker location compared to baseline. In parallel, there was an increase in lying lateral, regardless of side. In another experiment, Pairis-Garcia *et al.* (2015) showed that meloxicam can mitigate pain caused by induced synovitis, as shown by a higher frequency of standing and a lower frequency of lying in treated sows compared to saline-treated sows. Based on the recording of postural time budgets, Grégoire *et al.* (2013) also found that lame sows spend more time lying down than non-lame sows. Bos *et al.* (2015) used a feed reward collection test to investigate the extent to which the mobility of multiparous sows is affected by different degrees of lameness. During a 15-min session, sows with varying degrees of gait abnormality had to walk a predefined distance (9.3 m) to get a palatable feed reward, and the outcome variable was the number of rewards per testing session. The authors found that lame and severely lame sows collected fewer rewards than non-lame and mildly lame sows, indicating that the former could experience a reduced access to valuable resources, especially during gestation, when they are housed in groups. Although not yet fully investigated in naturally occurring cases of lameness, it is clear that these behavioural changes can have negative effects on the sows' health, welfare and productivity.

Many studies investigated the prevalence of lameness in breeding sows, with results that vary depending on the specific study design and management system. **Table 1.2** presents an overview of some of the published studies on sow lameness prevalence carried out in the European Union in the past ten years. As can be seen from that overview, the reported prevalences in studies involving ten commercial herds or more vary from 8.8% (Heinonen et al, 2006, Finland) to 41.7% (Quinn, 2014, Ireland). These striking differences likely depend on several factors including genetics, housing, management system, parity, reproductive stage, and scoring methodology. Recently, some authors reported that group housing of pregnant sows – compulsory by law in the EU since January 2013 – increases the risk of lameness (Calderón Díaz *et al.,* 2014; Maes *et al.,* 2016). The increased incidence of lameness in group-housed sows is primarily attributed to a higher frequency of agonistic interactions among unfamiliar animals around the time of mixing. Apart from group housing, many factors can play a role in increasing or limiting the risk of lameness, (Maes *et al.,* 2016; Bos *et al.,* 2016). These factors and their interactions are not well investigated so this is a growing and important area of research for sow welfare.

One important prerequisite to investigate the physiological and behavioural aspects of sow lameness is to have reliable gait assessment methodologies. This is also critical for several other reasons. Firstly, there is a requirement for good reliability both in research settings and within farm welfare assessment or assurance schemes (Main and Green, 2000; Mullan *et al.,* 2011; Welfare Quality®, 2009). Secondly, reliable scales make it possible to know the real incidence and prevalence of lameness and to verify the impact of corrective or preventive measures. Thirdly, the availability of reliable methods has the potential to increase awareness about locomotion disorders (Whay, 2002), thus possibly leading to a higher treatment rate and better preventive measures.

The sections that follow present an overview of the current techniques for lameness assessment in sows. The available methodologies are described according to the biomechanical component studied: gait, postural behaviour or weight distribution. In the second part, the concepts of nociception and hyperalgesia are explained, with specific examples taken from the literature on hyperalgesia due to lameness in livestock. Finally, some of the potential implications of hyperalgesia on animal welfare are briefly discussed.

Table 1.2 Studies published in the last 10 years on lameness prevalence in grouped housed sows and gilts in the European Union**.**

Reference	***Country***	***Mean prevalence***	***Type of study***	***N. of herds and animals***	***Flooring***	***Bedding***	***Scoring system***
Heinonen *et al.* (2006)	FI	8.8%	Cross-sectional	21 herds (n= 646)	- Slatted -Partially slatted -Solid	Not in all systems (when present: different types and variable quantities)	Verbal rating scale (3 levels)
Kilbride *et al.* (2009)	UK	11.8% maiden gilts 14.4% pregnant gilts 16.9% pregnant sows	Cross-sectional	88 herds (n=2411)[1]	- Outdoor paddocks - Solid concrete floors - Partly slatted floors - Fully slatted floors	- Straw - No bedding	Ordinal (0-5, based on Main *et al.,* 2000)
Jensen *et al. (*2010)	DK	29.1% across parities (14.3% severe)	Cross-sectional	34 herds (n=2989)	N.A.	N.A.	Verbal rating scale (3 levels)
Pluym *et al.* (2011)	BE	9.7% across parities	Cross-sectional	8 herds (n=421)	N.A.	N.A.	Binary (lame/non lame)
Pluym *et al.* (2013a)	BE	5.9% across parities	Longitudinal	5 herds (n=491)	- Partly slatted, concrete	No	Binary (lame/non lame) based on definitions of Welfare Quality®
Calderon Díaz *et al.* (2013)	IRL	*Gilts et al. (start of study)* 30% (CON)-34% (RUB) *2nd parity sows* 44% (CON)-45%	Longitudinal	1 herd (n=164)	- Fully slatted,concrete (CON) - Fully slatted, rubber covered (RUB)	No	Binary (lame/non lame; originally based on 0-5 ordinal system of Main *et al.,* 2000)

Table 1.2 Studies published in the last 10 years on lameness prevalence in grouped housed sows and gilts in the European Union**.**

Reference	*Country*	*Mean prevalence*	*Type of study*	*N. of herds and animals*	*Flooring*	*Bedding*	*Scoring system*
Knage-Rasmussen *et al.*, (2014)	DK	24.3% across parities	Cross-sectional	44 herds, of which 36 loose-housed (n=1304)[2]	N.A.	N.A.	Binary (lame/non lame)
Quinn (2014)	IRL	38.9% replacement gilts 41.1% pregnant gilts 41.7% pregnant sows	Cross-sectional	68 herds (1 pen/herd) replacement gilts n=525 pregnant gilts n=518 pregnant sows n=604	- Solid - Partially slatted - Fully slatted	No bedding	Binary (lame/non lame; originally based on 0-5 ordinal system of Main *et al.,* 2000).
Willgert *et al.* (2014)	UK	4.5% outdoor reared 4.7% indoor reared	Cross-sectional	76 herds (n=1520)	- Solid - Partly slatted - Fully slatted	- Straw/wood shavings - No bedding	Binary (lame/non lame)
Bos *et al.* (2016)	BE	*d 50 gestation* 30.5% (concrete) 16.8% (rubber coated) *d 108 gestation* 35.6% (concrete) 22.7% (rubber coated)	Longitudinal	1 herd (6 groups of 21±4 sows)	- Partly slatted, concrete - Partly slatted, with rubber covered slats + rubber mats covering 50% of each lying area	- No bedding	Continuous tagged visual analogue scale (tVAS) as described in Chapter 2

N.A. = information not available

[1] Only the data relative to maiden gilts, pregnant gilts and pregnant sows are reported here. The study also included 1,623 finishing pigs.

[2] Only the data relative to sows raised in conventional farms are reported here. The study also included data on lameness prevalence in sows raised in 9 organic farms.

[3] Only the data relative to group-housed sows are reported here. The study also included data on lameness in a group of stall-housed sows

1.2 Lameness assessment based on gait and postural variables

In all species, the major difficulty of lameness assessment lies in its multidimensional nature. Lameness is often detected and studied by observing gait. The simplest locomotion scoring methods rely on the ability of trained observers to visually identify various degrees of deviation from a “normal” gait. However, some authors reported only limited reliability with visual scoring systems (Channon *et al.,* 2009; D'Eath, 2012; Main *et al.,* 2000). Depending on the underlying pathologies, postural behaviour and weight distribution between the limbs can also be affected. Automated lameness detection can detect postural and weight distribution changes in static and dynamic conditions: commercial solutions are already available for the dairy sector (StepMetrixTM, BouMatic, Madison, WI, US) and advancements are being made towards solutions for the pig sector (Pluym *et al.,* 2013b; Sun *et al.,* 2011, Stavrakakis *et al.,* 2013, 2015a, 2015b).

1.2.1 Visual locomotion scoring

Locomotion scoring is the evaluation of an animal's ability to walk normally. There is agreement in the literature on the typical and most frequent signs of lameness in pigs: these include reluctance to move, reduced walking speed, shorter or uneven stride length, vocalisations (squealing), swaying from side to side (Grégoire *et al.,* 2013; Karlen *et al.,* 2007; Okholm Nielsen, 2011). Head bobbing may be present but, in contrast to cows, it is considered generally difficult to identify due to the short necks of pigs, which limits the vertical movement of the head (Main *et al.,* 2000). A hunched posture (arched back) can sometimes be observed (Grégoire *et al.,* 2013). In severe cases, there is minimal or no weight bearing on the affected limb(s) or the animal can refuse to move (Main *et al.,* 2000). Postural variables such as asymmetric stance, fore or hind limbs turned out or stiffness can also be observed and scored while assessing locomotion, and are correlated with elbow and knee joint lesions (Kirk *et al.,* 2008). While in dairy cattle some locomotory signs, such as back arch, head bob, tracking up or reluctance to bear weight are associated with specific hoof pathologies (i.e. sole ulcers; Flower and Weary, 2006), no clear associations between locomotion traits and specific claw lesions in pigs have been identified so far. As a consequence of pain, lame animals can alter their social behaviour; the scoring system developed by Main *et al.* (2000) takes this into account and includes behavioural variables, such as inquisitiveness and level of activity within the group, in the total lameness score. Visual locomotion scoring can be carried out by means of live observation or analysis of video clips. Scores are typically assigned either on binary scales (yes/no), ordinal scales or continuous scales.

Ordinal scales

Ordinal scales are reportedly easier to learn and use than other types of scales (Hughes, 2008). However, due to the limited number of categories and descriptors, there is the risk to oversimplify, forcing the user to choose a wording or a category that may not correspond to the real degree of the observed condition. In fact, conditions such as pain or locomotion impairment vary in a continuous rather than in a discrete manner, and consequently the use of ordinal scales creates an arbitrary classification not necessarily based on agreed cut-offs between categories. In turn, this can lead to confusion and misclassifications on the part of observers (Hughes, 2008). Additionally, ordinal data require non-parametric statistical analysis, but as the measured variable (i.e., lameness) varies along a continuum and not along categories, this results in loss of statistical power (Hughes, 2008).

The ordinal scales described in the literature for visual locomotion scoring in pigs and sows have a number of categories that varies from 2 (yes/no; binary scales) to 10 (see **Table 1.3** for an overview of published scales). A low number of categories is sometimes used when an accurate description of the degree of lameness is not the main focus of investigation (Heinonen *et al.,* 2006; Mullan *et al.,* 2011), for example in studies that focus on prevalence or incidence rather than severity, or in welfare assessment standards, which typically combine several indicators of animal welfare problems (RSPCA, 2012; Welfare Quality©, 2009). Although existing scoring systems largely agree on the clinical signs of lameness, few of them have been tested for repeatability. Consistency of scoring, both inter- and intra-observer, is essential for the correct identification of individual animals requiring treatment (Mullan *et al.,* 2011) and for the assessment of the effectiveness of preventive of remedial actions. In addition, the successful implementation of farm animal assurance schemes heavily depends on the assessors' reliability because non-compliance with the established standards can lead to a negative audit results (D'Eath, 2012; Mullan *et al.,* 2011). Inter-observer repeatability (IOR) is reported for some locomotion scoring scales for growing pigs and sows. Some authors considered the found IOR to be moderate (D'Eath, 2012; Main *et al.,* 2000). A number of factors play a role in determining IOR, such as the professional profile of the assessor, (e.g., animal technician, farmer, veterinarian, researcher; D'Eath, 2012), the prevalence of the condition (which determines the degree of exposure of the assessors to it; Mullan *et al.,* 2011), the familiarity with the scoring system (D'Eath, 2012;

Main *et al.,* 2000; Mullan *et al.,* 2011), the testing situation (e.g., lighting conditions, stocking density; Petersen *et al.,* 2004) and the number of animals scored within a session (Geverink *et al.,* 2006). Periodic re-training of the assessors is recommended to reach and maintain an acceptable level of IOR (D'Eath, 2012; Main *et al.,* 2000). Whenever the performance of the assessor is evaluated against the scores of an expert or trainer, which are then considered as a “gold standard”, it is important that the reliability of the scoring within and between trainers is also tested (Mullan *et al.,* 2011). The use of few categories is proposed as a method to increase inter-rater agreement, i.e., the likelihood that two or more observers give exactly the same score (Channon *et al.,* 2009; D'Eath, 2012). However, unlike IOR, perfect agreement does not take into account the degree of disagreement when observers give different scores. Additionally, besides reducing resolution, using a low number of lameness categories does not necessarily improve IOR (Tuyttens *et al.,* 2009). Even with a “lame/non-lame” categorisation it is possible to obtain what can be considered only a moderate IOR (Petersen *et al.,* 2004).

Table 1.3. Published locomotion and lameness scoring systems for pigs.

Scoring system	*Scores*	*Observed characteristics*	*Comments*
Dewey *et al.* (1993)	0 1 2 3 4 5 6 7 8 9	Normal gait stiff gait: mild stiff gait: moderate stiff gait: severe lame: mild lame: moderate lame: severe requires assistance to stand and then can walk can stand with assistance but then falls cannot stand (even) with assistance	Sows. Study on the clinical and post-mortem examination of sows culled for lameness.
Main *et al.* (2000) – schematic	0	Bright, alert, responsive, inquisitive; active in group; stands squarely on all fours; even stride; easy accelerations and turnings	Finishing pigs. Assessment of the repeatability of a scoring system integrating different parameters besides gait, such as the behaviour in the group, the response to human presence and the posture while standing.
	1	Everything as in score 0 but abnormal stride length and movements no longer fluid (stiffness)	
	2	May show mild apprehension to boisterous pigs; uneven posture; shortened stride; lameness detected; caudal swagger; still agile	
	3	Bright but less responsive; last to leave the pen; apprehensive to boisterous pigs (remains separate from group activity); uneven posture; unwilling to bear weight on affected limb; shortened stride; caudal swagger; will still trot and gallop	
	4	May be dull; unwilling to leave familiar environment; will try to remain separate from other pigs in the group; affected limb elevated off floor while standing; visibly distressed; pig may not place limb on floor when moving	
	5	Dull and unresponsive; not leaving the group; may appear distressed by other pigs in the group but unable to respond; will not stand unaided; does not move	
Geverink *et al.* (2006)	0 1 2 3	Normal gait Difficulties walking, but still using all legs Severely lame, minimum weight-bearing on affected limb Non weight-bearing on the affected limb or unable to walk	Finishing pigs and breeding sows. Study on the repeatability of a scoring system for on-farm pig welfare monitoring.
Karlen *et al.* (2007) schematic	0	Normal ability to stand and move; symmetrical limb movements	Sows. Lameness scoring system integrated with other measures to compare the welfare of
	1	Normal ability to stand and move; legs bearing weight similarly but compromised movement	
	2	Moderately lame: obviously reduced ability to stand; movement diminished or difficult;	

Table 1.3. Published locomotion and lameness scoring systems for pigs.

Scoring system	*Scores*	*Observed characteristics*	*Comments*
	 3	unwillingness to bear weight on affected leg(s); frequent weight shifting Severely lame: compromised ability to stand and move; one or more non-weight-bearing limbs; (swollen joints); stiffness; frequent vocalizations if made to move.	sows housed in conventional stalls vs. in groups on deep litter.
Kilbride *et al.* (2009)	0 1 2 3 4 5	Even strides. Caudal body sways slightly while walking. Pig is able to accelerate and change direction rapidly. Abnormal stride length (not easily identified). Movements no longer fluent: pig appears stiff. Pig still able to accelerate and change direction. Shortened stride. Lameness detected. Swagger of caudal while walking. No hindrance in pig's agility. Shortened stride. Minimum weight-bearing on affected limb. Swagger of caudal body while walking. Will still trot and gallop. Pig may not place affected limb on the floor while moving. Does not move.	Finishing pigs, gilts and pregnant sows. Cross-sectional study on the prevalence of lameness in association with limb lesions and floor types in commercial farms.
Welfare Quality® (2009) protocol for sows	0 1 2	Normal gait, or the animal has difficulties walking but is still using all its legs, the stride may be shortened and/or there may be a swagger of the caudal part of the body when walking The animal is severely lame; it resists bearing weight on the affected limb There is no weight bearing on the affected limb or the animal is unable to walk	Sows, piglets and finishing pigs. Lameness evaluation at the farm level (part of an overall protocol).
ZinPro Corp. (2009)	0 1 2 3	Sow moves easily with little inducement. She is comfortable on all her feet. She moves relatively easy, but visible signs of lameness are apparent in at least one leg. She is reluctant to bear weight on that leg but still moves easily from site to site in the barn. Lameness is involved in one or more limbs. The sow exhibits compensatory behaviours such as dipping her head or arching her back. There is a real reluctance to walk and bear weight on one or more legs. It is difficult to move her from place to place on the farm.	Sows. Functional tool for the early detection of foot disorders and lesions, monitoring prevalence of lameness in a herd, comparing the incidence and severity of lameness between herds and identifying individual sows for functional claw trimming.
Mustonen *et al.* (2011)	0 1 2 3 4	None: no lameness Minimal: stiff, ataxic or swaying gait, shortened stride Slight: limp visible, but animal unconcerned and exercises normally Moderate: obvious limp present all the time (with head bobbing), animal having some difficulty with exercise, moderate kyphotic posture Severe: animal barely weight bearing/not weight bearing, severely lame but able to move, severe kyphotic posture	Sows. Study on the effectiveness of ketoprofen in the treatment of non-infectious lameness.

Table 1.3. Published locomotion and lameness scoring systems for pigs.

Scoring system	*Scores*	*Observed characteristics*	*Comments*
D'Eath (2012)	0	Normal: even strides, rear end sways slightly while walking, rapid acceleration and change of direction. Stands normally.	Sows. Study on the consistency over time, effect of sow characteristics and inter-observer reliability of repeated locomotion scoring at the herd level.
	1	Stiff: abnormal stride length, movements no longer fluent, pig appears stiff. Still able to accelerate and change direction. Stands normally.	
	2	Slight lameness: shortened stride, lameness detected, swagger or rear end while walking, no hindrance in pig's agility. Uneven posture while standing.	
	3	Lame: pig slow to get up (may dog sit), shortened stride, minimum weight-bearing on affected limb (standing on toes), swagger of rear end. May still trot and gallop	
	4	Limping: pig reluctant to get up, holds limb off floor while standing, avoids placing affected limb on the floor while moving	
	5	Downer: pig unresponsive, does not move and struggles to stand when encouraged to do so	
Grégoire *et al.* (2013)	1	Sow walks with even strides and no gait problem is observed	Sows. Study on the validation of quantitative techniques for the assessment of lameness.
	2	Abnormal stride length is detected. Movements are no longer fluent but no obvious lameness is detected	
	3	Stride is shortened and lameness is detected. Swagger of caudal body is noticed as sow walks	
	4	Sow does not place affected limb on the floor	
	5	Sow is unable to move.	

Continuous scales: the VAS

A visual analogue scale (VAS) is typically a horizontal line, usually 100 mm in length, anchored at the opposite ends by word descriptors such as, for instance, “perfect gait” and “downer animal” (**Fig. 1.1**). Observers indicate the score based on their evaluation of severity of the condition. The score is determined by measuring the distance in mm from the left hand end of the line to the point that the observer marks (Gould *et al.,* 2001). VASs are commonly used to rate the severity of a variety of diseases in human medicine, and have consistently proven to be reliable and sensitive in measuring parameters that are challenging to quantify and intrinsically subjective in nature, such as anxiety, pain, or other feelings (Hughes, 2008; Rausch and Zehetleiner, 2014). These parameters are also typically described as varying along a continuum rather than in a step-wise manner. VASs offer an unlimited discriminative range to observers and contain a greater amount of information compared to ordinal scales with few categories (Rausch and Zeheteleiner, 2014). For these reasons, the use of VASs is now common in veterinary medicine as well. For instance, an adapted version of a VAS, called DIVAS (Dynamic Interactive Visual Analogue Scale, part of a more composite assessment which includes palpation of the animal) is now frequently used, alongside other methods, to assess pain in veterinary patients (Barletta *et al.,* 2016; see also the Colorado State University canine acute and chronic pain scales[1]).

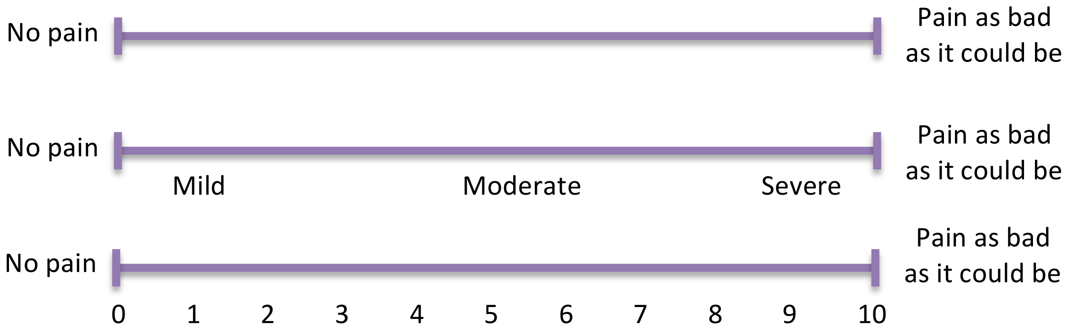

Figure 1.1. Examples of different types of visual analogue scale used by human patients in clinical settings to report their degree of pain. The scale can be blank (top), but descriptors (middle) or numbers (bottom) can be added to guide patients in their choices. Adapted from: http://www.nature.com/nrrheum/journal/v3/n11/full/ncprheum0646.html. (accessed 20.11.2016).

By contrast, the use of VASs to assess gait and lameness in farm animals is infrequent, although some examples are described in research studies on dairy cattle (Borderas *et al.,* 2008; Flower and Weary, 2006; Flower *et al.,* 2008; Tuyttens *et al.,* 2009). One of these

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[1] http://csu-cvmbs.colostate.edu/Documents/anesthesia-pain-management-pain-score-canine.pdf

studies comparing the performance of a “tagged” VAS versus a 3-point ordinal scale even found a better intra- and inter-OR with the former (Tuyttens *et al.,* 2009). Another advantage of measuring gait on a VAS is that the generated data are continuous and, if normally distributed, can be analysed with parametric statistics, which have more statistical power (Hughes, 2008). On the other hand, VASs typically require explanations to be used correctly, as they are believed to require greater cognitive skills than ordinal scales (Hughes, 2008). If the assessor is relatively inexperienced with the tool or if the descriptors at the opposite ends of the scale are not clear, confusion may occur (Benito-de-la-Víbora *et al.,* 2008). In order to limit this type of variation, descriptors can be “mapped” onto the VAS; this helps to make the evaluation of lameness severity more accurate (Fuller *et al.,* 2006). Mapping anchor points to visual analogue scales has been proven to facilitate pain reporting in human patients. For descriptors it remains important to disambiguate the exact meanings, as these may be unclear to users and influence their interpretation and scoring (Aicher *et al.,* 2012; Hughes, 2008). The study described in **Chapter 3** was the first to develop and test such a scale for locomotion scoring in pigs.

1.2.2. Kinematics and footprint analysis

Kinematic analysis quantifies the features of gait in the form of time-related, linear (distance-related) and angular measurements that describe the movements of the body segments and joint angles (Clayton and Schamhardt, 2001). This can be obtained by fixing a series of reflective markers at pre-determined locations and filming the animal's gait (**Fig. 1.2**). Various parameters can be simultaneously analysed by means of automatic tracking software (videographic or optoelectronic systems). However, kinematics is a complex technique with many critical points. Exact software calibration is required to ensure the correct calculation of the distances (Ceballos *et al.,* 2004). Placement of the markers is also critical and should be repeatable: markers can be difficult to place on some joints or bone prominences such as the hip or scapula. In addition, skin movements can displace markers during the recording (Bobbert *et al.,* 2007; Kim *et al.,* 2011). In most cases, each side of the animal has to be evaluated separately. This implies walking the animal at a steady pace and constant speed and may necessitate habituating the animal to the set up. This is the reason why in some species, such as horses and cows, a treadmill has been used to standardise gait speed (Keegan *et al.,* 2000; Meyer *et al.,* 2007). Another solution is to video record both sides at the same time with two cameras or to use a 3-dimensional kinematic system (Chateau *et al.,* 2001). Recent research studies have tried to identify “iceberg” kinematic indicators that can reliably detect lameness in pigs using simplified techniques.

Figure 1.2. Positioning of reflective markers at various anatomical landmarks in a pig (top) prior to kinematic gait analysis (bottom). Source: Newcastle University/Prairie Swine Centre.

Abnormalities in the movement of the pig's body during walking are associated with lameness, and in particular the vertical movement of head and neck is most affected, with an increase of +15–58 mm in lame compared to normal pigs (Stavrakakis *et al.,* 2013, 2015a). Stavrakakis *et al.* (2015b) tested the validity of the Microsoft Kinect™ sensor for the assessment of normal gait in pigs. The Microsoft Kinect™ is a line of motion sensing devices originally developed to enable videogame users to interact with their consoles with their body movements. The authors found that the Microsoft Kinect™ device – programmed with a custom-built algorithm - can distinguish sound from lame pigs by tracking the elevation of the neck region (tagged with a large reflective marker) during walking. Marker-free tracking is less reliable and will require further research (Stavrakakis *et al.,* 2015b). Another technique that holds some potential is high-speed bi-planar fluoroscopic kinematography, with or without reflective markers (FluoKin[2]). This technique was originally developed for companion animals in the laboratory of P. Boetccher and C. Mülling of Leipzig University (Germany), but it also enables precise studies and analysis on claw floor interaction in live pigs. One pilot study on locomotion patterns in pigs indicates that the main claws undergo high mechanical challenges during footing, with clearly visible significant concussions in the distal limbs (Pig Progress, 2016). Another critical phase is the roll over just before taking off the claw from the ground. Here the tip of the toe, where there are no protecting fat cushions, is exposed to high mechanical stress. The FluoKin analysis so far supports the hypothesis that the majority of claw lesions observed have primarily mechanical causes or a strong mechanical component in their development (Pig Progress, 2016).

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[2] http://www.fluokin.de/

As an alternative to kinematic analysis by means of reflective markers, pressure-sensitive runways that record and analyse kinematic gait variables in real time were developed for cows (Maertens *et al.,* 2011; Van Nuffel *et al.,* 2009; **Fig. 1.3**). Maertens *et al.* (2011) reported that the Gaitwise system could identify lame cows with an overall sensitivity of 76–90% and a specificity of 86–100% when compared to the gait scores obtained by observers, which were used as silver standards. Similar applications were also studied in pigs, and the recorded parameters, such as stride length, walking speed, swing and stance time, foot height and joint angles, were used to determine kinematic "patterns" that are indicative of gait abnormalities (von Wachenfelt *et al.,* 2008). Although currently restricted to research settings because of the time required to set up the procedure, this technique was successfully applied to the study of the impact of different floor conditions (clean, wet, greasy or rubbery) on pig gait (Thorup *et al.,* 2008; von Wachenfelt *et al.,* 2010). Kinematics already showed some potential as an instrument for the identification of lameness in sows (Grégoire *et al.,* 2013). Similarly to dairy cattle (Blackie *et al.,* 2011; Van Nuffel *et al.,* 2009), gait components such as stride length, stance time and walking speed change significantly depending on the degree of lameness as determined by visual gait scoring. Recently, measurement of gait asymmetry by pressure mat analysis was sensitive enough to detect the analgesic effects of buprenorphine when used to treat moderate to severe clinical lameness in young pigs (Meijer *et al.,* 2015). In another recent study using the GAITFour pressure mat walkway system, Pairis-Garcia *et al.* (2015) found that flunixin meglumine and meloxicam were effective in mitigating pain sensitivity in sows after lameness induction.

Figure 1.3. Pressure sensitive runway developed by Van Nuffel *et al.* (2009) to study gait in dairy cattle.

Footprint analysis is another method that makes it possible to study the positioning of the four feet simultaneously and to quantify parameters such as the foot area in contact with the ground and the distance between contralateral feet. Many methodologies for footprint analysis were used in various species, including inking or painting the feet and the use of photosensitive or carbon paper (Buddle *et al.,* 1994a). Techniques to record videos from below a see-through floor were also studied (DigigaitTM, CatwalkTM). However, these methodologies are not always adapted to the pig because of its size, weight and relative difficulty of handling. The use of a corridor with a clay-covered floor was tested to obtain sow footprints (Grégoire *et al.,* 2013), but no relationship between the footprint pattern and the degree of lameness (scored visually) could be established. According to the authors, this was most likely due to the slipperiness of the clay, which modified the sow's gait (Thorup *et al.,* 2008). Therefore, the type of flooring used to record footprints should be carefully chosen to avoid interference with gait measurements.

1.3. Lameness assessment based on activity patterns

1.3.1. Postural time budget

Postures are often used in animal behaviour studies to evaluate overall activity or more specific behaviours like maternal abilities in sows (Pedersen *et al.,* 2003). In particular, postures can be used to evaluate comfort of housing systems for gestating sows (Anil *et al.,* 2002; Tuyttens *et al.,* 2008). The measure of the time spent standing is particularly interesting, because lame sows having difficulty to stand up or with a painful limb would reduce their time spent standing. Many studies found a direct relationship between postures and locomotion disorders. Using photocells to record standing and lying postures of tethered sows, Cariolet and Dantzer (1984) and Madec *et al.* (1986) found that time spent standing was decreased in sows with severe claw conditions or lameness. Buddle *et al.* (1994b) investigated two criteria related to postural behaviour at the sow and the group levels: latency to lie down after morning feeding and percentage of sows still standing one hour after feeding. They found that lame sows spent less than half the time standing after the meal than non-lame sows (28 vs. 71 min). These results were confirmed by Grégoire *et al.* (2013) with a latency to lie down after feeding of 33 vs. 49 min, and a percentage of time spent standing over 24 h of 6.3 vs. 14.5% for lame and non-lame sows, respectively. Technically, postural time budget is traditionally measured using direct observation or video recording followed by scan sampling. These methods are reliable to detect changes in posture due to lameness (Whalin *et al.,* 2016), but they have the disadvantage of being time consuming. To try and overcome this problem, automated posture recording systems were developed in sows. Using one accelerometer fixed to a rear limb (**Fig. 1.4**). Ringgenberg *et al.*

(2010) were able to determine the percentage of time spent in the standing position in gestating and lactating sows with a sensitivity of 99.5% and a specificity of 99.7% (this was calculated by means of the degrees of tilts of the vertical axis, recorded at a 5-s rate). Accelerometers may also potentially discriminate and record the different activities of sows (such as sitting, lying, or walking), but correct recognition of activities remains difficult because most active behaviours have similar acceleration patterns (Cornou and Lundbye-Christensen, 2008). Traulsen *et al.* (2016) recently developed ear tags with sensors to automatically record acceleration data and activity patterns in group-housed sows. The authors found that the positioning and acceleration measurements obtained with these ear sensors can be used to describe the activity patterns of the sows. They concluded that – pending further refinement of the methodology – these data could be used in the future to automatically detect lameness in group-housed sows based on a change in the activity patterns of each individual animal.

Figure 1.4. Positioning of the accelerometers to measure postural time budget and posture changes in sows. Reproduced from Ringgenberg *et al.* (2010).

1.3.2. Posture change

Many studies focused on posture changes, especially standing up and lying down, to study housing comfort or lameness. Posture changes are usually observed visually and can be measured using categorical variables (Grégoire *et al.,* 2013) or continuous variables such as the time taken to change posture (Anil *et al.,* 2002; Marchant and Broom, 1996), the frequency of posture changes per day (Anil *et al.,* 2002; Cariolet and Dantzer, 1984) and the frequencies of various behaviours while in motion, such as slips, stops, stepping, and uncontrolled movements (Bonde *et al.,* 2004). In theory, posture changes are influenced by lameness, especially when caused by pathologies affecting the joints or involving a high degree of pain. However, many environmental factors can interact in determining posture change patterns, such as the floor type and slipperiness (Elmore *et al.,* 2010), the space available for movement (Marchant and Broom, 1996), or the level of reactivity to humans (Clouard *et al.,* 2011; Kilbride *et al.,* 2009). Observing voluntary posture changes can be time consuming, as some specific movements may occur as little as 10 times per day per pig (Anil *et al.,* 2002; Cariolet and Dantzer, 1984). On the other hand, standing up can be stimulated, but the efficiency of the stimulus is critical and can vary between sows, especially in function of their reactivity to humans (Buddle *et al.,* 1994a, Grégoire *et al.,* 2013). Indeed, the percentage of sows that do not stand up after being stimulated can be higher than 50% (Grégoire *et al.,* 2013), and may thus not be very indicative of the presence of lameness.

1.4. Lameness assessment based on weight distribution

1.4.1. Weight distribution between limbs

Lame animals tend to bear less weight on the affected limb as a strategy to reduce pain by redistributing the weight among sound limbs (Pastell and Kujala, 2007). Consequently, measuring weight distribution is helpful to assess which limb(s) are lame. A first evaluation of weight bearing can be carried out visually. Indeed, Main *et al.* (2000) used weight distribution in their detailed visual gait scoring system for sows, with a grading scale of 0 or 1 (pigs stand squarely on all four limbs), 2 (uneven posture), 3 (uneven posture, will not bear weight on affected limb), 4 (affected limb elevated off floor) and 5 (will not stand unaided). However, this scale is qualitative and does not provide quantitative information about the degree of deviation from normal weight distribution. This limitation can be overcome by using force plates and pressure-sensitive walkway mats, which can measure ground reaction forces and pressures, respectively, and in particular the vertical forces that are developed while the animal is in motion. Peak vertical force (N/kg) and pressure (N/cm^2) correspond to the maximal load applied during stance time (i.e., the gait phase that lasts from heel strike to toe off) with and appear to be repeatable (Lascelles *et al.,* 2006). Weight distribution can be calculated by dividing the peak vertical force of one limb by the total peak vertical forces of all limbs (Kim *et al.,* 2011). Moreover, different symmetry indexes can also be calculated from peak vertical forces (Mölsä *et al.,* 2010; Oosterlinck *et al.,* 2010). A decrease in peak vertical forces, as well as an asymmetry in peak vertical forces and contact area, has been associated with lameness in dogs and cattle (Oosterlinck *et al.,* 2011; Schulz *et al.,* 2011). In an experimental lameness model, Karriker *et al.* (2013) demonstrated a reduction of the peak pressure of the affected limbs when the sows walked on a pressure mat (GaitFour$^{©}$, CIR Systems, Inc., Havertown, PA), indicating that this method can be used to detect lameness. However, one critical point in the assessment of weight distribution in moving animals is that the pace (speed and acceleration) along the walkway and the type of surface must be controlled because they influence the vertical component and stance time (Khumsap *et al.,* 2001; Thorup *et al.,* 2007). To avoid these constraints while still collecting information on the weight loaded on each of the four limbs, another possibility is to assess the weight distribution in static animals. In horses and cows, a force plate with 4 separate scale platforms gave promising results for the detection of laminitis or lameness, particularly by measuring the asymmetry in the weight applied within contralateral pairs of limbs (Chapinal *et al.,* 2010; Hood *et al.,* 2001; Pastell and Kujala, 2007). Pastell and Kujala (2007) reported a sensitivity of 100% and a specificity of 57.5% for the identification of lame cows with this methodology. Various models of force plates for sows are currently at an advanced stage of development. Sun *et al.* (2011) presented promising results for the measure of weight distribution from an embedded microcomputer-based static force plate. The force plate consisted of 4 quadrants with a single load cell placed in the middle of each quadrant to record the weight of all limbs. The results indicated that all sows averaged more weight on the front limbs than on the hind limbs, regardless of lameness status. The authors suggested that a key signature that would classify sows as lame (in either the front or hind limbs) could be any large deviation in front-to- hind weight distribution. However, when inducing lameness in one limb, it was reported that the sow redistributed the weight on the 3 unaffected limbs (Johnson *et al.,* 2011), but mostly on the contralateral limb (Karriker *et al.,* 2013).

The SowSIS (Sow Stance Information System; **Fig. 1.5**) developed by Pluym *et al.* (2013b) integrates force stance variables derived from force plate analysis and visual stance variables derived from image processing. The preliminary results from on-farm testing indicated that sows with hind-limb lameness were reluctant to bear weight on the affected limb and put more weight on the contralateral sound hind limb. Sows showed more frequent but shorter kicks (i.e., lifting limb off the ground) with their lame limb compared to the other limbs. One critical point is to ensure that the animal stands properly on the scale (i.e., feet placed on their respective platforms and without too many movements) to avoid erroneous data collection.

Figure 1.5. The SowSIS measuring device described in Pluym *et al.* (2013). The the bottom plate contains four force plates that continuously record force stance variables, once the sow is standing inside the modified crate. Visual stance variables are also recorded with a mounted photocamera.

Future research is also required to better understand the variables that characterise lame sows, such as the link between weight distribution pattern and underlying pathologies. In this way, it will be possible to establish threshold values to detect lame animals with a high specificity and sensitivity. These systems are being developed further with a view to integrating them within electronic sow feeding stations (Maselyne *et al.,* 2014; MCNeil, 2015). This approach sounds promising: one study (McNeil, 2015) found that the parameters recorded by a force plate installed under an electronic sow feeder (ESF) in one dynamic group of 120 multiparous sows could identify lameness almost 5 days before it was visually assessed. The authors concluded that these embedded microcomputer-based force plates could help identify lameness before the clinical signs become evident. Early diagnosis may be useful to reduce treatment costs and the losses in productivity that are associated with patent lameness. In the future, the influence of factors other than lameness, such as parity or stage of gestation, on weight distribution will need to be further assessed.

1.4.2. Weight shifting and stepping

Shifting weight from one limb to another can be a strategy to relieve a lame limb (Neveux *et al.,* 2006; Rushen *et al.,* 2007). Measuring weight shifting is a method adopted by the Welfare Quality$^{©}$ protocol to assess lameness in tied cows, whose movements are restricted (Leach and Whay, 2008). Indeed, it was shown that weight shifting, particularly in the hind limbs, was more prevalent in cows judged as lame with visual locomotion scoring (Leach and

Whay, 2008; Leach *et al.,* 2009). In sows, Wells (1984) described the shifting of weight as a potential expression of polyarthritis and Jørgensen (2000) described stepping of hind limb as clinical sign of claw lesions. One method to assess weight shifting is to visually record the number of events per minute. Anil *et al.* (2007) showed a reduced weight shifting in lame sows after pain medication, concluding that weight shifting is a good indicator of lameness. However, information is still lacking regarding the relationship between the amount of weight shifting and the degree of lameness severity in sows.

Stepping is a more pronounced behaviour than weight shifting because it implies that the animal completely removes the weight from one limb by lifting it, thus also changing the centre of gravity. The relevant acceleration can be measured on the vertical axis. Ringgenberg *et al.* (2010) successfully used accelerometers attached to the pelvic limbs of sows to automatically detect stepping in a standardised manner. A step was characterised by acceleration on the vertical axis below 0.6 *g* or above 1.4 *g*. Grégoire *et al.* (2013) confirmed that accelerometers can successfully be used to measure stepping, and found that lame sows presented a higher recorded number of steps than sound animals. Future research is needed to define a potential threshold value of stepping frequency, hopefully permitting an early detection of lameness. However, Leach and Whay (2008) draw attention to the fact that before establishing threshold values it will be necessary to discriminate between shifting and stepping behaviours related to lameness, and nervousness and anticipation of feeding.

1.4.3. Weight pressure on claws

The pressure, or compression stress, applied on each toe can be measured by combining a pedobarograph (optical pressure-sensitive platform) and a force plate. Webb (1984) showed that in pigs most weight is loaded on the outer digits of the front and hind feet. De Carvalho *et al.* (2009) found similar results for the hind limbs using a pressure mat, but suggested a better balance between inner and outer digit of the front limbs compared to the hind limbs. Systems measuring pressure patterns, contact area and force applied on the sow's feet are useful to gain a better understanding of the biomechanical reasons why injuries tend to occur in given areas of the claws (Webb, 1984; **Fig. 1.6**). Indeed, the greater load on the outer digit may partially explain its higher prevalence of lesions (Anil *et al.,* 2007; de Carvalho *et al.,* 2009). Moreover, these instruments are also very useful to understand the impact of different floor systems (i.e., use of rubber mat or different slat width) on pressure distribution and relief on the claws.

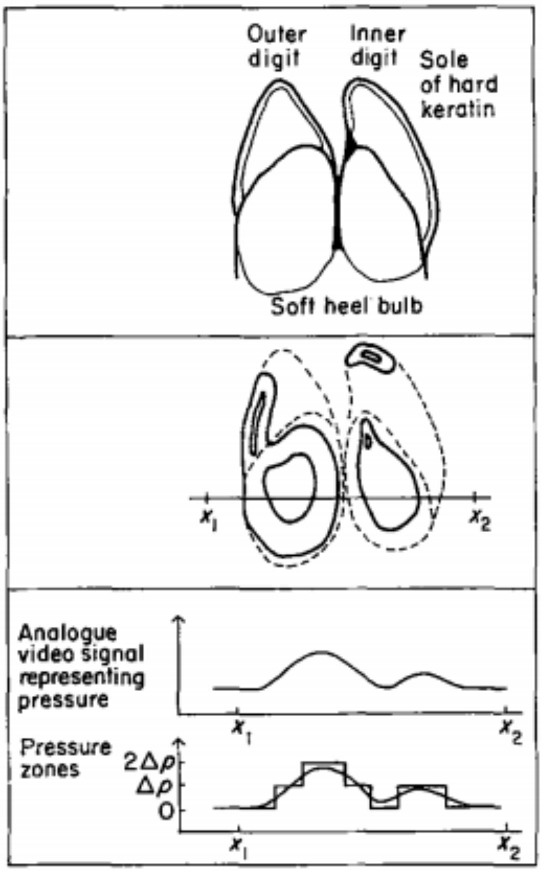

Figure 1.6. Diagram of the underside of a pig's foot showing the relationships between (top) the anatomy and (middle) a typical pressure pattern. The scheme for digitizing the video analogue pressure pattern signal is shown in the bottom part of the image (from Webb, 1984).

1.5. Nociception

Nociception is "The neural process of encoding noxious stimuli. Consequences of encoding may be autonomic (e. g. elevated blood pressure) or behavioural (motor withdrawal reflex or more complex nocifensive behaviour). Pain sensation is not necessarily implied" (IASP, 2012). A simplified and schematic representation of different nociceptive pathways is included in **Figs. 1.7 and 1.8.**

The sections that follow provide some basic definitions of the terminology that will be used throughout this thesis, and explain the most common methods that are used to measure nociception in animals.

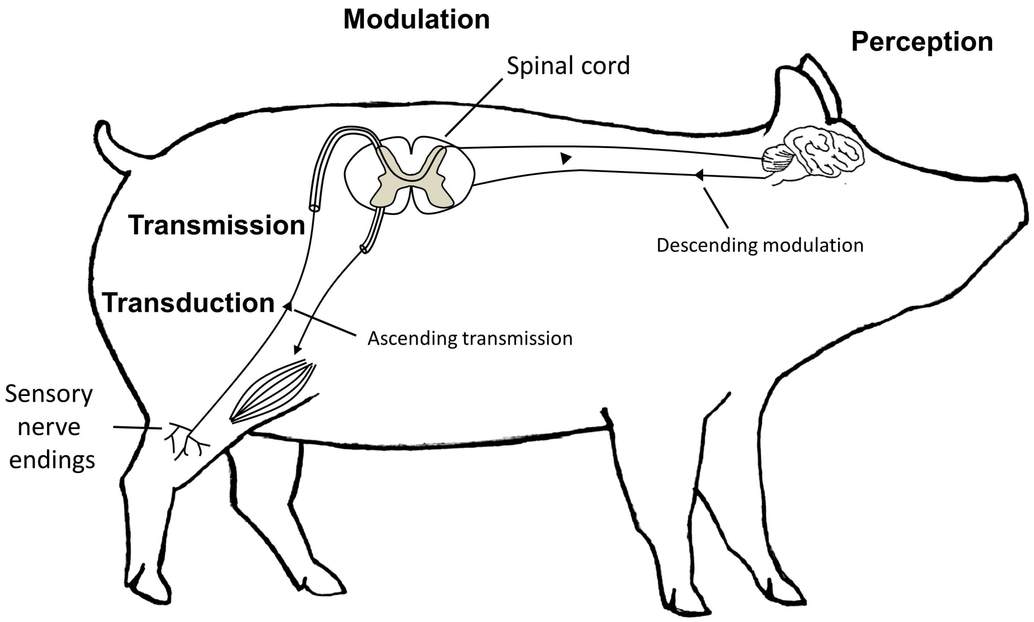

Image: Di Giminiani, 2016 (in press)

Figure 1.7. Schematic representation of nociceptive pathways. Specialised peripheral sensory neurons (nociceptors) are activated by noxious temperature, pressure or hazardous chemicals, and transduce these stimuli into electrical signals that are transmitted to the central nervous system (spinal cord and supraspinal centres). Pain perception occurs in specific areas of the brain cortex and in the deeper limbic structures, which are associated with the affective, sensory-discriminative, emotional and behavioural components of the pain experience. The descending modulation of pain involves the release of neurotransmitters that can inhibit the transmission of pain impulses (inhibition) or amplify the transmission of pain impulses. Image kindly provided by Pierpaolo Di Giminiani (2016, in press).

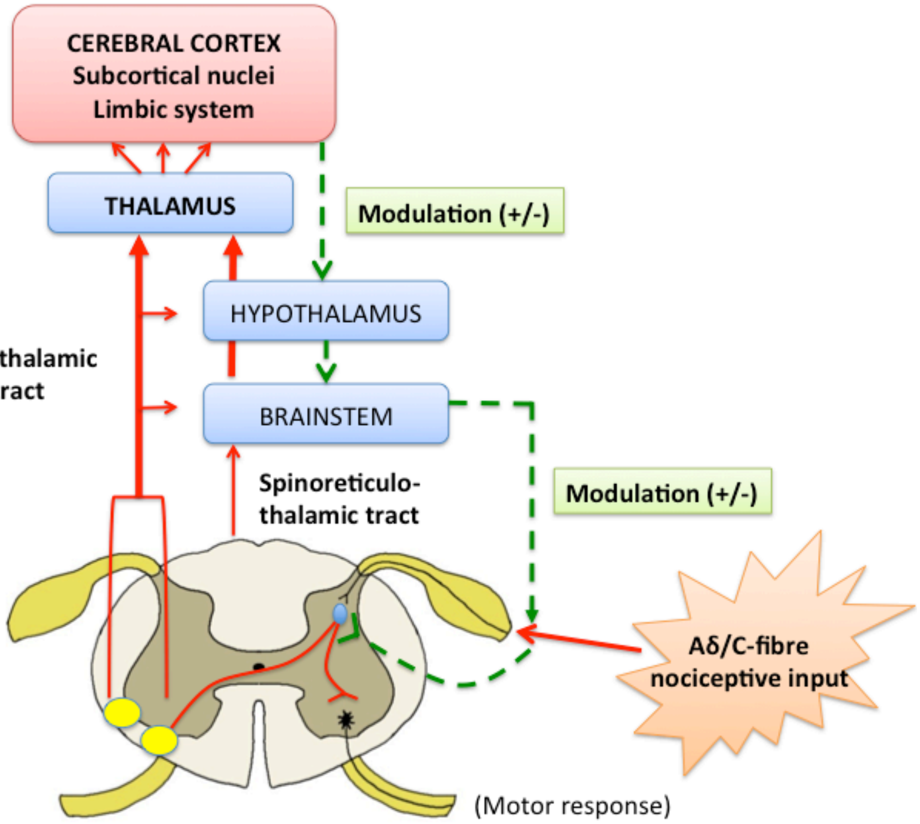

Figure 1.8. Detail of the most important nociceptive pathways (adapted from Barker *et al.*, 2012). Ascending nociceptive stimuli reach the brainstem, hypothalamus and thalamus and are then further elaborated by specialised areas of the cerebral cortex, the limbic system, and subcortical nuclei. The brain and subcortical structures can modulate pain (descending modulation), but can also create pain perception.

1.5.1. Definitions

The International Association for the Study of Pain (IASP, 2012) provides the following definitions that are also valid for the purposes of this thesis:

Nociceptive stimulus: "An actually or potentially tissue damaging event transduced and encoded by nociceptors."

Noxious stimulus: "An actually or potentially tissue-damaging event."

Nociceptor: "A sensory receptor that is capable of transducing and encoding noxious stimuli." Nociceptors are free ending nerve fibers with different characteristics that innervate all tissues except the brain. They are highly specialised, meaning that their activation inevitably results in painful sensations. They are also unique in two ways: firstly, they are able to respond to different stimuli (chemical, thermal, mechanical; see below); secondly, they can be modulated, thus becoming more or less responsive to stimuli (Julius and Basbaum, 2001). The quality of the pain sensation depends on the tissue in which the activated nociceptor(s) is located, as well as on the nature and intensity of the stimulus (Julius and Basbaum, 2001; Willis and Westlund, 1997). In simplistic terms, nociceptors can be divided into *mechanical*, responding to pressure, *thermal*, responding to heat (> 45° C) and noxious cold, *chemical*, responding to pH extremes, environmental irritants and internal neuroactive substances, and *polymodal*. Additionally, there are *silent* or *sleeping* nociceptors which are activated only when sensitised by tissue injury. Nociceptive sensations are carried by two types of fibres (**Fig. 1.9**), namely Aδ (lightly myelinated, generatin, sharp and intense pain) and C (slow, unmyelinated, generating persistent, dull pain; Julius and Basbaum, 2001). C-fibres end exclusively in free nerve endings, while Aδ-fibres can also terminate in a specialised receptor (Gregory, 2004).

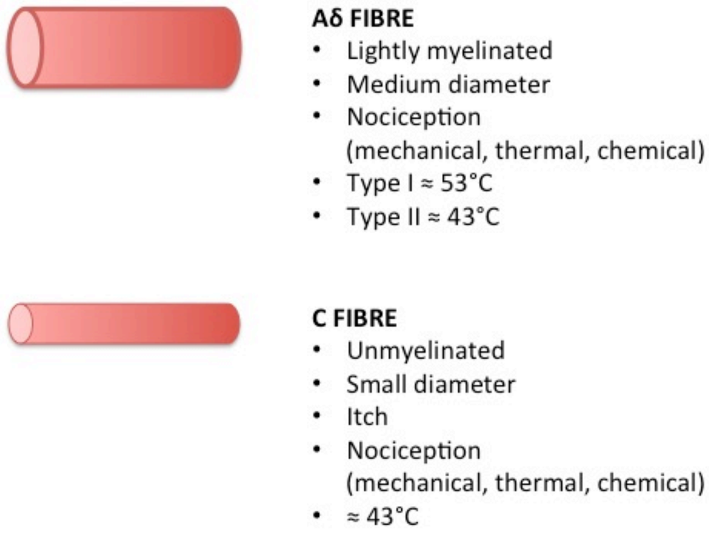

Figure 1.9. The two main types of peripheral nociceptors and their characteristics (adapted from Julius and Basbaum, 2001).

The prolonged stimulation of nociceptors in a given tissue or area, which may be caused for example by an injury or an inflammatory process, can induce sensitisation phenomena, meaning that nociceptors become more reactive to stimuli, or in other words they acquire a lower activation threshold. Sensitisation can be limited to the periphery or can induce more long-lasting changes in the central nociception pathways, located in the spinal cord. These two mechanisms have important effects on pain perception (Julius and Basbaum, 2001).

Sensitisation: "Increased responsiveness of nociceptive neurons to their normal input, and/or recruitment of a response to normally subthreshold inputs. Clinically, sensitization may only be inferred indirectly from phenomena such as hyperalgesia or allodynia".

Peripheral sensitisation: "Increased responsiveness and reduced threshold of nociceptive neurons in the periphery to the stimulation of their receptive fields". This is the result of the exposure of nociceptor terminals to the products of tissue damage, also known as the "inflammatory soup" (Julius and Basbaum, 2001). This phenomenon can be amplified locally because nociceptors are able to release pro-inflammatory neurotransmitters that in turn stimulate the production of more irritants by neighbouring cells (Julius and Basbaum, 2001).

Central sensitisation: "Increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input (i.e., an input that would not normally evoke a response). This may include increased responsiveness due to dysfunction of endogenous pain control systems. Peripheral neurons are functioning normally; changes in function occur in central neurons only". This means that, under certain circumstances, pain can result from the spontaneous activation of central nociceptive pathways without involving the stimulation of peripheral nociceptors (Willis and Westlund, 1997).

Pain: "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."

Hyperalgesia: "Increased pain from a stimulus that normally provokes pain. […] This is a clinical term that does not imply a mechanism. For pain evoked by stimuli that usually are not painful, the term **allodynia** is preferred, while hyperalgesia is more appropriately used for cases with an increased response at a normal threshold, or at an increased threshold, e.g., in patients with neuropathy. Current evidence suggests that hyperalgesia is a consequence of perturbation of the nociceptive system with peripheral or central sensitization, or both. Hyperalgesia may be seen after different types of somatosensory stimulation applied to different tissues". Hyperalgesia and allodynia are phenomena or great clinical importance as they are major components of neuropathic pain (peripheral neuropathies and central pain disorders), the pharmacological treatment of which can be challenging (Jensen and Thinnerup, 2014). A diagram illustrating how hyperalgesia and allodynia influence nociception is presented below (**Fig. 1.10**).

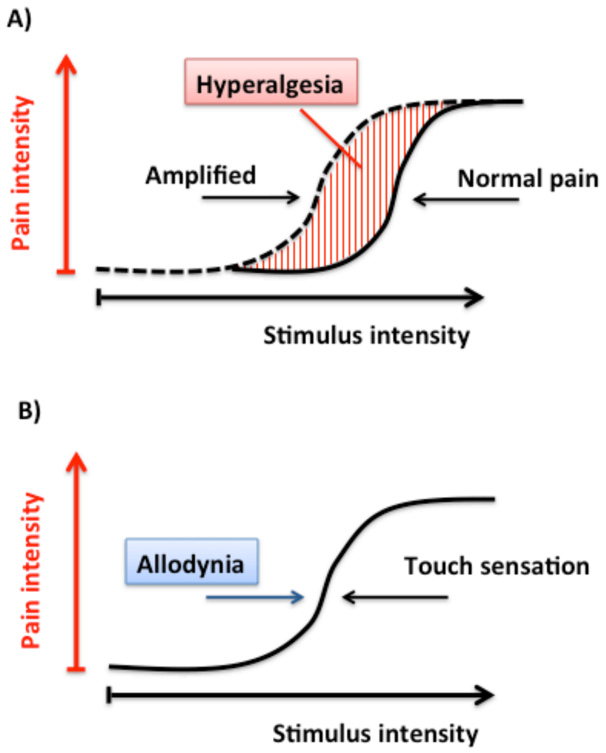

Figure 1.10. Stimulus-response function in the case of hyperalgesia (A) and allodynia (B); adapted from Sandkühler, 2009). Hyperalgesia is a heightened response to stimuli that are normally painful, meaning that the affected subject has a lower threshold to nociceptive stimuli (e.g., reacts sooner to pressure, or to a chemical insult). Allodynia occurs when a subject reports painful sensations as a result of normally innocuous stimuli (e.g., light touch, brushing of skin with a feather). Both allodynia and hyperalgesia result from an initial nerve damage, often amplified by peripheral or central sensitisation processes.

A further distinction can be made between primary and secondary hyperalgesia (Ali *et al.,* 1996; Hsieh *et al.,* 2015; Meyer *et al.,* 2005; Sandkuhler, 2009):

- **Primary hyperalgesia**: hyperalgesia at the site of injury
- **Secondary hyperalgesia:** hyperalgesia in an area adjacent to or remote of the site of injury

Primary hyperalgesia is due to sensitisation of peripheral nociceptive nerve endings and can be present both to mechanical and thermal stimuli. **Secondary hyperalgesia** is not caused by the sensitisation of nociceptive nerve endings but rather by changes in the processing of sensory information in the neurons of the dorsal horn of the spinal cord (central sensitisation; Ali *et al.,* 1996; Meyer *et al.,* 2005). Due to the different types of nerve fibers involved (Hsieh *et al.,* 2015), secondary hyperalgesia develops only to punctate mechanical stimuli, and not to thermal stimuli (Hsieh *et al.,* 2015). Hyperalgesia and allodynia are symptoms of an increased vulnerability of an injured tissue. As such, they represent behavioural symptoms of an underlying pathology and they have a protective function for the organism. However, they may persist long after the injury or inflammatory process has healed. Persisting hyperalgesia has been demonstrated, for instance, in lame dairy cattle even after hoof trimming even after systemic treatment with antibiotics (Laven *et al.,* 2008). In this case, hyperalgesia is maladaptive rather than protective, thus becoming a disease in its own right (Gregory, 2004; Lautenbacher and Fillingim, 2004).

1.5.2. Measuring nociception in animals

Pain perception requires elaboration by a conscious subject (IASP, 2012). While human patients are capable of describing and grading painful sensations, a major challenge when studying pain perception in animals is that it cannot be measured directly. Therefore, other quantifiable and correlated substitutes of pain sensation are commonly used in research studies involving animals, but also in non-verbal human patients, such as very young children (Hughes, 2008). These substitutes include withdrawal reflexes, vegetative and hormonal responses, vocalisations, grimaces, or in other words, phenomena that can be quantified (in various ways) and that represent species-specific responses to nociceptive stimuli (Hughes, 2008). Similarly, tests that are used to measure nociception do not directly measure pain, but rather changes from baseline nociceptive thresholds induced by presumably painful pathologic conditions. Throughout this thesis, the presence of a heightened sensitivity to nociceptive stimuli (hyperalgesia) will be used as an indirect indicator, or surrogate indicator, of an exacerbation in the pain sensation experienced by animals – in our specific case sows affected by lameness. This does not presuppose that the degree of hyperalgesia can be correlated in a linear way with the pain sensation that an animal is experiencing.

In animals, nociception and its changes are measured using a choice of standardised tests. For a long time, the main focus of nociception studies has been the extrapolation of data on pain mechanisms from animals to humans. These tests were originally developed in laboratory settings and tailored on mice (Mogil, 2009). More recently, several methods have been developed to investigate pain perception in companion animals and livestock, both for the purposes of fundamental research and for clinical practice (Le Bars, 2001). One of the methods commonly used is nociceptive threshold testing, which consists of the application of a quantifiable stimulus to a given anatomical region until a clear response – defined in the experimental protocol – is obtained (Love *et al.,* 2011). Recent studies using and refining this technique to measure nociception in animals have involved, among others, cats (Dixon *et al.,* 2007, 2010; Slingsby *et al.,* 2011; Steagall *et al.,* 2007), dogs (Coleman *et al.,* 2014; Dixon *et al.,* 2010; Gorney *et al.,* 2016; Kaka *et al.,* 2015; Lane *et al.,* 2016; Schütter *et al.,* 2016), horses (Haussler and Erb, 2006; Haussler *et al.,* 2007; Taylor *et al.,* 2016), donkeys (Grint *et* *al.,* 2015; Lizarraga *et al.,* 2015), cattle (Peters *et al.,* 2013; Raundal *et al.,* 2014, 2015; Tadich *et al.,* 2013), pigs (Di Giminiani *et al.,* 2013a, 2013b, 2014, 2015, 2016a, 2016b; Fosse et al, 2010, 2011; Janczak *et al.,* 2012; Mohling *et al.,* 2014; Sandercock *et al.,* 2009), sheep (Bortolami *et al.,* 2015; Musk *et al.,* 2014; Stubsjøen *et al.,* 2010; Walkowiak *et al.,* 2015), and broiler chickens (Caplen *et al.,* 2013; Hothersall *et al.,* 2014).

The most commonly used nociceptive thresholds tests measure responses to electrical, thermal and mechanical stimuli, with or without prior induction of local inflammation with chemical agents (e.g., capsaicin, amphotericin-B). Electrical, thermal and mechanical nociceptive threshold tests differ methodologically and in the neurophysiological responses that they evoke, and therefore they are not interchangeable. As thermal and mechanical methods are most frequently used in livestock, they will be described in more detail.

Thermal nociceptive threshold tests

Thermal stimuli are used to activate superficial cutaneous nociceptors, by means of contact heat (plates) or radiant heat (e.g., laser beams). Normally these tests measure either the latency to respond after application to the skin of a stimulus at a constant temperature, or the maximum ramped temperature at which an animal responds (Di Giminiani *et al.,* 2013a, 2013b; Dixon *et al.,* 2002, 2016; Grint *et al.,* 2015; Love *et al.,* 2011). Thermal nociception threshold tests are advantageous because they can be carried out with small, lightweight equipment and even remotely with a laser beam, and thus can be used to study thermal nociception and its response to analgesics in small species (e.g., mice, rats) and in species in which restraint can significantly alter behavioural responses (e.g., cats, Dixon *et al.,* 2002; Steagall *et al.,* 2007; **Fig. 1.11**). Thermal nociceptive threshold tests are also used in association with mechanical nociceptive threshold tests to investigate changes in nociception induced by surgical procedures, acute inflammatory processes and chronic pain, as well as the effects of analgesics. However, compared to mechanical nociception tests, special attention is required as prolonged heat exposure can more easily cause skin lesions (Dixon *et al.,* 2016; Grint *et al.,* 2015).

Figure 1.11. Thermal and mechanical nociceptive threshold testing in an unrestrained cat. In this example, the thermal probe was held in place by means of an elasticated band. In this experiment, the mechanical nociceptive threshold was also tested via the cuff placed around the cat's forelimb. An inflatable bladder pushed three ball bearings mounted on short pins against the skin. Source: Steagall *et al.* (2007).

Mechanical nociceptive threshold tests

Mechanical nociceptive threshold tests measure the animals' reaction to a punctate stimulus (blunt or sharp) which is applied perpendicularly to the anatomical area of interest with increasing force, thus generating increasing pressure, preferably at a constant rate. The stimulus can be delivered by means of different instruments, such as von Frey filaments (**Fig 1.12**), either hand-held or remotely operated, and pressure algometers (**Fig 1.13**).

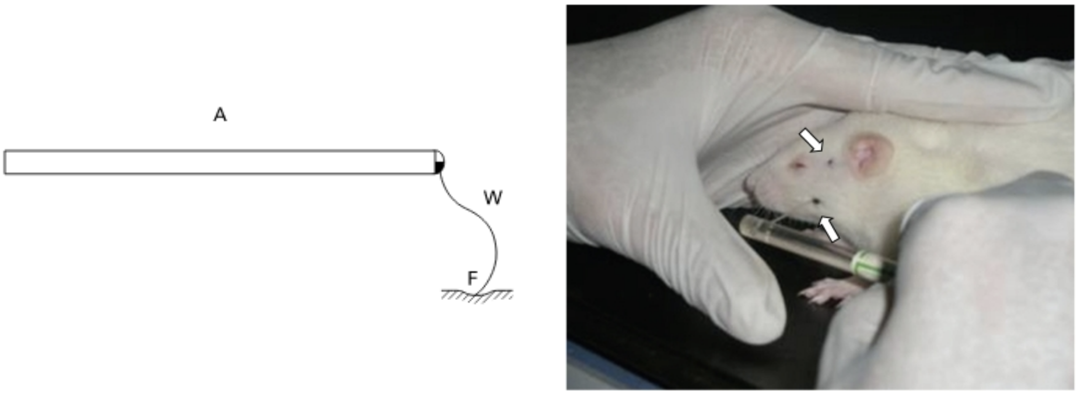

Figure 1.12. Left: diagram illustrating the functioning of the von Frey monofilament (original drawing by von Frey, 1896; reproduced from Huang *et al.*, 2011). F= end point; W=inflection point. When the tip of a fiber of given length and diameter is pressed against the skin (end point) at a 90 degree angle, the force of application increases as long as the operator continues to push the probe against the skin, until the fiber bends (inflection point). After the fiber bends, continued advance creates more bend, but not more force of application. This principle makes it possible to apply a reproducible force to the skin surface when using a hand-held probe (Source: BioSeb.com). Recent versions of von Frey monofilaments allow for the electronic recording of results. **Right:** assessment of mechanical nociceptive thresholds in the orofacial region of the rat with a von Frey monofilament. The arrows indicate where the tip of the monofilament was used to probe the rat's skin. Source: Huang *et al.* (2011).

Figure 1.13. Different models of hand-held pressure algometers, for use in small and large animals. The algometer is a force transducer provided with one or mores tips of variable materials, shapes and diameters and which is manually pushed against a given anatomical landmark – preferably at a constant rate – until a codified behavioural response is obtained from the animal. The average of several readings at the time of the reaction, either expressed as units of force or pressure, is the mechanical nociception threshold of the landmark tested. Image sources: Top left Wagner Instruments. Top right, bottom left and right: TopCat Metrology.

Scientists have also developed pneumatic devices that can be fastened to the appendicular skeleton or the trunk of the animals and operated remotely, thus standardising the application of the stimulus while minimising restraint (**Fig. 1.14**). The most common procedure when measuring MNT in animals and humans is to take consecutive measurements at the same anatomical site at predefined, arbitrarily decided intervals, varying from seconds to minutes. The interval is decided based on the species and on the perceived or known danger of causing tissue damage. Care must also be taken to minimise the occurrence of sensitisation of nerve endings due to the temporal summation of the nociceptive stimuli, as temporal summation can alter nociceptive function (Lautenbacher and Fillingim, 2004). The results are commonly expressed in terms of the maximum average force applied or the pressure (force/area) that is capable of producing a codified response.

Fig. 1.14. Limb-strapped pneumatic devices connected to a digital algometer with tubing for remote measuring of mechanical nociceptive thresholds. The pressure within the plastic tube pushes the pin within the pneumatic device against the skin of the animals at a constant rate, until a behavioural response is obtained. At that point, pressure can be manually removed and the pin immediately retracts. Source: TopCat Metrology.

1.5.3. Effects of lameness on nociception, and implications for animal welfare

Lameness induces changes in pain perception that may outlast the initial injury or inflammatory challenge (Laven *et al.,* 2008; Whay *et al.,* 1995). Mostly, these changes go in the direction of an increased sensitivity to mechanical and thermal noxious stimuli (i.e. hyperalgesia). In some cases, more difficult to assess in animals, prolonged pain might even lead to perceiving non-painful stimuli as painful (i.e., allodynia).

Studying changes in the nociceptive system of lame animals can help to clarify the relationship between various levels of locomotion impairment and the degree of pain experienced by the animal. No less importantly, changes in nociception help to better understand the efficacy of different analgesics in attenuating pain. These types of studies are being carried out in a range of species (Caplen *et al.,* 2013). Hyperalgesia is also worthy of investigation as it may be a more sensitive indicator than gait score when it comes to the effects of lameness. For example, in one study dairy cattle presented mechanical hyperalgesia even 28 days after receiving treatment for lameness, but there was no statistically significant effect of lameness score on nociceptive thresholds (Whay *et al.,* 1998). In a study on a group of free-range pigs, Etterlin *et al.* (2015) found no association between the severity of presumably painful osteochondrotic lesions and gait score measured visually on a 4-point scale. The authors concluded that visual gait scoring could be an insensitive tool for the evaluation of joint pathologies in finishing pigs. They hypothesised that their findings could have been influenced by several concomitant factors: ordinal gait scoring scales, especially those with few categories, may have a low sensitivity; animals may hide signs of pain; pain may be relieved by physical activity and by stronger joint supportive tissue in free-range livestock; bilateral lesions, such as osteochondrosis, may mask lameness (Etterlin *et al.,* 2015). The study did not involve the measurements of nociceptive thresholds. It is possible that mechanical nociceptive threshold testing would have been helpful in determining the presence of hyperalgesia in the animals affected by osteochondrosis.

The cognitive and emotional effects of hyperalgesia are not thoroughly investigated in pigs, but there is evidence from human medicine and from laboratory animals that hyperalgesia leads to a significantly compromised quality of life. One model of hyperalgesia in human patients is the disabling pain associated with knee osteoarthritis (Imamura *et al*., 2008). This condition can induce central sensitisation, demonstrated by a generalised hyperalgesia, the only treatment for which is total knee replacement. Patients affected by knee osteoarthritis scored lower than controls for vitality, mental health and social functioning (Imamura *et al*., 2008). In another study on female patients affected by fibromyalgia, endometriosis, low back pain or rheumatoid arthritis, Laursen *et al*. (2005) also found generalised hyperalgesia and a clear correlation between mechanical nociceptive thresholds and the pain scores indicated by the patients on a VAS. Gormsen *et al*. (2010) demonstrated a high prevalence of anxiety and mental distress among patients with chronic pain. There is no reason to exclude the possibility that hyperalgesic animals may experience the same adverse emotional and cognitive effects. In fact, there is substantial evidence of anxiety and depressive-like states in mice with experimentally induced chronic neuropathic pain (Narita *et al*., 2006; Norman *et al*., 2010). These mice show lower reactivity in the classical laboratory tests that are used to test vitality and anxiety, such as the forced swimming test, light-dark exploration, and the elevated plus-maze test (Benbouzid *et al*., 2008; Suzuki *et al*., 2007), so much so that researchers talk about chronic pain-depression co-morbidity (Bravo *et al.,* 2012). In effect, both neuropathic hyperalgesia and the related anxiety states respond to treatment with antidepressants (Hache *et al*., 2012; Matsuzawa-Yanagida *et al*., 2008). Far less is known about the emotional and cognitive effects of pain in farm animals, but this is a growing field of research. Recently, Neave *et al*. (2013) found that calves experiencing post-disbudding pain showed signs of negative judgement bias, meaning that they categorised an ambiguous event as being potentially hazardous. But judgement bias is a complex phenomenon to investigate. For instance, it seems that pigs can be more or less optimistic depending on their personality traits and on external circumstances (Asher *et al*., 2016). Pigs with a more proactive personality, as shown by their exploratory behaviour, become more “pessimistic” if they are housed in a barren environment, and more “optimistic” if housed in an enriched environment. In other words, personality and moods interact, thus influencing cognitive bias (Asher *et al*., 2016). Some authors suggest that the investigation of cognitive bias in farm animals could become an important component in the assessment of animal welfare (Baciadonna & McElligott, 2015). In conclusion, the relationship between pain and animal wellbeing in its fullest sense is an interesting and growing area of research, which will eventually lead to a more profound understanding of the many ways in which pain negatively affects farm animal welfare.

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Chapter 2 - Research Objectives

Current methods to visually assess sow lameness are quite variable and it is not clear which method is most suitable to reliably score sow gait. In addition, the effect of lameness on nociception in sows is not known. Finally, very little is known about the factors influencing the mechanical nociceptive threshold testing methodology in sows. The general aim of the thesis was to investigate these aspects and better understand how lameness in sows should be addressed in the future.

There were three specific objectives:

(1) Devising a tagged visual analogue scale (tVAS) for the visual assessment of sow gait and comparing its inter-and intra-observer repeatability with those of a 2-point and a 5-point categorical scale, as well as the performance of naïve observers compared to trained observers (**Chapter 3**).
(2) Investigating (a) whether sow lameness as measured on the tVAS is associated with changes in mechanical nociceptive thresholds and (b) what factors, beside lameness, affect mechanical nociceptive thresholds in sows (**Chapter 4**).
(3) Studying factors affecting the intra-individual and intra-session variability when measuring mechanical nociceptive thresholds in healthy sows (**Chapter 5**).

The results, the limitations and the areas where further research is required are discussed in **Chapter 6.** A schematic representation of the structure of this thesis is presented in **Fig. 2.1**.

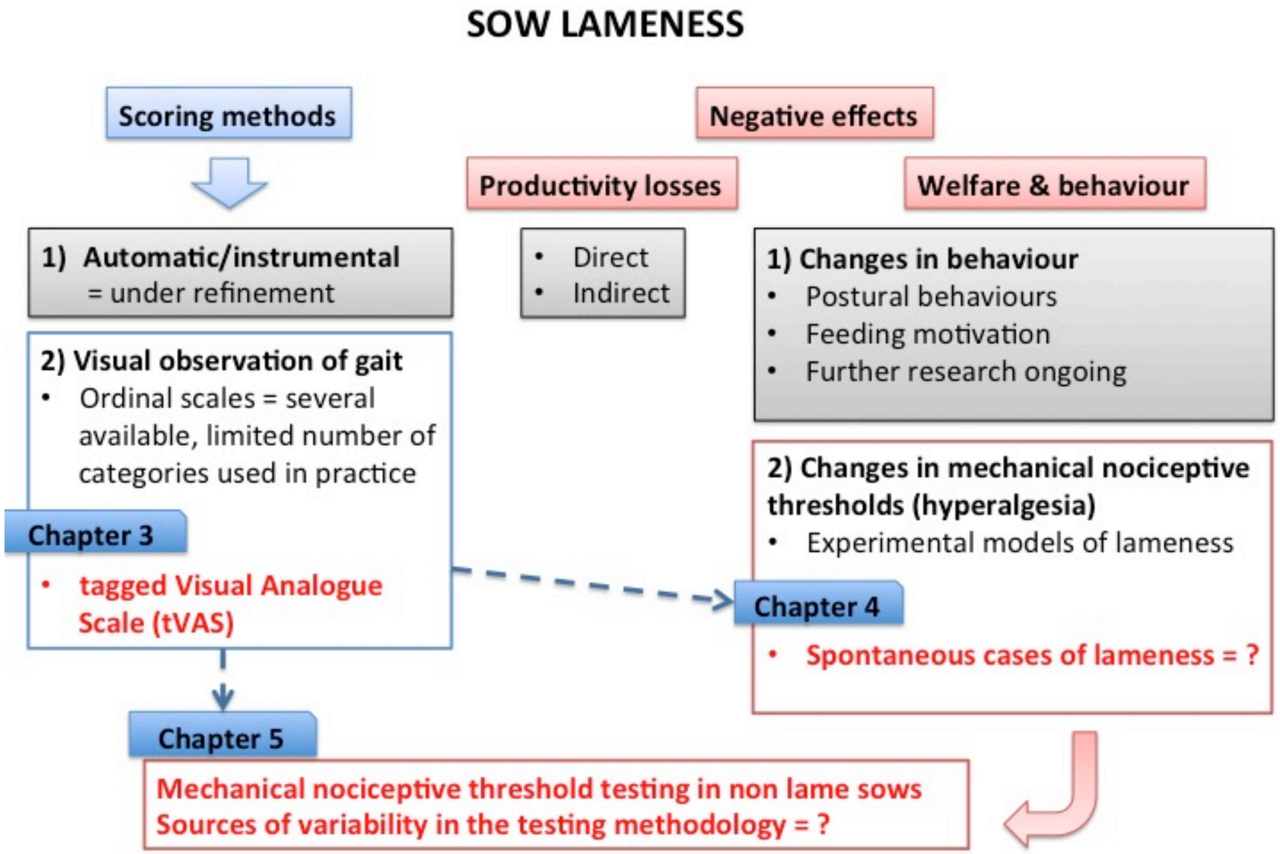

Figure 2.1. Schematic representation of the different parts of this thesis. The grey blocks indicate areas of research not covered by this thesis, but that are relevant to the topic. **In bold**: information that was already available in the literature at the time this PhD started. **In red:** the questions that the thesis aimed to answer. **Dashed blue arrows:** the preliminary step of this thesis was to develop a tagged visual analogue scale (tVAS) for the assessment of gait in sows. This tVAS would then be used to select the sows for the experiments described in Chapters 4 and 5.

Chapter 3 - Comparison of the inter- and intra-observer repeatability of three gait-scoring scales for sows

Adapted from: E. Nalon, D. Maes, S. Van Dongen, M. M. J. van Riet, G. P. J. Janssens, S. Millet and F. A. M. Tuyttens (2014). Comparison of the inter- and intra-observer repeatability of three gait-scoring scales for sows. Animal, 8, 650-659.

Abstract

Most gait-scoring scales for pigs have a limited number of categories, supposedly to improve repeatability. However, reducing the number of categories could lead to loss of information if the observers' discriminative capacities are underused. With a recently estimated within-herd prevalence of sow lameness of 8.8%-16.9% in the European Union and the associated losses, the availability of reliable tools for the timely detection of initial cases warrants attention. This study investigated the intra- and inter-observer repeatability (intra-OR and inter-OR) of three gait-scoring scales for sows: a continuous 'tagged' visual analogue scale (tVAS, measured in mm), a 5-point, and a 2-point ordinal scale (5P and 2P), all with the same descriptors. Veterinary medicine students (n=108) were trained to use the scales and then asked to score 90 videos (30 per scale) of sows with normal and abnormal gait. Thirty-six videos were shown once and 18 were randomly shown three times, of which one was mirrored horizontally. The students' opinions on the scales were also collected. Intra- and inter-OR were higher with the tVAS than the 2P scale (inter-OR: 0.73 vs. 0.60;$P < 0.05$. Intra-OR: 0.80 vs. 0.67;$P < 0.05$). Intra-OR was higher with the 5P (0.81) than the 2P scale (0.67;$P < 0.05$). For all three scales, repeatabilities were lower ($P < 0.05$) for non-lame sows (gait score of ≤ 45mm on the tVAS) than for sows showing some signs of lameness (gait score > 45 mm). Video order (first 45 vs. last 45 clips), mirroring, users' opinions on the scales, and previous declared experience in handling pigs or scoring lameness in other species had no effect on repeatabilities. Correlations between the students' and experts' scores were high (tVAS = 0.92; 5P = 0.91; 2P = 0.88) but the association for the 2P was not linear and the frequency distribution showed lower correlations for a group of students. This study confirms recent evidence that it is possible to design high-resolution gait scoring scales that do not reduce observer repeatability. Visual gait-scoring scales with fewer than five categories are likely to entail loss of information on lameness in individual sows.

Implications

Lameness in sows constitutes an animal welfare challenge and an economic concern. In practice, lameness is mostly assessed visually by means of ordinal gait scoring scales with few categories. Using few categories reportedly increases inter-observer repeatability; however, repeatability depends on many extrinsic factors besides the scale itself. This study presents evidence that it is possible to develop continuous, high-resolution gait-scoring scales for sows that are repeatable and make full use of the trained observers' discriminative abilities. If used in practice, such scales could contribute to a more accurate identification of locomotor problems in individual sows and entire herds.

Introduction

Lameness has a considerably negative impact on the welfare and productivity of sows (Heinonen *et al.,* 2013; Pluym *et al.,* 2013) and has been included as a welfare indicator in welfare assessment protocols (Welfare Quality®, 2009) and in farm assurance schemes (Global Animal Partnership, 2015; Royal Society for the Protection of Cruelty to Animals, 2015). Recent studies estimated the prevalence of sow lameness between 8.8% and 16.9% in the European Union (Heinonen *et al.,* 2006; KilBride *et al.,* 2009; Heinonen *et al.,* 2013). Sow lameness is associated with economic losses, both in terms of early removal of sows from the herd and treatment costs. Willgert (2011) estimated the potential total treatment costs to be between 19£ (early case) and 266£ per affected sow (approximately 21€ - 296€ or 23USD-323USD[3]). Thus, the timely and reliable detection of sow lameness may increase farm profitability as well as animal welfare.

In most practical settings, lameness is detected by visually inspecting the sow's gait. Typically, a trained observer assigns a score on an ordinal scale, corresponding to the perceived severity of the condition (Main *et al.,* 2000; Welfare Quality®, 2009; Grégoire *et al.,* 2013). Several of the ordinal gait-scoring scales for pigs developed for research purposes have many categories and detailed descriptions of the different behavioral components of lameness (Chapter 1). However, the aggregation of categories or their retrospective simplification has been recommended by some authors as one of the methods to increase inter-and intra-observer repeatability when assessing lameness in practical settings (Brenninkmeyer *et al.,* 2007; Channon *et al.,* 2009; D'Eath, 2012) and the use of few gait-scoring categories is indeed common in animal welfare assessment protocols for pigs

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[3] Exchange rate of November 2016 (InforEuro- European Commission)

(Welfare Quality®, 2009; RSPCA, 2015; BPEX, 2013).

The number of scores available in any given scale is important because it determines the smallest degree of discrimination possible: scales with only two or three response levels offer limited opportunity to fully exploit the trained observer's discriminative capacities (Hjermstad *et al.,* 2011). From an epidemiological and animal welfare perspective, the reduction of the number of gait-scoring categories, particularly when these are reduced to a simple "lame/non-lame" classification, is likely to entail the loss of potentially important information on the lameness status of individual sows and of entire sow herds. It should also be noted that many factors besides the number of categories influence intra- and inter-observer repeatability (intra-OR and inter-OR), among which training and experience (Main *et al.,* 2000; Brenninkmeyer *et al.,* 2007) and the use of clear and specific descriptors (Welsh *et al.,* 1993; Flower and Weary*,* 2006) are important. At the opposite end of the spectrum relative to the simplification of scoring systems are visual analogue scales (VASs). These can be used in both humans and animals to assess physiological phenomena (including pain and lameness) that are considered to range across a continuum of values (Tuyttens *et al.,* 2009; Hjermstad *et al.,* 2011; Viñuela-Fernández *et al.,* 2011). The much larger range of available scores means that it is possible to record a change on a VAS when a change in category would not be achieved (Averbuch and Katzper, 2004). In addition, VASs that are "tagged" or "labeled" with descriptors (tVASs) retain some of the advantages of ordinal scales with clearly defined categories because observers are helped to make consistent choices (Lansing *et al.,* 2003; Averbuch and Katzper, 2004).

To investigate factors affecting intra- and inter-OR when scoring sow lameness from video, this study compared the results obtained with a tVAS, a 5-point and a 2-point ordinal scale (5P and 2P) with identical descriptors. In addition, we tested the effects on intra- and inter-OR of lameness severity, video orientation (original vs. mirrored horizontally), video sequence (first 45 vs. last 45 clips), users' opinions on the scales and declared experience in lameness evaluation. Finally, the users' opinions on each of the three scales are presented.

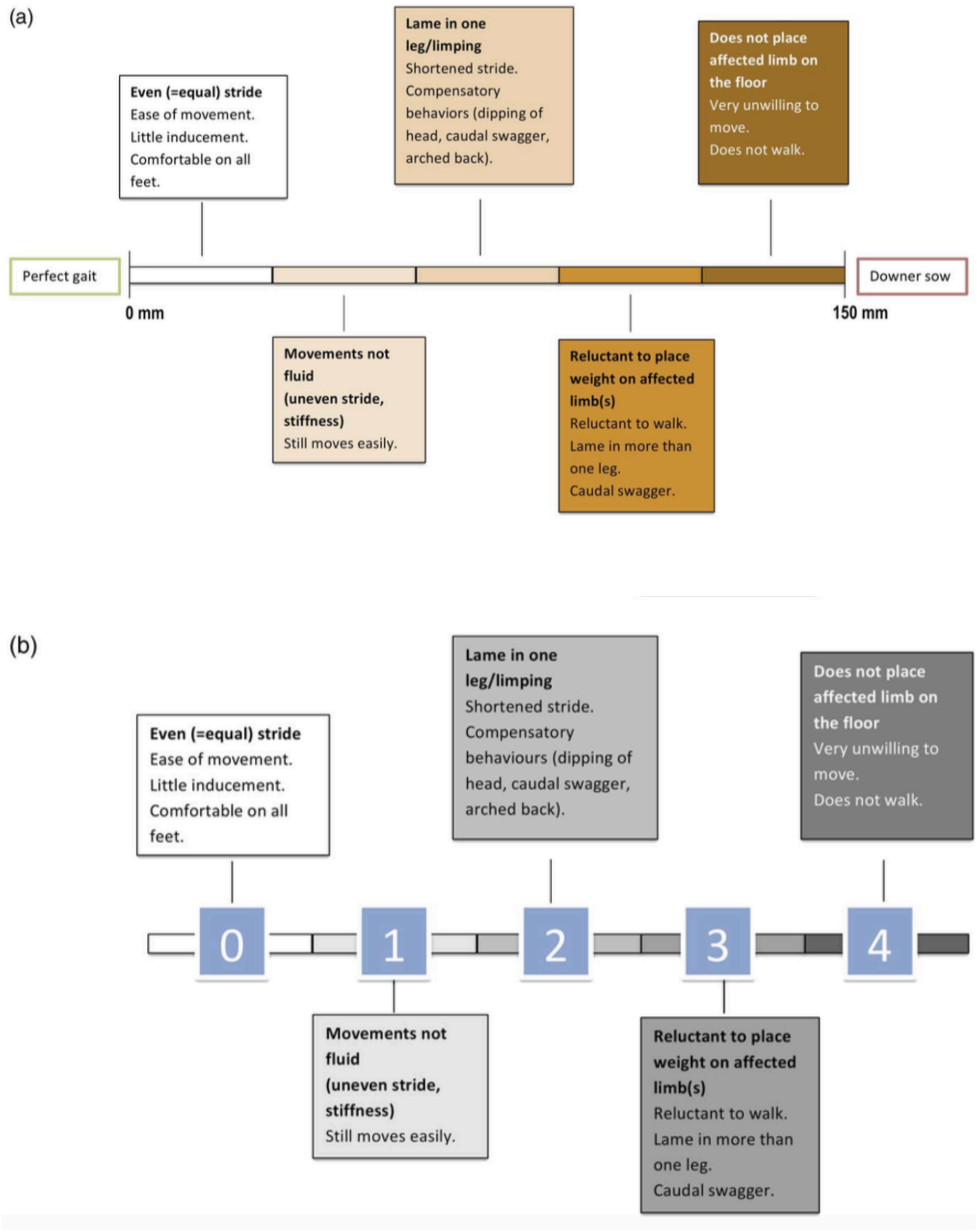
Materials and methods

Ethics statement

The filming of sows was carried out within an experimental protocol approved by the Animal Experiment Ethics Committee of the ILVO (approval n. 2011/146 and subsequent modifications).

Scoring scales

A 150 mm ‘tagged’ visual analogue scale (tVAS) with colour codes identifiying different degrees of sow lameness was developed (**Fig.3.1***A*). The tags consisted of 5 text boxes with a series of descriptors derived from the specific literature on pig lameness assessment (Main *et al.,* 2000; ZinPro Corp., 2009; Grégoire *et al.,* 2013). These text boxes were distributed along the full length of the tVAS and were matched to five coloured areas (each 30 mm in length) that visually guided the users from one extreme of the tVAS (“perfect gait”) to the other (“downer sow”).



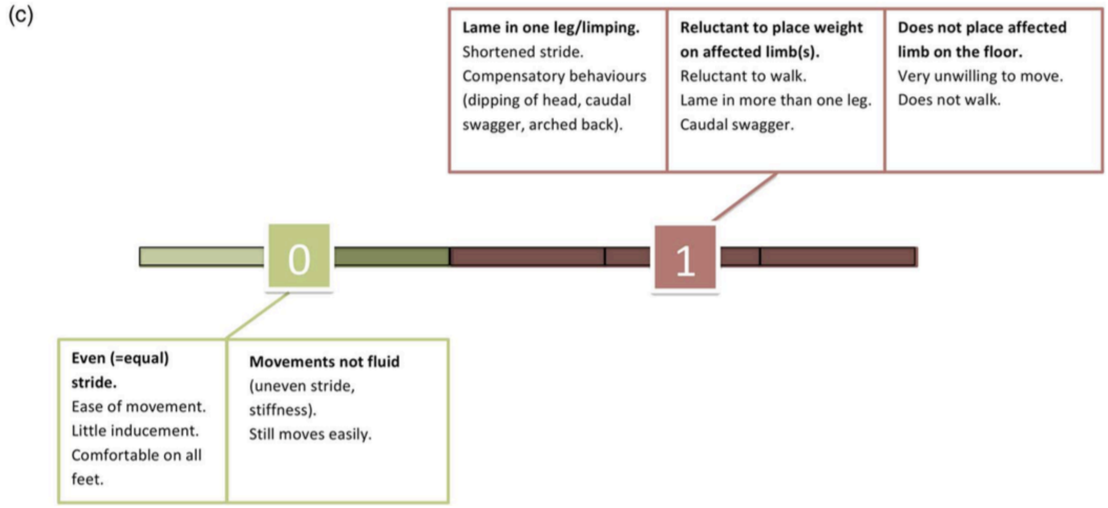


Figure 3.1.

A The tagged visual analogue scale (tVAS) created for the experiment. Observers can place a vertical mark anywhere along the 150-mm bar. The left-most extreme corresponds to a "perfect gait" and the right-most extreme to a "downer sow" (meaning a sow that is not capable of standing unaided). The different descriptor boxes and colors serve to help observers make full use of the length of the tVAS. Descriptors were adapted from Main *et al.* (2000), ZinPro Corp. (2009) and Grégoire *et al.* (2013).

B The 5-point ordinal scale mapped onto the tVAS. Five lameness categories (0 to 4) were graphically superimposed on the tVAS of Fig. 1A but descriptors remained identical.

C The 2-point categorical scale derived from the tVAS. Two lameness categories (0 and 1) were graphically superimposed on the tVAS of Fig. 1A. Descriptors were maintained but aggregated so as to fall under one of the two categories.

Two categorical scales were derived from this tVAS, namely a 5-point and a 2-point scale (5P and 2P). In the 5P, numbers from 0 to 4 were superimposed to the tVAS in correspondence with the descriptors (**Fig.3.1*B***). In the 2P, the numbers 1 and 2 were superimposed centrally to the 0-60 mm and 61-150 mm areas of the tVAS and descriptors were aggregated so as to fall under one of the two categories (**Fig.3.1*C***). The 2P and 5P only allowed full scores.

Participants and introductory setting

The experiment took place at the Faculty of Veterinary Medicine of Ghent University (Merelbeke, Belgium) on 14 April 2012. One hundred and eight 3rd-year undergraduate veterinary medicine students (89 female, 19 male) participated in the study. The students were first given a 25 min lecture on lameness in sows, illustrated by video examples of different severity. The tVAS, 5P and 2P were then introduced and the relevant descriptors were explained one by one, also by means of video examples. A 20 min interactive training session followed, in which the students used the three scales to score nine videos of sows with varying degrees of lameness (including downers, i.e., non-deambulating animals). Finally, the students discussed their results with the experts. The videos used during the introductory part and the training session were different from those used in the actual experiment. The introductory lecture, training session and experiment proper, consisting of three scoring sessions, took place in the same afternoon.

Selection of videos

Fifty-four 30 s videos of commercial hybrid sows (Ra-Se) with varying degrees of lameness were used for the study. The videos were taken from a gait-scoring library consisting of ca. 150 clips filmed in the open at the ILVO experimental pig farm (Melle, Belgium) between 2011 and 2012. Each sow was filmed while walking back and forth along a 60 m concrete run. The sows were encouraged to walk by a technician who walked alongside them, using sound cues or waving arms as necessary. The three severely lame sows in the sample were made to walk the shortest distance possible to obtain a clear view of all sides on video. The filming technique was semi-standardized, in that distance and perspective were not fixed; however, the videos were edited to obtain 30 s clips in which all sides of the sows were shown for a fixed amount of time (front: 10 s, back: 10 s, left side: 5 s, right side: 5 s).

In preparation for the experiment, two experienced observers (the first author and a technician) viewed and independently scored the edited clips with the tVAS. The criterium for including a video into the experiment was a maximum disagreement of 30 mm (i.e., the length of one category on the tVAS as marked by different colors and descriptors) between the scores attributed by the two observers. The mean of the experts' scores was used as a "gold standard", or the "true" score for each clip. To establish mean reference values for the other two scales, the videos were then independently re-scored one week apart by both experts with the 5P and the 2P. The intra-class correlation coefficients (95% CI) of the experts' scores were 0.88 (0.81 -0.93) with the tVAS, 0.86 (0.79 - 0.91) with the 5P and 0.62 (0.43 - 0.81) with the 2P. The selected clips were chosen to represent a wide range of lameness severity. However, there were only three severe cases (range 91-120 mm) and no very severe cases (range 121-150 mm) within the ILVO herd and therefore not all degrees of lameness were represented in the sample (**Table 3.1**).

Table 3.1. Proportion of videos used in the experiment based on lameness severity (as determined by the experts).

Score range on tVAS	*Concise description (not for experimental purposes)*	*N. of videos*
0-30 mm	Normal	21
31-60 mm	Stiff	15
61-90 mm	Lame (moderate)	15
91-120 mm	Lame (severe)	3
121-150 mm	Lame (very severe to downer)	0

tVAS = tagged visual analogue scale.

To establish the students' intra-OR for the three scales, 18 videos (six per session) were shown three times in a randomized order. In order to reduce memory effects we manipulated one of the repeats (Engel *et al.,* 2003) by mirroring the videos horizontally. However, to verify the potential effect of mirroring on intra-OR, we also presented each video for a third time, again in its original format.

Experimental set-up

First, information was collected on previous experience in (1) handling pigs, (2) scoring lameness in pigs and (3) scoring lameness in other species. The questions were of the yes/no type and no further information was collected on the nature or duration of the declared experience. Subsequently, the students were asked to score 90 videos in three separate 30 min sessions (30 videos per session), separated by 15 min breaks. All the available degrees of lameness were equally represented within each session.The students were randomly assigned to one of three groups and were given a scoring guide and three printed scoring sheets, stapled in a different order for each group. During each scoring session the three groups scored with a different scale, so that by the end each group had scored 30 videos per scale (**Table 3.2**). The paper sheets were completed in pen; students were instructed to place one single vertical mark on the tVAS or to cross the number on the ordinal scale corresponding to their score. Scores could be changed by clearly signalling the old and new value. The results were automatically transferred to a computer with a digital caliper (Mitutoyo ABSOLUTE 500-733-10; LCD resolution: 0.01mm; repeatability: 0.01mm). The measurements were expressed in millimeters and approximated to the first decimal place.

Table 3.2. Order in which the three groups of students (n=108) assessed 90 videos on sow lameness using the tVAS, 5P and 2P scale in 3 different scoring sessions.

	Session I	***Session II***	***Session III***
	(min 0-30)	***(min 45-75)***	***(min 90-120)***
Videos	1-30	31-60	61-90
Group			
A (n=36)	2P scale	5P scale	tVAS
B (n=36)	5P scale	tVAS	2P scale
C (n=36)	tVAS	2P scale	5P scale

tVAS = tagged visual analogue scale.

The seating order ensured that neighbouring students belonged to different groups, thus scoring with different scales. In addition, the students were instructed to score independently and not to discuss scores during the experiment. Each video was shown twice with 3 s in between successive viewings so that students had time to assign their score. At the end of the experiment, the students were also asked to rank the scales in terms of which one would in their opinion yield the most (rank = 1) to least (rank = 3) consistent scores between and within observers. Finally, an open question could be filled in concerning the advantages and disadvantages of using the tVAS for lameness assessment in sows.

Statistical analysis

The same modeling technique, assuming approximate normality, was applied to all scales. According to the central limit theorem, the distribution of the average scores for 2P and 5P could be assumed to approximate normality because of the large sample size. As a rule of thumb, in the binomial case (e.g., the 2P, which shows the strongest deviations from normality) the approximation will be sufficient if the expected number of observations in each combination of levels is larger than five. Thus, even for true probabilities of success (p) of a binomial distribution of 10%, a sample size of 50 will be sufficient to approach approximate normality, a sample size that was exceeded in this experiment.

Intra- and inter- OR were calculated from the variance components of a mixed model with sow, student and their interaction as random factors (Viñuela-Fernández *et al.,* 2011). Estimates of variance components were obtained using Monte Carlo Markov Chains (MCMC) in a Bayesian framework. With this approach the posterior distributions are approximated by a large (N=10 000) number of samples, which can then be used to construct credibility intervals, the so-called Highest Posterior Density (HPD-) intervals. The prior distributions used were the default inverse-Wishart distributions representing weak prior information. MCMC iterations were applied to construct HPD-intervals for the derived parameters, i.e., the proportions of variances and differences in these proportions. MCMC were then used to estimate credibility intervals for intra- and inter-OR and their differences among the scoring scales. These intervals can then be used to decide if differences are 'statistically significant' in a frequentist interpretation. Thus, if zero is not within a credibility interval, the difference will be indicated as statistically significant.

To gain a better insight into the factors influencing intra- and inter- OR, students' performances were further compared according to i) two levels of gait abnormality based on the experts' scores on the tVAS (none/mild vs. moderate/high), ii) presentation of the repeated videos (normal vs. mirrored horizontally), iii) order of the videos in the sequence (first 45 vs. last 45 clips, possibly influenced by fatigue), iv) declared experience in pig handling and lameness evaluation and v) users' opinions on the scales.

Finally, two exploratory analyses were performed to examine in more detail the differences in intra- and inter-OR among the three scales. Individual sows' scores were averaged across observers (dependent variable) and regressed against the experts' scores. This made it possible to investigate the strength of the association between the students' and experts' scores and the possible deviations from linearity, which would indicate expert/student disagreement within a particular range of lameness scores. Correlation coefficients – either parametric or non-parametric, depending on linearity – were calculated. In addition, frequency distributions and descriptive statistics were obtained by calculating the correlation between the students' and experts' scores across sows for each scoring scale. All analyses were performed in R (R Core Team, R Foundation for Statistical Computing, version 2.15.2, 2012, freely available at: http://www.R-project.org).

Results

Descriptives

All scoring sheets were returned; eleven were incomplete but the filled-in data were included in the analysis. The Q&A section was completed by all but two participants. Thirty percent of the students had previous experience in handling pigs. While 29.6% declared to have some experience in lameness scoring in other animal species, only 2.8% had already assessed lameness in pigs.

Inter- and intra-observer repeatability with the three scoring scales

While intra- and inter-OR were similar for the 5P and tVAS scales, values were lower for the 2P (**Table 3.3**). In particular, the 5P had a significantly higher intra-OR than 2P and the tVAS had both a higher intra- and inter-OR than the 2P. Intra-OR was higher than inter-OR for all scales. When scoring with the tVAS, students showed a higher inter- and intra-OR when assessing sows with more overt signs of locomotor problems, i.e., those ranging from stiff to lame (there were no severe cases in the sample). This difference was significant if 45 mm (mean of the experts' scores) was used as a cut-off on the scale to separate sows with normal to slightly stiff gait from sows with higher degrees of lameness. There was no evidence of differences in inter- or intra-OR based on any of the other parameters considered: the effect of video order in the sequence (first 45 vs. last 45 clips), the users' declared experience in handling pigs or scoring lameness in other species, the direction of the repeated clips (original vs. mirrored horizontally), and the users' opinion on the scoring scales (**Table 3.4**). As the proportion of students who declared having some experience with scoring lameness in pigs was very low (3 out of 108), this factor was not included in the analysis. The same comparisons were performed for the 5P and 2P scales with very similar results (values not shown).

Table 3.3. Inter- and intra-observer repeatability of the students' lameness scores with the three scoring scales. Results are expressed as medians and 95% HPD-interval of the posterior distributions obtained using MCMC. Differences among the three scales and their 95% HPD-intervals are also provided as pairwise comparisons.

Scoring scale	***Inter-observer repeatability***	***Intra-observer repeatability***
2P	0.60 (0.50 – 0.69) ^{a}	0.67 (0.59 – 0.75) ^{a}
5P	0.71 (0.63 – 0.79) ^{ab}	0.81 (0.75 – 0.86) ^{b}
tVAS	0.73 (0.65 – 0.81) ^{b}	0.80 (0.75 – 0.85) ^{b}
Pairwise comparisons[1]		
5P vs. 2P	0.11 (-0.02 – 0.23)	**0.14 (0.04 – 0.24)**
tVAS vs. 2P	**0.13 (0.005 – 0.25)**	**0.1 (0.03 – 0.23)**
tVAS vs. 5P	0.02 (-0.09 – 0.13)	-0.01 (-0.10 – 0.06)

^{a,b} Values within a column with different superscripts differ significantly atP <0.05.

HPD = highest posterior density; tVAS = tagged visual analogue scale; 2P = 2-point ordinal scale; 5P = 5-point ordinal scale.

[1] Statistically significant differences – i.e., those with HPD intervals not containing 0 – are in bold (P <0.05).

Table 3.4. Inter- and intra-observer repeatabilities (medians and 95% HPD-intervals) of the students' lameness scores with the tVAS. The table reports the students' intra-and inter-OR for sows with relatively high (>60mm or >45mm) or low (≤60mm or ≤45mm) degrees of lameness and the effects of other possibly influencing factors. Differences and their 95% HPD-intervals are also provided.

	Inter-observer repeatability	***Intra-observer repeatability***
Effect of degree of lameness		
tVAS ≤60 mm	0.49 (0.37 – 0.60)	0.59 (0.49 – 0.69)
tVAS>60 mm	0.60 (0.44 – 0.76)	0.68 (0.55 – 0.80)
Difference	0.11 (-0.08 – 0.30)	-0.09 (-0.25 – 0.08)
tVAS ≤45 mm	0.38 (0.26 – 0.53)	0.47 (0.34 – 0.59)
tVAS>45 mm	0.67 (0.56 – 0.80)	0.76 (0.66 – 0.84)
Difference	**0.28 (0.11 – 0.46)**	**0.28 (0.12 – 0.44)**
Effect of (repeated) video orientation		
Original - Original (repeated)	0.74 (0.66 – 0.82)	0.78 (0.72 – 0.84)
Original - Mirrored horizontally	0.76 (0.64 – 0.89)	0.78 (0.65 – 0.88)
Difference	0.02 (-0.12 – 0.16)	-0.0 (-0.13 – 0.13)
Effect of video order in the sequence		
First 45 clips	0.70 (0.60 – 0.81)	0.77 (0.69 –0.86)
Last 45 clips	0.75 (0.64 – 0.84)	0.83 (0.75 –0. 90)
Difference	0.05 (-0.09 – 0.20)	0.05 (-0.06 – 0.16)
Effect of experience in handling pigs		
Yes (30.0% of respondents)	0.75 (0.66 – 0.82)	0.81 (0.74 –0. 87)
No	0.72 (0.64 – 0.80)	0.78 (0.72 – 0.84)
Difference	-0.02 (-0.13 – 0.09)	-0.04 (-0.12 – 0.05)
Effect of experience in scoring lameness in species other than pigs[1]		
Yes (26.9% of respondents)	0.73 (0.65 – 0.81)	0.81 (0.75 – 0.86)
No	0.73 (0.66 – 0.81)	0.78 (0.72 – 0.84)
Difference	0.0 (-10.1 – 10.2)	0.03 (-0.07 – 0.11)
Effect of users' opinion on easiness of scoring with the tVAS		
Difficult (score above median)	0.74 (0.66 – 0.81)	0.79 (0.72 – 0.85)
Easy (score equal to or below median)	0.74 (0.66 – 0.82)	0.80 (0.74 – 0.86)
Difference	0.00 (- 0.12 – 0.11)	0.02 (-0.11– 0.07)

HPD = highest posterior density; tVAS = tagged visual analogue scale; 2P = 2-point ordinal scale; 5P = 5-point ordinal scale.

Statistically significant differences – i.e., those with HPD intervals not containing 0 – are in bold (P <0.05).

[1] Differences based on declared experience in scoring lameness in pigs were not calculated because condition applied to only 2.8% of students.

Associations between the students' average lameness scores and the experts' scores were high and comparable for all scales (**Fig. 3.2**). For the 5P and tVAS, the association was linear and the regression line was located close to the bissectrice, indicating a nearly perfect agreement between the students' and experts' scores. For the 2P, however, the association was not linear, i.e., the data points were not scattered symmetrically around the estimated linear regression line. The discrepancy became most apparent for the expert score of 0.5, which indicates sows on which the two experts disagreed (5 videos in total; in 4 cases, one expert consistently scored "0" and the other "1"). For those sows, student scores were on the average lower than the intermediate experts' score of 0.5. This tendency was not present with the other scales. The deviation from the line of perfect agreement, expressed as percentage, was 29%, 22% and 22% for the 2P, 5P and tVAS, respectively.

Figure 3.3 provides frequency distributions of correlation coefficients between the students' and experts' scores across the different sows for each scoring scale. These distributions confirm the earlier results in that the 5P and tVAS scales show very comparable characteristics, while correlation coefficients are smaller for the 2P. In addition, the distribution of the correlation coefficients for the 2P shows a wide tail to the left, indicating that a relatively large group of students showed high agreement with the experts' scores but that, for a small group, correlations dropped very strongly.

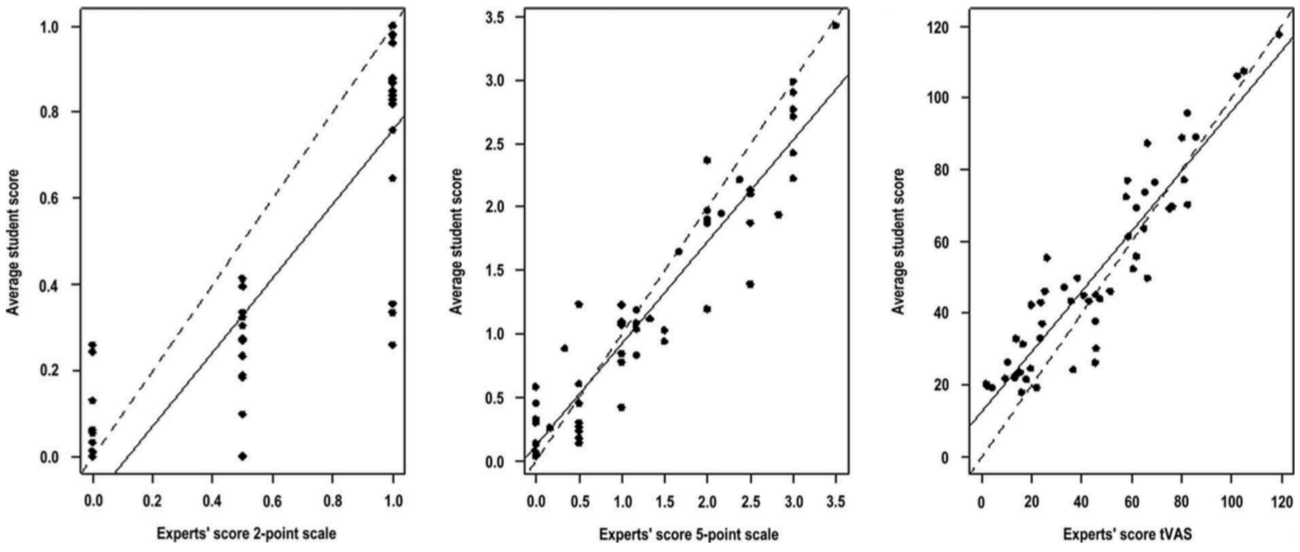


Figure 3.2. Associations between experts' scores and the averages students' scores for the three scoring scales. The regression line (solid) and bissectrice (dashed line) are provided. Non-parametric Spearman Rank correlations equaled 0.88, 0.91 and 0.92 for the 2P, 5P and tVAS, respectively.

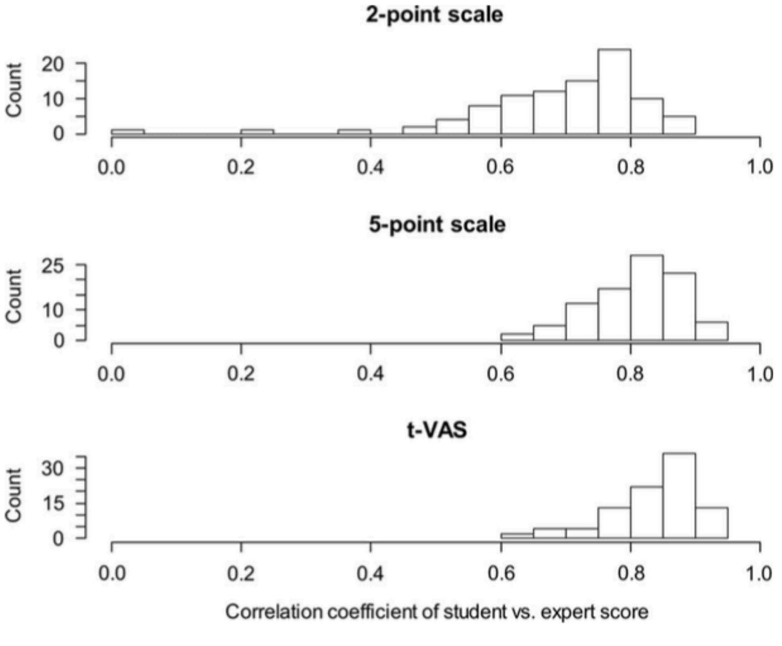


Figure 3.3. Frequency distributions of the parametric correlation coefficients of students' vs. experts' scores for each student with the three scoring scales. The averages of these correlation coefficients (and their standard deviations) were 0.70 (0.13), 0.81 (0.07) and 0.83 (0.07) for the 2P, 5P and tVAS, respectively.

Students' questionnaire on the three scoring scales

At the end of the experiment, the students were asked to fill out a short questionnaire on the three scoring scales. The first three questions and a descriptive overview of students' responses are reported in **Table 3.5**. The tVAS and 5P were considered equally appropriate for the assessment of lameness status at the herd level, while the 2P scored lower. However, the students clearly indicated a preference for the tVAS for the clinical evaluation and follow-up of individual lame sows. Learning to use this instrument was perceived as quite difficult, while the 5P and 2P were considered easier to learn. The students were also asked to rank the scales in terms of which one would in their opinion yield the most (rank = 1) to least (rank = 3) consistent scores between and within observers. The 2P was ranked as the most consistent scale (rank 1) by 75% of participants, followed by the 5P (rank 2: 80.2%) and tVAS (rank 3: 83.3%). The main reported advantages of using the tVAS were a higher accuracy, precision or specificity (indicated by 70% of respondents; more than one response was possible). However, as these last questions were of the "open" type, the answers should be interpreted with caution as the students may not have been fully aware of the differences between accuracy, precision and specificity. Absence of strict boundaries between categories and a greater freedom of choice were also considered advantageous (27%). A minority of students indicated easiness of use or the visual nature of the tool as advantages (2%). By contrast, many students considered scoring with the tVAS more subjective or leading to more variability in the results (49%), as well as difficult to learn and to use in practice (40%). Some students found scoring with the tVAS time consuming (24%). A minority commented that the tVAS is possibly best used only for the follow-up of individual animals (4.6%).

Table 3.5. Students' opinions on the use of three scoring scales for lameness assessment in sows. The students answered each question by placing a vertical mark on a 118-mm visual analogue scale. The wordings at the extremes varied and are reported under each specific question.

	Question 1	***Question 2***	***Question 3***
	Rate how appropriate you think the three scales are to evaluate **lameness status in 20 sow herds by means of short farm visits** (max ½ day per farm)	Rate how appropriate you think the three scales are for accurately checking **the degree of lameness of an individual lame sow** before and after treatment	Rate **how easy you think it is to be trained to score consistently/reliably** with each of the three scales
Min score	0 mm= totally inappropriate	0 mm= totally inappropriate	0 mm= extremely easy
Max score	118 mm= most appropriate	118 mm= most appropriate	118 mm= not at all easy
Scale			
2P	55.6 (± 36)$^{a, 1}$	22.3 (± 20.1)^{a}	25.2 (± 17.9)^{a}
5P	80.9 (± 19.4)^{b}	79.7 (± 22.6)^{b}	62.2 (± 17.8)^{b}
tVAS	72.6 (± 31.6)^{b}	101.2 (± 19.2)^{c}	82.0 (± 23.5)^{c}
F-test:	$F_{2,285}$= 18.0,P <0.0001	$F_{2,285}$= 373,P <0.0001	$F_{2,285}$= 202,P <0.0001

tVAS = tagged visual analogue scale.

The students answered each question by placing a vertical mark on a 118-mm visual analogue scale. The wordings at the extremes varied and are reported under each specific question.

^{a,b,c} Values within a column with different superscripts differ significantly at P < 0.01. Questions have been summarised. Emphasis is original.

^{1} Scores are expressed in mm as means ± S.D.

Discussion

Our experimental setting offered a unique opportunity to compare the performance of three gait-scoring scales for sows by collecting a large amount of data from 108 freshly trained observers. Additionally, as descriptors were maintained across scales, the found differences can be exclusively ascribed to the intrinsic characteristics of the scales (i.e., number of categories; continuous vs. ordinal) rather than to the wordings used to define gait.

Our findings contradict the assumptions that lie behind the use of scales with few categories: in this study, inter-OR, intra-OR, and frequency distributions of correlation coefficients between the students' and experts' scores were lower with the 2P than with the tVAS and 5P. It follows that freshly trained observers could reliably discriminate between at least five different levels of sow lameness on an ordinal scale. Even more interestingly, repeatabilities on the tVAS were comparable with those of the 5P. These results are in line with what was previously described by other authors, i.e., that repeatability depends on the specific characteristics of both the observers and the scales. Tuyttens *et al.* (2009) reported that a modified VAS for gait scoring in dairy cattle – with visual anchoring points – had a superior inter-observer repeatability compared with a 3-point ordinal scale with the same descriptors. Viñuela-Fernández *et al.* (2011) observed a higher repeatability when students scored horse laminitis on a VAS compared with two ordinal scales. Finally, in their comprehensive review of the literature comparing the available clinical tools for the self-reporting of pain in human patients, Hjermstad *et al.* (2011) found that the most commonly used scales (i.e., a numerical rating scale with 11 categories, a verbal rating scale with 7 categories, and a 100-mm VAS) had comparable performances. It should also be noted that repeatability is not a fixed property of any given scale; on the contrary, it is influenced by the group of observers carrying out the assessment and by the circumstances of the observation (Streiner, 2013) as well as by the level of training and experience (Brenninkmeyer *et al.,* 2007; Viñuela-Fernández *et al.,* 2011). This study presented a number of unique characteristics: firstly, the observer population can be considered homogeneous for educational background and level of experience. Very few students declared to have previous experience in scoring lameness in pigs, and we did not collect detailed information on the nature and duration of this experience. Thus, for the purpose of this experiment, the student population can be considered to be relatively inexperienced. Additionally, the observations happened under the same circumstances and the students were trained just before scoring. Finally, the proportion of lame sows (> 60 mm on the tVAS) in the selected videos was 33.3% versus a reported on-farm prevalence of 8.8% to 16.9% (Heinonen *et al.,* 2006; KilBride *et al.,* 2009; Heinonen *et al.,* 2013). This can be considered an unusually high level of exposure, which could have influenced the results.

Comparison of our results with those of similar studies is complex, because various methods can be used to derive measures of repeatability. We adopted the method of Streiner and Norman (2008) as described in Viñuela-Fernández *et al.* (2011), which is appropriate whenever more than two observers are involved and which takes into account multiple sources of error variance. The minimum acceptable level of repeatability depends on the aims of the measurement and should be established on a case-by-case basis. It has been proposed that for most applications a repeatability of 0.60 can be considered as an acceptable minimum threshold, with 0.80 representing a high repeatability (Streiner, 2013). Elsewhere (Portney and Watkins, 2000, cited in Viñuela-Fernández *et al.,* 2011), values lower than 0.50 were considered as indicative of poor repeatability, between 0.50 and 0.75 of moderate repeatability, and above 0.75 of good repeatability. Thus, our results indicate that the 2P had only acceptable to moderate overall repeatabilities; the intra-OR of both tVAS and 5P was good to high and the inter-OR was moderate to high.

One limitation of this study was that the filming technique was not standardized; thus, recognizable elements in the videos (perspective, operator leading the sow, buildings, weather conditions etc.) might have artificially increased intra-OR. The choice to avoid standardized conditions was justified by the fact that these are rarely found in practical settings. Additionally, it should be noted that scoring from video allows for an optimal view of all sides of the sow, while in most on-farm situations this is not always the case. As the reported on-farm prevalence of sow lameness is lower than in the videos used in the present study, and we found lower inter- and intra-OR for normal to stiff sows, it is possible that repeatability would be lower under field conditions. On the other hand, the reported field prevalence of lameness depends in turn on the sensitivity of the scale and on the threshold chosen to classify an animal as "lame". Consequently, it is equally possible that using more sensitive scales with clear descriptors could result in increased recorded on-farm prevalences. For all of the above-mentioned reasons, the reported repeatabilities should be verified under field conditions.

The lower intra- and inter-OR observed for sows with minimal to slight gait abnormalities such as stiffness is consistent with the results of previous studies in pigs (D'Eath, 2012), dairy cattle (O'Callaghan, 2002; Flower and Weary, 2006; Brenninkmeyer *et al.,* 2007) and sheep (Welsh *et al.,* 1993). In general, inter-observer repeatability increases with increasing severity of lameness (Welsh *et al.,* 1993; Menzies-Gow *et al.,* 2010). This phenomenon can have negative implications for animal welfare, especially when sows with slight gait abnormalities are missed or routinely classified as "non-lame". In fact, sows that are at the early stages of lameness are the ones that can most benefit from timely veterinary treatment (Pluym *et al.,* 2013).

The majority of participating students indicated the tVAS as the most challenging scale to learn. However, these perceptions had no effect on repeatabilities and correlations with experts' scores: although students considered the tVAS as the least intuitive to use, performances were best with that scale. In a study on dairy cattle (Tuyttens *et al.,* 2009), users' preference for a specific scale influenced their performance: users that expressed a preference for the tVAS had a significantly higher inter-OR with that scale compared to an ordinal scale.

Conclusions

The tagged visual analogue scale (tVAS) and the 5-point ordinal scale (5P) developed within this study to assess lameness in sows were found to have similarly high inter-and intra-observer repeatabilities as well as a high correlation with the experts' scores. In addition, the tVAS was superior in all respects to the 2-point scale (2P), while the 5P was superior to the

2P in terms of intra-observer repeatability. Observers were less consistent when scoring the lowest degrees of gait abnormalities. None of the other factors included in our analysis (in particular fatigue, video presentation, and the observers' opinions on the scales) had an effect on performances. The use of a continuous scoring scale such as a tagged VAS, or at least a 5-point ordinal scale, is in our opinion advisable when scoring lameness in sows to make full use of the trained observer's discriminative abilities. Future research should examine the performance of the three scales under field conditions and with different types of observers.

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Chapter 4 - Mechanical nociceptive thresholds in lame sows: evidence of hyperalgesia as measured by two different methods

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Abstract

Lameness is a frequently occurring, painful condition of breeding sows that may result in hyperalgesia, i.e., an increased sensitivity to pain. In this study a mechanical nociception threshold (MNT) test was used (1) to determine if hyperalgesia occurs in sows with naturally- occurring lameness; (2) to compare measurements obtained with a hand-held probe and a limb-mounted actuator connected to a digital algometer, and (3) to investigate the systematic left-to-right and cranial-to-caudal differences in MNT. Twenty-eight pregnant sows were investigated, of which 14 were moderately lame and 14 were not lame.

Over three testing sessions, repeated measurements were taken at 5 min intervals on the dorsal aspects of the metatarsi and metacarpi of all limbs. The MNT was defined as the force in Newtons (N) that elicited an avoidance response, and this parameter was found to be lower in limbs affected by lameness than in normal limbs ($P < 0.05$). Forelimbs had higher MNTs than hindlimbs ($P < 0.001$). The hand-held probe systematically yielded lower values than the actuator ($P < 0.001$), and the MNT differed between morning and afternoon testing sessions ($P < 0.001$), as well as between days ($P < 0.001$). The findings provide evidence that lame sows experience hyperalgesia. Systematic differences between forelimb and hindlimb MNT must be taken into account when such assessments are performed.

Introduction

Studies indicate that 8.8-16.9% of sows bred within the European Union exhibit some degree of lameness (Heinonen *et al.,* 2013). Lameness results in major economic losses in pig production (Deen *et al.,* 2008; Willgert, 2011; Heinonen *et al.,* 2013; Pluym *et al.,* 2013), and is one of the principal reasons for the culling, euthanasia or death of otherwise productive gilts and young sows (Heinonen *et al.,* 2013; Pluym *et al.,* 2013). The welfare of lame sows is also a great concern as lame animals experience pain and are less able to cope with their environment and herd mates, resulting in a significantly reduced quality of life (Galindo and Broom, 2002; Metz and Bracke, 2005; Anil *et al.,* 2009; Heinonen *et al.,* 2013).

Limb and joint disorders can lead to hyperalgesia (Ley *et al.,* 1995, 1996; Whay *et al.,* 1998, 2005; Laven *et al.,* 2008; Brydges *et al.,* 2012), defined as 'increased pain derived from a stimulus that normally provokes pain' (IASP, 2012). This hypersensitive state, typical where persistent inflammation exists, is caused by the release of pro-inflammatory mediators at the site of injury and in the spinal cord, which alters sensory nerve function and nociception (Dolan *et al.,* 2011). Although hyperalgesia typically reflects underlying disease and serves to protect vulnerable tissues, it becomes a disease in its own right if it persists after its cause has been treated (Sandkühler, 2009). Therefore, investigating hyperalgesia is particularly important in species with a high prevalence of chronic diseases such as lameness, which is all too often under-diagnosed and under-treated (Kaler and Green, 2008; Vansickle, 2008; Whay *et al.,* 2011).

Various methods have been used to study pain and hyperalgesia, including mechanical nociception tests (Ley *et al.,* 1995, 1996; Whay *et al.,* 1998, 2005; Laven *et al.,* 2008; Dolan *et al.,* 2011; Love *et al.,* 2011). These tests involve the progressive application of a mechanical stimulus until a clear behavioural reaction, attributable to pain, is elicited. Typically, this involves pushing a metal pin against a pre- determined anatomical area. Upon triggering a visible reaction in the animal, the stimulus is removed and the mechanical nociception threshold (MNT) recorded. Different portable MNT testing devices have been described; some studies used hand-held pressure algometers (Haussler and Erb, 2006; Haussler *et al.,* 2007; Fosse *et al.,* 2011a,b; Janczak *et al.,* 2012; Di Giminiani *et al.,* 2013), while in other studies pressure transducers were connected to the operating unit by means of tubing and a 'limb-mounted' cuff (Dixon *et al.,* 2007, 2010; Kemp *et al.,* 2008). The choice of method has practical implications, including the possibility that the measurements will vary because of particular biomechanical conditions.

The objectives of the present study were (1) to compare MNTs in lame vs. non-lame sows and verify whether lame sows suffer from hyperalgesia; (2) to compare the measurements obtained with a hand- held probe with those of a limb-mounted actuator connected to the same digital algometer, and (3) to investigate left-to-right and cranial- to-caudal differences in MNTs which, if present, would need to be taken into account when interpreting this type of assessment.

Materials and methods

Animal selection and housing

Three groups of Ra-Se sows from the ILVO (Institute for Agricultural and Fisheries Research, Melle, Belgium) herd with a mean gestation stage (± SD) of 97.6 ± 2.9 days were studied (n=12, n=8, and n=8 in groups 1, 2 and 3, respectively) between September and December 2011. Parity ranged 1-10 (mean, 3.6 ± 2.2) and bodyweight 217 - 329 kg (mean, 267 ± 33.4 kg). Animals in each group were housed together in a single pen from the fourth week of pregnancy until 1 week prior to farrowing. The pen measured 10.7 x 3.5 m and had a solid concrete floor and deep straw bedding. The sows were fed a restricted diet (2.6 kg of standard commercial gestation feed) once daily. Water was available *ad libitum*. Mean (± SD) ambient temperatures in the pen were 16.5 (± 1.0) °C, 12.5 (± 1.3) °C, and 9.9 (± 0.6) °C during September, October, and December, respectively.

Sows were initially selected based on their locomotion score, which was assessed on the 150 mm “tagged” visual analogue scale (tVAS) described in Chapter 3. The descriptors on the tVAS illustrate the typical signs of normal and abnormal gait in sows (Main *et al.,* 2000; ZinPro Corp., 2009; Mustonen *et al.,* 2011; Grégoire *et al.,* 2013): at one extreme, fluid movements and equal strides, progressing to stiffness and unwillingness to walk, presence of an arched back, caudal swagger, head-dipping and, at the other extreme, the reluctance or refusal to bear weight in one or more limbs.

Two experienced observers (EN and a technician) scored sows walking along a 60 m flat concrete run. Sows scoring >30 mm on the tVAS (mean of the two observers) were classified as ‘lame’ and were matched with non-lame controls (tVAS ≤30 mm). The herd had no severely lame animals at the time of sow selection. Locomotion scoring was repeated on lame sows and each limb was classified as ‘not-lame’, ‘lame’ or ‘doubtful’. The latter term was assigned when lame limb(s) could not be clearly identified. All four limbs of sows with a gait score ≤30 mm on the tVAS were initially classified as non- lame. For all sows, gait scoring and lameness evaluation of the limbs were repeated at the beginning of each testing day to record possible changes.

Experimental setting and instrumentation

An individual gestation crate (2 x 0.5 m) was fixed to the floor in a quiet area of the pig accommodation where the group pen was located. The sows were led into this crate during MNT testing, where they remained in auditory contact with other sows. The lowest bar of the crate was removed to facilitate access to the limbs during measurements. A feeder was fitted to the front end.

A digital algometer (ProdPlus MT1, TopCat Metrology) provided with a probe and two limb-mounted actuators was used. Pressure within the limb-strapped actuator ('actuator') was increased manually using a syringe and depressed a 1 mm diameter brass pin against the sow's limb until the animal showed an avoidance response (i.e. lifting the limb off the ground or kicking). During training and testing, actuators were strapped to both contralateral limbs. Rate lights ensured that inflation occurred at the desired predetermined rate of 4 N/s. At 'threshold', pressure was released immediately. A controller within the digital algometer held the threshold reading in Newtons (N).

A hand-held version of the same instrument supplied with a 1 mm diameter brass tip 'probe' (ProD-Plus, TopCat Metrology) was used to compare measurements. This probe was manually pushed against the limb at 90° and 4 N/s. At threshold, the operator withdrew the probe and the peak reading of force (N) was stored and displayed automatically.

Training and testing protocols

The experimental protocol was approved by the Ethics Committee of the ILVO (Reference 2011/146). The sows were trained daily for over one week. Once inside the modified gestation crate, straw and food rewards were provided. Straps with dummy actuators were then attached to the fore- and hindlimbs (**Fig. 4.1**) for 15 min; no pressure was applied during training. The sows' reactions were recorded daily and training was considered successful when the straps and dummies could be put in place with minimal effort and when the sows showed no signs of discomfort. All sows achieved this learning objective.

Fig 4.1. Detail of 'actuator' attached to right forelimb of a sow. During training and testing, a 'dummy' actuator was always attached to the contralateral limb.

The three groups of sows were tested in September, October and December 2011, respectively. On each of three testing days held 1 week apart, MNTs were measured on all limbs with both the probe and actuator in two sessions (morning and afternoon) in order to minimise stress. All measurements were carried out by the same experienced technician. The dorsal aspects of the metatarsi and metacarpi were marked in black to indicate the location of the stimulus. Four sows were tested daily.

On the morning session of the first day, actuators were strapped to the metacarpi and three repeated measurements were taken at 5 min intervals. Within the same session, three repeated measurements were taken with the probe at 5 min intervals on the dorsal metatarsal regions. Food rewards were given before each measurement to minimise 'stepping' behaviour due to restlessness. A cut-off value of 25 N was established to prevent tissue damage (Fosse *et al.,* 2011b). Sows that did not show a response at cut-off were assigned a threshold of 25 N. At the end of the test the actuators were removed and the sow was released back into the group. In the afternoon of the same day the actuators were strapped on the metatarsi and the probe was applied to the metacarpi. The testing order of the sows and the initial position (metacarpal vs. metatarsal) of the actuators were changed on each testing day. The order in which the limbs were tested was also changed between sessions.

Statistical analysis

Factors associated with the mechanical nociception threshold were identified fitting a linear mixed regression model with ‘sow’ and ‘limb within sow’ as random effects and ‘session within limb’ as a repeated effect using SAS 9.3 software (PROC MIXED, SAS Institute). A compound symmetry correlation structure was used to account for the clustering of repeated measurements within a session. The model with the mechanical threshold as continuous outcome variable included ‘lameness at limb level’ (predictor of main interest, 3 levels), ‘day’ (3 levels), ‘measurement nested within day’ (9 levels), ‘method’ (2 levels), ‘time of day’ (2 levels), ‘limb position’ (4 levels), ‘parity’ (3 levels), ‘gait score’ (2 levels), and ‘group’ (3 levels), as categorical independent variables and ‘bodyweight’ as a continuous variable. Additionally, all first-order interaction terms were tested. Significance was assessed at $P < 0.05$.

To evaluate the proportion of variation occurring at the different levels of the data hierarchy, a four-level null model (intercept only) with ‘sow’ (RANDOM statement) and ‘limb’ (REPEATED statement) as random effects, was fitted. The adequacy of the model was tested by examining normal probability plots of residuals and plots of residuals vs. predicted values to check whether the assumptions of normality and homogeneity of variance had been fulfilled. There were no patterns indicating heteroscedasticity.

Results

All 28 sows completed the study. Lame animals (n = 14) had a mean (±SD) gait score across all testing days of 68.1 ± 18 mm (range: 34-113 mm) on the tVAS, corresponding to ‘moderate’ lameness, *versus* 13.3 ± 8 mm (range: 2-29 mm) for non-lame sows (n = 14). Because of technical problems with the equipment, the first group of sows was not tested with the actuator on the third day and the third group was tested on two days instead of three. In 11% of measurements (n = 203), the mechanical stimulus did not elicit a behavioural response when the cut-off threshold of 25 N was reached, suggesting that this threshold was possibly too conservative for adult sows.

Factors affecting MNTs

The MNTs (± SEM) in lame limbs were lower than in non-lame limbs (12.84 ± 0.84 N vs. 14.89 ± 0.51 N; $P < 0.05$) (**Table 4.1**).

Table 4.2. Multivariable multilevel linear regression model describing the factors associated with the mechanical nociception threshold of 1680 measurements from 28 sows.

Independent variable	***n*** *measurements*	***β***[a]	***SEM***[b]	***LSM***[c]	***P-value***
Lameness (0-1-2) at limb level					0.02
Non-lame (0)	1266	Ref.	…	14.9	…
Doubtful (2)	201	0.18	0.57	15.1	0.75
Lame (1)	213	-2.07	0.77	12.9	0.01
Day					< 0.001
Day 1	672	Ref.	…	13.5	…
Day 2	672	0.73	0.29	14.3	0.04
Day 3	336	1.41	0.38	14.9	< 0.001
Measurement (Day)[d]	1680	…	…	…	0.83
Method					< 0.001
Actuator	768	Ref.	…	15.9	…
Probe	912	-3.23	0.26	12.6	< 0.001
Time					< 0.001
Morning	768	Ref.	…	14.9	…
Afternoon	912	-1.24	0.27	13.6	< 0.001
Limb position					< 0.001
Left front	420	Ref.	…	16.6	…
Left hind	420	-3.81	0.62	12.7	< 0.001
Right hind	420	-4.36	0.62	12.2	< 0.001
Right front	420	-1.03	0.65	15.5	0.12
Parity[e]					0.40
1	5	Ref.	…	15.4	…
≥ 2 and ≤ 4	16	-1.67	1.26	13.7	0.20
> 5	7	-1.66	1.44	13.7	0.26
Weight	84	...	...	...	0.41
Gait score (0-150mm) at sow level					0.51
≤ 30 ("non-lame")	39	Ref.	…	14.4	…
>30 ("lame")	45	-0.35	0.53	14.1	0.51
Group					0.20
Group 1- September	720	Ref.	…	13.4	…
Group 2 - October	576	0.37	1.27	13.8	0.77
Group 3 - December	384	2.14	1.19	15.6	0.08
Group × lameness[f]	…	…	…	…	0.02

[a] Regression coefficient

[b] Standard error of the mean

[c] Least square means

[d] Estimates are not shown

[e] Number of animals in each parity range

[f] Estimates are not shown

The MNT of ‘doubtful’ limbs (15.06 ± 0.68 N) did not differ from that of non-lame limbs. The thresholds differed between days (P <0.001); MNTs increased from the first to the third day (day 1, 13.55 ± 0.56 N; day 2, 14.28 ± 0.56 N; and day 3, 14.97 ± 0.61 N). MNTs were lower in the afternoon than in the morning sessions (P <0.001). However, no significant differences were observed between measurements within a day (P=0.83). Forelimbs had remarkably higher MNTs than hind limbs (P <0.001). No substantial left-to-right differences were observed. The probe yielded systematically lower MNTs than the actuator (P <0.001; **Fig. 4.2**).

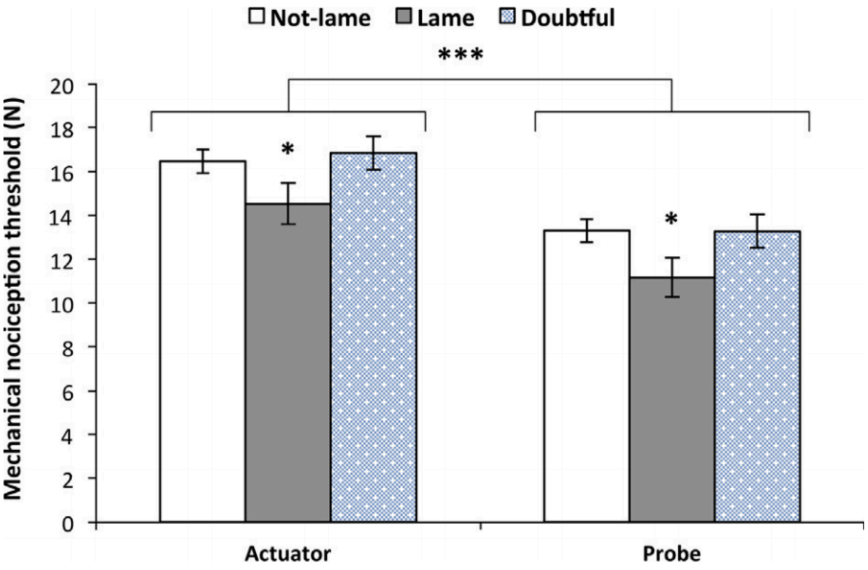

Fig. 4.2. Mean mechanical nociception thresholds (±SEM) measured with limbmounted actuator and with the probe on ‘not-lame’, ‘lame’ and ‘doubtful’ limbs of sows. *** P < 0.001; * P < 0.05.

The mean (± SEM) MNT as measured with the actuator was 14.53 (± 0.94) N for lame vs. non-lame (16.47 ± 0.54 N) limbs. Using the probe, the mean MNT was 11.17 (± 0.90) N for lame, and 13.31 (± 0.53) N for non-lame limbs. MNTs were not affected by bodyweight, parity or gait score. Although MNTs were not significantly different between groups, the difference between lame and non-lame limbs was more pronounced in the second and third groups than in the first (P=0.02). Variation primarily resided at observation level (68.9%; **Table 4.2**). Almost 14.1% of the variation occurred at sow level, whereas 17.0% occurred at limb level. These proportions only changed slightly when adding the fixed effects. Of the total variance, 22.2% was explained by these fixed effects.

Table 4.3. Variance components at the sow, limb, and observation level of the null and full model.

Data hierarchy	***Null model***		***Full model***	
	Variance estimate	***%***	***Variance estimate***	***%***
Sow	6.3	14.1	3.8	10.9
Limb	7.6	17.0	3.5	10.1
Observation	30.7	68.9	27.4	79.0
Total variance	44.6	100.0	34.7	100.0

Discussion

This study used a mechanical nociception threshold test to investigate hyperalgesia in lame sows and the factors affecting MNTs. Lame sows had lower MNTs in lame limb(s), revealing local sensitisation to noxious stimuli (hyperalgesia). This condition can be treated (Dolan *et al.,* 2011), although studies in dairy cattle have shown that (depending on the type, location and severity of the original lesion or inflammatory process) once hyperalgesia develops, it can persist for weeks after treatment (Fitzpatrick *et al.,* 1998; Whay *et al.,* 2005; Laven *et al.,* 2008). Consequently, timely intervention with non-steroidal anti-inflammatory agents is advisable (Friton *et al.,* 2003; Mustonen *et al.,* 2011).

In our study, adult lame sows had substantially lower MNT in the affected limbs than non-lame sows, although the difference in threshold depended on the specific group. The fact that the difference in MNT between lame and non-lame limbs was less pronounced in the first than in the other groups may, in part, be explained by the differences in ambient temperature. Lame sows had a mean MNT of 16,500 kPa and non-lame animals a mean of 19,167 kPa. These values are in line with those of Di Giminiani *et al.* (2013), who found median MNTs (after conversion) of around 22,215 kPa in non-lame, 60 kg, male pigs tested on the upper hindlimbs (forelimbs were not examined). Other studies have measured the MNTs of younger piglets (1 - 8 weeks old) on the volar surfaces of the metacarpi and metatarsi and reported much lower MNTs, ranging (after the appropriate conversions) from 1,231 to 5,420 kPa (Sandercock *et al.,* 2009; Fosse *et al.,* 2011b; Janczak *et al.,* 2012). These results would seem to indicate that bodyweight (and probably also age) has an effect on MNTs in pigs (Janczak *et al.,* 2012; Di Giminiani *et al.,* 2013). In addition, MNTs are specific to anatomical location (Haussler and Erb, 2006; Di Giminiani *et al.,* 2013), and may vary significantly even between adjacent points in the same anatomical region (Stubsjøen *et al.,* 2010).

The device used and the shape (flat, rounded or sharp) of the probe tip(s) appear to be important in determining MNTs. Occasionally, specific probe tips or mechanical adaptations to the equipment are required to obtain clear-cut behavioural responses (Fosse *et al.,* 2011b; Janczak *et al.,* 2012). Ideally, the rate of application of the stimulus should always be reported because it can affect both the response threshold and the operator bias (Di Giminiani *et al.,* 2013). The area of the probe tip can be used to 'normalise' the results of different studies according to the formula: pressure = force/area. However, preliminary evidence suggests (Taylor and Dixon, 2012) that the relationship between MNT and tip area is not always linear. Consequently, comparisons based on normalised pressures should be interpreted with caution. In conclusion, a standardisation of MNT measurement appears to be required to establish a range of physiological (and pathological) MNTs for pigs of different ages and sizes and to compare the results of different studies.

To our knowledge, this is the first study in which MNTs obtained with a hand-held probe are compared with those measured with a limb-mounted actuator. The probe gave consistently lower readings than the actuator. Using the probe possibly results in a higher predictability of the stimulus because the sows can see the operator approaching the limb and thus might react faster. We also noticed a certain degree of lateral slippage when using the probe, which led us to repeat some measurements. Slippage is prevented when using the actuator because this is securely strapped to the limbs. In addition, although our algometer was equipped with an indicator of acceleration rate, it is possible that the acceleration itself or the angle of application of the force were less constant with the probe than with the actuator. This could have determined a different pattern of activation of the nociceptors resulting in lower MNTs.

Forelimbs had higher MNTs than hindlimbs, possibly because of the uneven cranial-to-caudal weight distribution in pigs. Haussler *et al.* (2007) found similar results in horses and noted that pressure algometry of the appendicular skeleton requires an animal to repeatedly redistribute its bodyweight in order to withdraw a limb. In pigs, 54% of bodyweight is supported by the forelimbs, which are closer to the centre of gravity (Thorup *et al.,* 2007). It is plausible that this redistribution of weight from the forelimbs comes with a higher energy demand, resulting in a longer latency to react and higher MNTs. As reported for other species (Haussler and Erb, 2006; Stubsjøen *et al.,* 2010), left-to-right differences were not significant in our study. The lower MNTs in the afternoon, compared to the morning, sessions might be due to either 'stimulus conditioning' (Le Bars *et al.,* 2001) or to physiological within-day variation. Daily fluctuations in ambient temperature may influence nociceptive thresholds via both animal- dependent factors (e.g., skin temperature and perfusion), and through interference with the measuring equipment (Love *et al.,* 2011).

The highest variation in MNTs depended on factors related to observation. Some of the variation in MNTs between observations was explained by day-to-day variation and differences between morning and afternoon sessions. Almost half of the variation at 'sow' and 'limb' levels could be explained by differences in limb position, lameness and the measuring method used. In mechanical nociception testing, the repetition of the mechanical stimulus, as well as the high pressure applied, can cause a decrease or increase in the sensitivity of the stimulated part of the body (Le Bars *et al.,* 2001). We did not find any systematic tendency towards sensitisation (i.e. decreasing thresholds) or habituation (i.e. increasing thresholds) within the same session. Conversely, MNTs increased from the first to the third day of testing. Janczak *et al.* (2012) also described this phenomenon, but in that study the increased bodyweight of the piglets was considered to be the principal influencing factor. In our study, the effect of bodyweight on the MNTs, and the changes in bodyweight between days were statistically negligible. It is therefore possible that the progressive increase in MNTs was the effect of habituation.

There was no effect of gait score on MNTs. If the MNTs of lame and non-lame animals based on this parameter had differed, even after factoring out the effect of the lame limb, this might have constituted an indication of a more generalised hyperalgesic state. However, it is possible that visual locomotion scoring may not be sensitive enough to detect hyperalgesia. For example, Whay *et al.* (2005) found that while the MNTs of lame cows increased after analgesic treatment, no improvement could be detected in their locomotion scores. In another study, Ley *et al.* (1996) found that, although lame cows had lower MNTs than non-lame animals in affected limbs, there was no significant correlation between MNTs and the qualitative severity of lameness as determined by visual gait scoring.

Conclusions

The results of our study demonstrate that lame sows have an increased sensitivity to pain in the affected limb(s), an indication of hyperalgesia. This is likely to aggravate the local pain due to the original lesion(s), thus further compromising animal welfare. Prompt veterinary treatment of all lame sows is therefore advised in order to minimise the scale and duration of hyperalgesia. We found no evidence of more generalised sensitisation to painful stimuli, although further study is required to support this finding. Our results indicate that systematic time-of-day, methodological (probe vs. actuator) and cranial-to-caudal limb differences need to be considered when comparing MNTs between and within groups of sows.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Chapter 5 - Factors affecting mechanical nociceptive thresholds in healthy sows

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Abstract

The objective of the study was to describe anatomical and methodological factors influencing mechanical nociceptive thresholds (MNTs) and intra-site variability in healthy sows. Eight pregnant, healthy, mixed-parity sows (176-269 kg) were selected for a prospective study (randomized validation) in which repeated MNT measurements were taken (1) with a hand-held probe and a limb-mounted actuator connected to a digital algometer; (2) at nine landmarks on the limbs and tail and (3) at 1- and 3-minute intervals. Data were analysed using linear mixed regression models. The MNTs (± SEM) of the limbs were lower with the probe (14.7 ± 1.2 N) than with the actuator (21.3 ± 1.2 N;$P < 0.001$), in the pelvic versus the thoracic limbs (16.7 ± 1.2 versus 19.2 ± 1.2 N;$P < 0.001$), and in the lateral versus the dorsal metatarsi and metacarpi (17.6 ± 1.2 versus 18.4 ± 1.2 N; $P = 0.002$). MNTs were higher in all subsequent measurements compared with the first ($P < 0.001$) and in the morning compared with the afternoon ($P = 0.04$). We found no evidence of MNT differences based on interval between consecutive measurements (1 versus 3 minutes). Variability was lower in the thoracic limbs [mean back-transformed $\log_{10}$ coefficient of variation (CV) ± SE = 25.5 ± 1.5% versus 30.6 ± 1.5% in the pelvic limbs; $P < 0.001$], with the actuator (22.7 ± 1.5% versus 33.4 ± 1.5% with the probe; $P < 0.001$), and on the left (CV = 26.9 ± 1.5% versus 29.3 ± 1.5% on the right; $P = 0.01$). Tail data (probe only) were analysed separately: mean MNT (± SE) was 11.7 (± 1.8); MNT increased in days 3–6 of testing compared with day 1 ($P < 0.001$). The mean CV (± SE) was 38.9% (± 1.1%). In conclusion, MNTs and intrasite variability in healthy sows were affected by several factors, indicating that this methodology requires considerable attention to detail.

Introduction

Animals have been used for decades to study nociception – the neural process of encoding noxious stimuli (IASP 2012) – and to derive information about the physiological pathways that generate pain (Le Bars *et al.,* 2001; Mogil 2009, 2012). Inflammatory processes can induce pathological changes in pain perception pathways, such as allodynia, defined as pain resulting from a stimulus that does not normally provoke pain (IASP 2012), and hyperalgesia, defined as increased pain from a stimulus that normally provokes pain (IASP 2012). Both conditions can become debilitating and compromise quality of life in human patients (Laursen *et al.,* 2005; Imamura *et al.,* 2008) and they can presumably negatively affect animal well-being. For this reason, a number of nociception studies focused on normal versus abnormal processing of noxious stimuli in many species of veterinary interest. The principal aim was to investigate the presence of hyperalgesia – which may develop both during and long after the original inflammation process – and to verify the efficacy of anti-inflammatory and analgesic drugs (Whay *et al.,* 2005; Dixon *et al.,* 2007; Haussler *et al.,* 2007; Caplen *et al.,* 2013; Tapper *et al.,* 2013; Mohling *et al.,* 2014).

During the past 15 years, livestock species are under increasing scrutiny, as they are often affected by painful inflammatory conditions causing hyperalgesia that may be overlooked. Limb disease is one example: lame dairy cows (Whay *et al.,* 1998), sheep (Ley *et al.,* 1995; Colditz *et al.,* 2011) and pigs of different ages and gender had lower mechanical nociceptive thresholds (MNTs) in the affected limbs, whether tested at the site of the lesion or elsewhere on the limb (Sandercock *et al.,* 2009; Fosse *et al.,* 2011a,b; Chapter 4; Tapper *et al.,* 2013; Mohling *et al.,* 2014). Recently, MNT testing of the limbs has been described as a potentially objective tool to assess the efficacy of analgesic protocols in sows affected by lameness (Tapper *et al.,* 2013; Pairis-Garcia *et al.,* 2014). However, the methodology currently suffers from a number of limitations: MNTs in pigs differ substantially depending on the configuration of the instrument used (Chapter 4), the size and shape of the probe tip (Fosse *et al.,* 2011b), the age and weight of the animals (Janczak *et al.,* 2012; Di Giminiani *et al.,* 2013), anatomical location (Di Giminiani *et al.,* 2013; Chapter 4), as well as familiarity with the procedure (Di Giminiani *et al.,* 2015). Furthermore, MNT can be influenced by the time of day (pigs, Chapter 4; dogs, Coleman *et al.,* 2014), anticipation or sensitization phenomena (humans, Jones *et al.,* 2007; dogs, Coleman *et al.,* 2014) and distraction (donkeys, Ruscheweyh *et al.,* 2011; Grint *et al.,* 2014). The low repeatability of consecutive MNT measurements at the same anatomical site is also an issue (horses, Haussler *et al.,* 2007; sheep, Stubsjøen *et al.,* 2010; dogs, Coleman *et al.,* 2014). In conclusion, before MNT testing is proposed as a valid and reliable research tool, 'it should be fully evaluated in normal animals for consistency, repeatability, and the factors influencing threshold responses need to be known' (Coleman *et al.,* 2014).

In our study we investigated methodological and anatomical factors affecting MNT in healthy sows, namely the measuring method (hand-held probe versus remotely controlled actuator), the anatomical location (thoracic versus pelvic limbs, right versus left side of the body, dorsal versus lateral metacarpi and metatarsi, and ventral aspect of the tail), and the interval (1 versus 3 minutes) between repeated stimuli. We also investigated factors affecting variability when taking repeated measurements at the same site.

Materials and methods

Ethical statement

The experiment was approved by the Ethical Commission of the Institute for Agricultural and Fisheries Research (ILVO) (authorization no. 2011/146 and subsequent modifications).

Animals and housing

The experiment was carried out at the ILVO, Melle, Belgium in March and April 2012. Eight pregnant hybrid sows (Ra-Se Genetics, Belgium) from the same experimental herd were studied. Sample size was determined with Win Episcope 2.0 based on a two-tailed, paired-sample test (hand-held probe and remotely controlled actuator) and assuming a population mean of 12.6 and 15.9 N, respectively, and an expected standard deviation (SD) of 6.7 N (Chapter 4), at a 95% confidence level and 85% power. The sows were chosen from a pool of 20 belonging to the same static mid-gestation group (the only one available at the time of the trial). This enabled us to train and test the animals for 6 weeks consecutively. Average parity was 5.6 (range 2–11), and gestation stage 54 (±11) days. The average body weight was 233 kg (±33; range 176–269). The selected sows were group-housed in a 10.7 × 9 3.5 m pen with a solid concrete floor and deep straw bedding, and were fed a restricted diet (2.6 kg of standard commercial gestation feed) in individual troughs once daily. On each testing day, the experimental sows were given half of the ration separately from the rest of the group before the test in the morning (around 9:30 hours), and the remaining half at the end of the testing day (around 15:00 hours). Water was available *ad libitum*.

Selection protocol

The sows were selected based on their locomotion score on a continuous 150 mm 'tagged' visual analogue scale with descriptors associated to various anchor points placed at 30 mm intervals. The descriptors ranged from 'perfect gait' to 'downer sow' (Chapter 3). Out of the initial pool of 20 animals in the same gestation group, the eight sows with the lowest average gait scores (mean = 11.5 ± 5.3 mm, range 5-20; all within the non-lame interval) were selected. An experienced swine veterinarian examined the sows to exclude underlying disease, e.g., claw infections, which could affect the experimental data. The sows were re-examined before the beginning of the trial and were considered clinically normal and not lame. None of the sows became lame during the experiment.

Experimental setting

An individual gestation crate (2 × 0.5 m) was fixed to the floor in a quiet area of the barn in which the group pen was located. The lowest bar of the crate was removed to allow easy access to the limbs during the measurements; a wooden feeder was fitted to the head end. The sows were led into this crate for MNT testing, where they remained in auditory contact with their pen mates.

Instrumentation

Mechanical nociceptive thresholds were measured with a digital algometer (ProdPlus; TopCat Metrology Ltd, UK) provided with a probe for hand-held use ('probe') and two limb-mounted actuators for remote control ('actuator') connected to the same digital algometer. The actuators were fixed to the sows' limbs by means of custom-made boots with adjustable Velcro straps. For 'actuator' measurements, pressure within the limb-mounted actuator was increased manually via a 20 mL air-filled syringe and nondistensible polythene tubing (2 m long, internal diameter 1 mm), which pushed a hemispherical tipped brass pin (diameter 1 mm) against the sow's limb. The digital algometer is equipped with dynamic rate lights that ensure application of ramped force at a predetermined rate of 4 N s^{-1} (equivalent to 5095 kPa s^{-1}) with a tolerance of 0.5 N. A green light indicates that the application rate is too slow, and red that it is too fast; no light signals the correct application rate. Indicator lights on the algometer apply to both probe and actuator use. These rate lights enabled the experimenter to train himself in the technique. The MNT was defined as the minimal force that elicited a clear avoidance response (lifting the foot off the ground or kicking). For tail measurements, the tail was gently held by its distal part, and the response was either a tail flick (lateral movement) or a tail clamp (vertical movement). At threshold, the pressure was released immediately, either by withdrawing the probe tip ('probe' measurements) or by disconnecting the tubing from the syringe ('actuator' measurements). The reading in Newtons (N) remained recorded on the algometer display until reset. For 'probe' measurements, the algometer – fitted with a brass tip of the same profile and diameter – was manually pushed against the limb at a 90° angle and at 4 N s^{-1}. At threshold, the operator withdrew the probe and the peak reading of force (N) was held and displayed automatically. Based on the results of a previous study (Chapter 4), in which 25 N was shown to be a potentially conservative humane threshold for adult sows (resulting in 11% non-responses), the humane end point was increased to 30 N, equivalent to 38,216 kPa (30 N/ 0.785 mm^2).

Training protocol

The sows were trained daily for 1 week. To keep the sows positively occupied, straw and food rewards (pieces of apple and raisins) were provided in the food trough of the individual crate. Straps with dummy actuators were then attached to the thoracic and pelvic limbs and kept in place for 15 minutes; no pressure was applied during training. The sows' reactions were recorded every day, and training was considered successful when the sow no longer attempted to retract the limb when the straps were being attached. All sows succeeded.

Testing protocol

Two sows were tested on each test day, one in the morning and one in the afternoon. The initial testing order of the sows was randomized. The sows were matched in pairs based on parity (two pairs of sows having ≤ four parities and two pairs of sows having > four parities) and each sow was tested again at exactly 1-week intervals (two consecutive days per week for three consecutive weeks, adding up to 6 days of testing per sow). The whole experiment lasted 6 weeks: sows 1-4 were tested during the first 3 weeks, while sows 5-8 were tested during the last 3 weeks. Sows were weighed each week on the first testing day.

Before the start of each testing session, the dorsal and lateral aspects of the metatarsi and metacarpi of all limbs (approximately 3 cm proximal to the coronary band) and the ventral aspect of the tail (approximately 4 cm from the base) were marked with a black spot to indicate the stimulus site. The sows did not have visible lesions on the limbs. For each sow and testing day, five repeated measurements (i.e. stimuli) were taken with the probe and the actuator at nine different anatomical locations, namely the dorsal and lateral aspects of the metacarpi and metatarsi, and the ventral aspect of the tail (**Fig. 5.1**). The tests were not blinded, but the operator was instructed not to look at the digital screen of the algometer while taking the measurements. On each testing day, either a 1 or a 3-minute interval between successive stimuli was used (random allocation). The tail was only tested with the hand-held probe, as the configuration of the actuators used for the limbs in this experiment could not be adapted to this anatomical area.

To minimise a possible interference by habituation or sensitisation, care was taken to randomise and then rotate or switch on each testing session: 1) the time of day (morning versus afternoon) in which the sows were tested on each testing day; 2) the initial position of the actuators (metacarpal versus metatarsal); 3) the first region to be tested; 4) the anatomical aspect (dorsal versus lateral); 5) the sequence direction (clockwise versus counter-clockwise); and 6) the interval (1 versus 3 minutes) between repeated stimuli. To reduce testing time, repeated measurements were taken in rounds (measurement 1 at all positions, followed by measurement 2 at all positions, and so on). Each testing session lasted approximately 1 hour and 20 minutes. The sows were provided with food rewards dispersed in straw at regular intervals, and they were rooting for food in correspondence with each measurement phase. This ensured that the animals stood still and allowed us to recognize true avoidance responses. Tests that did not elicit a response at the end point were allocated a threshold of 30 N and the lack of response was recorded.

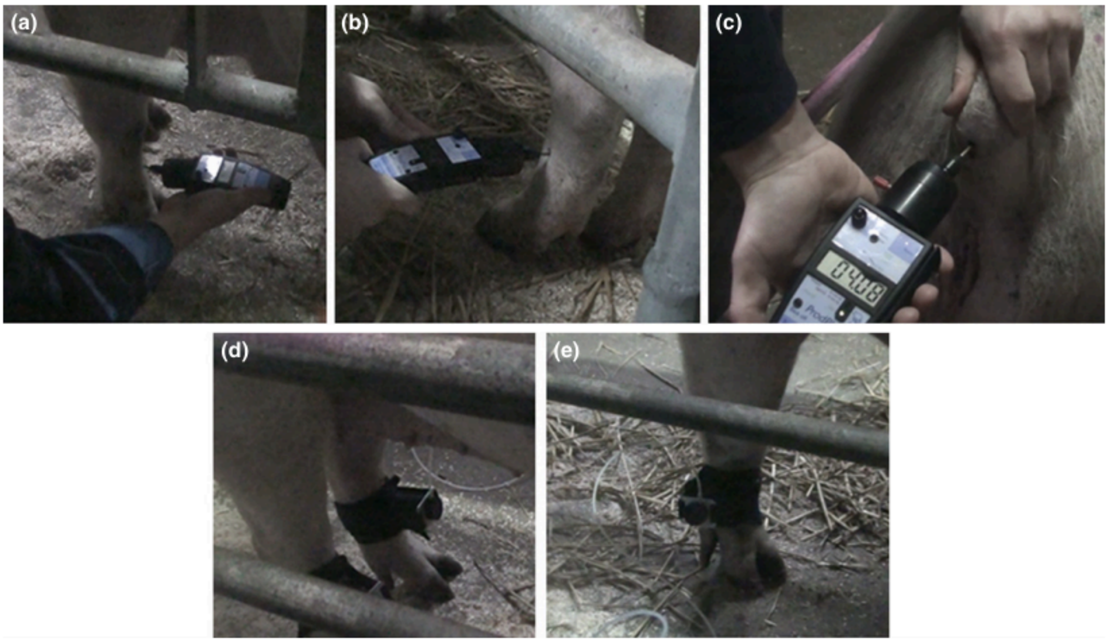

Figure 5.1. Location of the stimuli when applied with the probe (top) and the actuator (bottom). The images show the position of the probe when applied on the dorsal (a) and lateral (b) aspects of metatarsus and ventral aspect of tail (c), and with the limb-mounted actuator on the dorsal metatarsus (d) and lateral metacarpus (e). The dorsal and lateral metatarsi and metacarpi were tested with both probe and actuators, while the tail was only tested with the probe.

Data and statistical analysis

Prior to statistical analysis, observations were examined for impossible values (e.g., typing errors). No data were excluded for this reason. For statistical analyses, when the humane end point (30 N) was reached, the MNT was recorded as 30 N. Bland and Altman agreement analysis (Bland & Altman, 1986) was used to evaluate the agreement between the within-sow MNT values obtained with the probe at the tail and limbs. The agreement limits were defined as $\text{Diff}_{\text{legtail}} \pm 2\ \text{SD}_{\text{diff},}$ with $\text{Diff}_{\text{legtail}}$ indicating the difference between the tail MNT and the MNT of each limb position for each sow. An agreement plot of the within-sow difference between the mean tail MNT and the mean MNT of each limb position was used to spot outliers, defined as differences lying outside the agreement limits.

To explore animal-related and methodological factors affecting MNT, a linear mixed regression model was fitted, with threshold as outcome variable and sow and limb as random effects using SAS (version 9.3; SAS Institute Inc., NC, USA; PROC MIXED). A first-order autoregressive correlation structure was used to account for the clustering of repeated measurements within a limb. The regression model-building process involved several steps (Dohoo *et al.,* 2001). Initially, univariable associations were tested between the continuous outcome variable MNT and the categorical independent variables: method (actuator versus probe), anatomical site (lateral versus dorsal, right versus left, thoracic versus pelvic), time (morning versus afternoon), day (six levels), and measurement (five levels). Statistical significance at this stage was assessed at $P < 0.15$. Secondly, Spearman correlation coefficients were calculated among the significant independent variables separately. When two independent variables had a correlation coefficient $\geq|0.6|$, only one was selected for further analyses. None of the variables was excluded for this reason. In the third and final step, a multivariable model was fitted. A backward stepwise approach was used to produce the final model, including only factors that were statistically significant at $P < 0.05$. A similar approach was used to determine the factors that were associated with the tail MNTs obtained with the probe.

To explore animal-related and methodological factors affecting within-session and within-site variability, a linear mixed regression model was fitted, with the coefficient of variation (CV) as outcome variable and sow as random effect. The CV, which shows the extent of variability in relation to the mean of a population, was based on the five repeated measurements per site and was defined as $\text{CV}_{\text{site}} = \sigma_{\text{site}}/\mu_{\text{site}}$, where σ_{site} is the SD of the five repeated measurements per anatomical site and μ_{site} is the average of the five repeated measurements per anatomical site. The model for the limbs included method (actuator versus probe), three anatomical site variables (lateral versus dorsal, right versus left, thoracic versus pelvic), day (six levels), time (morning versus afternoon), and interval (1 versus 3 minutes) as categorical independent variables, and body weight as a continuous independent variable. A similar model was built to determine the association between the above factors and the CV of the tail MNTs. In all models, a $\log_{10}$ transformation of the CV values was performed to normalize the distributions.

For all linear mixed models, the goodness of fit measures included −2 × log-likelihood (−2LL), Akaike's information criterion (AIC) and the Bayesian information criterion (BIC). Residuals were evaluated graphically against the predicted values. To evaluate the proportion of variance occurring at the different levels of the data hierarchy, two-three-level null models (only including intercept and measurement) were fitted, with sow and either limb or tail as random effects. The variation at the sow and limb/tail level are presented as variance components.

Results

Descriptive statistics

All sows completed the study (4080 measurements in total). The percentage of non-responses was 18.7% (n = 764) for the limbs and 8.8% (n = 21) for tail measurements. There were marked differences among sows in the number of non-responses: the mean (SD) was 93.1 (± 59.9) times with a median of 85 and a range of 168. A total of 69% of non-responses occurred with the actuator, compared with 31% with the probe. The mean (±SD) MNT across methods was higher in the thoracic limbs than in the pelvic limbs, and lowest in the tail (**Fig. 5.2**).

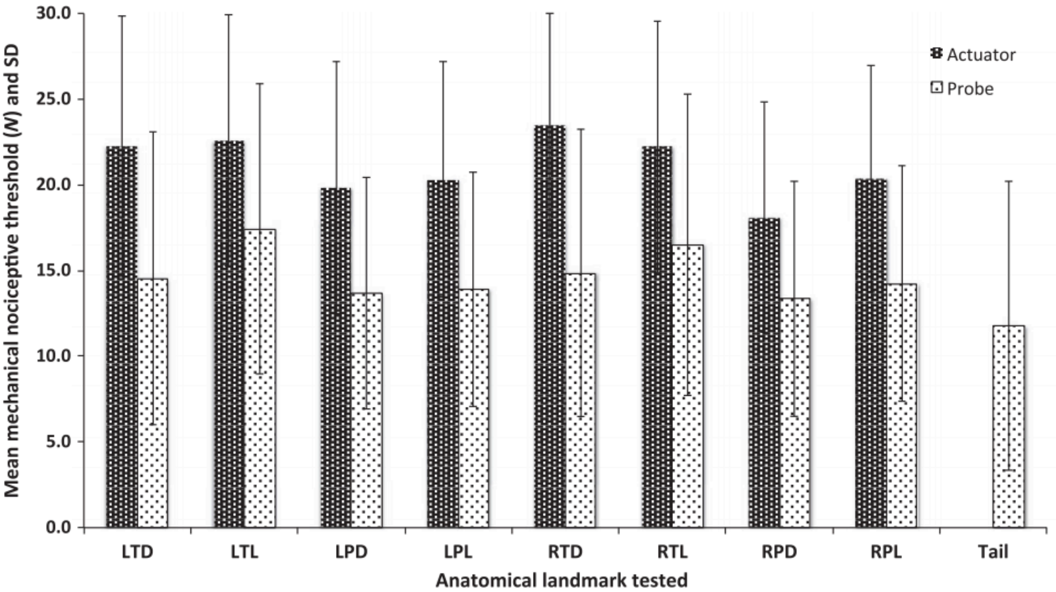

Figure 5.2. Mean mechanical nociceptive thresholds (MNTs) in adult healthy sows expressed in

Newtons (N) and their standard deviations (SDs) per anatomical location and method used (hand-held probe and limb-mounted actuator). Note that the maximum threshold (30 N) was assigned in the case of absence of response, so the reported MNTs should not be interpreted as representing reference values. LTD, left thoracic dorsal (left dorsal metacarpus); LTL, left thoracic lateral (left lateral metacarpus); LPD, left pelvic dorsal (left dorsal metatarsus); LPL, left pelvic lateral (left lateral metatarsus); RTD, right thoracic dorsal (right dorsal metacarpus); RTL, right thoracic lateral (right lateral metacarpus); RPD, right pelvic dorsal (right dorsal metatarsus); RPL, right pelvic lateral (right lateral metatarsus); Tail (ventral aspect).

The variance components for the two different null models for MNT are presented in **Table 5.1**. For the limb and tail MNT, 10.7% and 28.5% of the variation, respectively, resided at sow level. The difference between tail and limb MNT varied substantially between sows (**Fig. 5.3**). Similarly, there was a low but significant between-sow variation in the MNT of each anatomical site. The highest variation resided at the observation level for both limb and tail MNT (**Table 5.1**). Within session repeatability across methods produced a CV of 28.1% for the five repeated measurements within a limb and 38.9% for the tail.

Table 5.1. Variance components at the sow and limb/ tail level for the null models of limb and tail mechanical nociception thresholds in healthy sows. Note that the absence of a behavioural response at the humane endpoint of 30 N was assigned a threshold of 30 N, so the reported data should not be interpreted as representing reference values.

	Limb		***Tail***	
Data hierarchy	***Variance estimate ± SE***	***%***	***Variance estimate ± SE***	***%***
Sow	7.58 ± 4.28	10.7	21.10 ± 12.77	28.50
Limb/tail	0.56 ± 0.01	0.8	0.29 ± 0.07	0.39
Observation	62.49 ± 1.92	88.5	52.75 ± 5.43	71.15
Total variance	70.63		74.14	

SE, standard error of the mean.

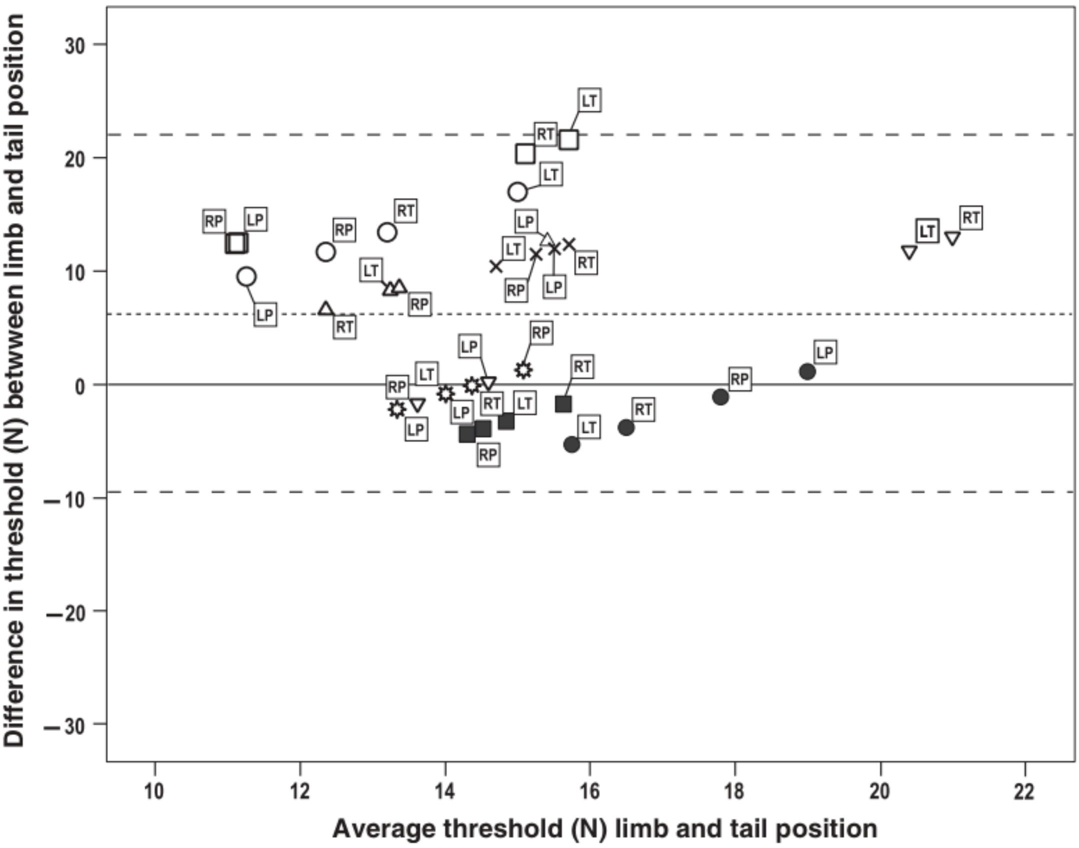

Figure 5.3. Bland–Altman plot showing within-sow average mechanical nociceptive thresholds (MNTs) expressed in Newtons (N) for each limb position and tail, plotted against the difference in MNT between the specific limb position and the tail. Each symbol represents an individual sow. Note that the maximum threshold (30 N) was assigned in the case of absence of response, so the reported MNT should not be interpreted as representing reference values. RT, right thoracic limb; LT, left thoracic limb; RP, right pelvic limb; LP, left pelvic limb. The dotted line at 6.2 N represents the average difference between the MNT of the limb positions and the tail. The dashed lines represent the agreement limits (average difference between values obtained at the tail and a specific limb position ± 2 × standard deviation of the difference between the values obtained at the tail and a specific limb position).

Factors affecting MNT

Mean MNTs across methods were higher for the thoracic than for the pelvic limbs (P < 0.001) and for the lateral versus dorsal aspects of the metatarsi/ metacarpi (P < 0.01; **Table 5.2**). The probe generated lower values than the actuator (P < 0.001). MNTs were higher in all measurements subsequent to the first one (P < 0.001), with a tendency towards stabilization in the fourth and fifth measurement. MNTs were higher in the morning than in the afternoon sessions (P < 0.05).

Table 5.2. Final multilevel, multivariable linear regression model describing the factors significantly associated with mechanical nociceptive thresholds in the limbs of healthy sows. Note that the absence of a behavioural response at the humane endpoint of 30 N was assigned a threshold of 30 N, so the reported data should not be interpreted as representing reference values.

Independent variable	***Measurements (n)***	***Estimate***	***SE (N)***	***LSM (N)***	***P-value***
Intercept	3840	20.5	1.2	…	< 0.001
Method					< 0.001
Actuator	1920	Reference	…	21.3	…
Probe	1920	-6.6	0.3	14.7	< 0.001
Measurement					< 0.001
1	816	Reference	…	15.9	…
2	816	1.7	0.3	17.6	< 0.001
3	816	2.5	0.3	18.5	< 0.001
4	816	2.9	0.3	18.9	< 0.001
5	816	3.0	0.3	18.9	< 0.001
Thoracic *versus* pelvic					< 0.001
Thoracic	1920	Reference	…	19.2	…
Pelvic	1920	-2.5	0.4	16.7	< 0.001
Dorsal *versus* lateral					0.002
Dorsal	1920	Reference	…	17.6	…
Lateral	1920	0.8	0.3	18.4	0.002
Time					0.04
Morning	1920	Reference.	…	18.3	…
Afternoon	1920	-0.8	0.4	17.6	0.04

SE, Standard error (N); LSM, mean values from the model (least square means) (N).

Within-session variability was higher with the probe than with the actuator (**Table 5.3**; P < 0.001) and also depended on the anatomical location: the CV was higher in the pelvic limbs than in the thoracic limbs (P < 0.001) and in the right versus the left side of the body (P < 0.05). Furthermore, body weight tended to be positively associated with variability (P = 0.09).

Table 5.3. Final multilevel multivariable linear regression model describing the factors associated with the coefficient of variation of the mechanical nociceptive thresholds of the limbs in non-lame sows. Note that the absence of a behavioural response at the humane end point of 30 N was assigned a threshold of 30 N, so the reported data should not be interpreted as representing reference values.

Independent variable	***Observations****	***Estimate***	***SE***	***LSM***	***P-value***
Intercept	737	3.2	8.6	…	0.7
Method					< 0.001
Actuator	367	Reference	…	22.7	…
Probe	370	10.6	0.9	33.4	< 0.001
Thoracic *versus* Pelvic					< 0.001
Thoracic	316	Reference	…	25.5	…
Pelvic	374	5.1	0.9	30.6	< 0.001
Left *versus* right					0.01
Left	347	Reference	…	26.9	…
Right	343	2.4	0.9	29.3	0.01
Body weight †	48	0.1	0.0	…	0.09

* Number of instances in which the CV was calculated. † Number of measurements (six times per sow). SE, standard error; LSM, backtransformed mean log10-values of the coefficient of variation (least-square means).

Tail MNT reached a plateau on day 3 and was higher on days 3–6 than on day 1 (P = 0.04; **Table 5.4**). None of the factors in our model explained variability, although there was a tendency for the CV to be lower in the afternoon (**Table 5.5**).

Table 5.4. Final multilevel, multivariable linear regression model describing the factors significantly associated with mechanical nociceptive thresholds of the ventral aspect of the tail (as measured with the hand-held probe) in healthy sows. Note that the absence of a behavioural response at the humane end point of 30 N was assigned a threshold of 30 N, so the reported data should not be interpreted as representing reference values.

Independent variable	***Measurements (n)***	***Estimate***	***SE***	***LSM***	***P-value***
Intercept	240	6.88	2.04		0.001
Day					0.04
1	40	Reference	...	7.05	...
2	40	2.17	2.25	9.22	0.34
3	40	6.98	2.45	14.02	0.006
4	40	6.94	2.49	12.31	0.007
5	40	6.65	2.50	13.99	0.009
6	40	5.26	2.54	13.71	0.04
Measurement					0.56
1	48	Reference	...	11.54	...
2	48	1.21	1.25	12.75	0.34
3	48	-0.77	1.47	10.76	0.60
4	48	-0.04	1.49	11.51	0.98
5	48	0.48	1.32	12.02	0.72

SE, standard error; LSM, mean values from the model (least square means).

Table 5.5. Final multilevel, multivariable linear regression model describing the factors associated with the coefficient of variation of the mechanical nociceptive thresholds of the ventral aspect of the tail in healthy sows. Note that the absence of a behavioural response at the humane end point of 30 N was assigned a threshold of 30 N, so the reported data should not be interpreted as representing reference values.

Independent variable	***Observations****	***Estimate***†	***SE***	***LSM***	***P-value***
Intercept	46	1.52	0.06	...	< 0.001
Time					0.09
Morning	22	Reference	...	33.1	...
Afternoon	24	0.14	0.06	45.7	0.09

* Number of instances in which the CV was calculated. † Log_{10} - transformed. SE, standard error; LSM, back-transformed mean $\log_{10}$-values of the coefficient of variation (least square means).

Discussion

This study examined animal-related and methodological factors affecting MNT in healthy adult, mixed parity sows, including factors influencing within-session variability with repeated measurements. Metacarpal and metatarsal MNTs were used as 'sentinel' anatomical areas for consistency with previous studies in pigs and other species (Whay *et al.,* 1998, 2005; Stubsjøen *et al.,* 2010; Colditz *et al.,* 2011; Raundal *et al.,* 2014). We also tested the lateral aspect as a potentially more practical site for some circumstances. The tail was also included, as this site has not yet been investigated in adult sows, although Di Giminiani *et al.* (2013, 2015) used a von Frey monofilament at this location in juvenile pigs. We saw no external evidence of tissue damage (indentations or bruising) even after stimuli of 30 N. However, as the safety of higher forces has not been determined, we recommend a 30 N humane end point when a 1-mm diameter probe tip is used on adult sows. Unfortunately, this leads to a proportion of 'non-responders', which generate censored data, making appropriate statistical evaluation challenging. Survival modelling, which has been specifically developed to handle the censoring of observations, could have been another option, and was also carried out on the dataset. The results corresponded well to those obtained by the linear mixed regression modelling. However, as the goal of this study was not to establish normative values, we opted not to report the results of survival analysis.

Factors affecting MNT and intra-session variability

We accounted for several factors known to be crucial for repeatable and accurate MNT testing (Coleman *et al.,* 2014; Raundal *et al.,* 2014): the stimuli were applied at a controlled rate (4 N s^{-1}, equivalent to 5095 kPa s^{-1}); the precise location of the stimulus was clearly marked; the tips of the instruments used were small – thereby keeping the forces low – and they were identical in size and shape; the animals were habituated to the procedure; and the end points were consistent. The same experienced operator made all the measurements throughout the experiment. All these precautions notwithstanding, we observed considerable intra-site variability and we identified a number of methodological and animal-related factors that influenced MNT. The hand-held probe consistently yielded lower limb MNT than the actuator. This confirms previous findings (Chapter 4) and could be the result of the sows' anticipation of the stimulus, given that they were not 'blinded' to the probe's application. By contrast, there was no visual cue when the remotely controlled actuator was used. A similar anticipation effect has been documented in other species (horse, Haussler & Erb 2006; sheep, Stubsjøen *et al.,* 2010; dogs, Coleman *et al.,* 2014); however, in those studies, anticipation was indicated as a possible reason for apparent sensitization (i.e. a progressive decrease in MNT), which was not observed in our sows.

Variability between repeated measurements (as expressed by the CV) was high and in line with recent findings in dairy cattle (Raundal *et al.,* 2014). The actuator produced more repeatable results than the probe: perpendicularity was guaranteed with the actuator but was more difficult to control with the hand-held probe. However, it is unlikely that variable side-load, a known problem with traditional straingauge force transducers, was responsible in this case. A pneumatic transducer, as used here, is unaffected by off-axial loads. In addition, the solid brass tip is nondeformable, so the skin contact area and the tip profile are constant, as opposed to von Frey monofilaments (Bove 2006). Raundal *et al.* (2014) observed that cows may react to the sudden contact of the probe tip with the skin (i.e. touch) as well as to pain. In our study, the actuators were secured on the limbs with 'boots' and Velcro straps, providing a constant tactile baseline stimulus or contact sensation, which may have reduced variability. Applying a preload pressure of 1 N can also improve consistency of responses (Musk *et al.,* 2014). All precautions notwithstanding, it appears that some variability is inevitable across repeated measurements, and that using different devices will result in different MNTs. Differences in intra-individual, intra-site MNTs and variability across measuring devices are well documented in healthy human patients (Polianskis *et al.,* 2001; Rolke *et al.,* 2005). Intra-individual and intra-site human MNT CVs of up to 25% are reported even in standardized laboratory settings (Rolke *et al.,* 2005; Ylinen *et al.,* 2007).

Mechanical nociceptive thresholds tended to increase with successive measurements at the same site and to reach a plateau towards the fourth and fifth repetition, possibly indicating habituation to the stimulus. Other studies found decreasing MNT over consecutive measurements and/or testing days (horse, Haussler & Erb 2006; sheep, Stubsjøen *et al.,* 2010; dogs, Coleman *et al.,* 2014). However, adaptation (i.e. a progressive loss of sensitivity to the applied stimulus) can also occur in MNT testing and may depend on the type of nerve fibre that is stimulated and the interval between repeated stimulations (Slugg *et al.,* 2000; Le Bars *et al.,* 2001). As already suggested by Stubsjøen *et al.* (2010), we recommend discarding the first measurements until a stabilized MNT is reached. There was no effect on MNT of the two measurement intervals tested, and hence the 1-minute interval is advisable whenever minimizing testing time is desirable.

In common with our previous study (Chapter 4), we found that MNT was higher in the thoracic limbs than in the pelvic limbs. Intra-session variability was also lower in the thoracic than in the pelvic limbs. This is possibly related to the unequal weight distribution in the pig, whereby thoracic limbs take more weight (Thorup *et al.,* 2007), so that lifting a thoracic limb may require more incentive than a pelvic limb. Similar results have been found in horses (Haussler & Erb 2006), while donkey MNT showed no thoracic/pelvic difference (Grint *et al.,* 2014). There were marked individual differences among the sows in this thoracic–pelvic effect, so this result should be interpreted with caution.

Metacarpal and metatarsal MNTs were higher on the lateral than on the dorsal aspects, in line with the findings obtained by Raundal *et al.* (2014) in dairy cattle. This difference is probably attributable to a different distribution and type of afferent nerve fibres at the stimulated site and to the variety of anatomical structures present (i.e. cutis, subcutis, muscle, fat, bone; Le Bars *et al.,* 2001; Raundal *et al.,* 2014).

Measurements taken in the morning yielded higher MNTs than those taken in the afternoon. The difference may be ascribed to feeding motivation, as all sows were on a restricted diet and received the first and second half of their daily ration at 9:45 and 15:00 hours. In practice, this means that, when tested in the morning, the sows had just received their first ration, but when tested in the afternoon they were expecting their second ration. Although restrictively fed pregnant sows have a constant and very high feeding motivation (Hutson 1991), it is probable that when tested in the afternoon the sows were hungrier than in the morning. The motivation to escape from or react to a noxious stimulus and hunger are competing, as animals tend to react to one status of imbalance at a time (LaGraize *et al.,* 2004) and this may have influenced nociceptive thresholds.

The CV for measurements taken on the right side was higher than that for measurements on the left side of the body. Several factors may have contributed to this finding. Left- or right-side dominance may contribute to determine nociceptive responses in animals (Haussler *et al.,* 2007), although in that case we would expect systematic differences in MNT as well as in variability. We cannot exclude a priori the influence of environmental factors, which, however, are more difficult to quantify. During the experiment, the sows' left side was close to a wall, while the right side of the modified crate was parallel to a narrow corridor. The sows may have felt drafts, or a higher vulnerability, on the right side. As these factors are obviously very difficult to control when operating on a farm, the focus of future research should be on improving repeatability irrespective of the testing environment.

Tail MNTs were not significantly affected by order of measurement, but increased from the second day of testing. This result would appear to indicate habituation, although over a longer period of time rather than within the single testing session. The tail site presents considerable advantages over the limbs: physical restraint of the pigs is not necessary (Di Giminiani *et al.,* 2011), and results are not influenced by weight-bearing, nor by left–right or thoracic–pelvic differences. Additionally, this may be a convenient sentinel area to assess generalized changes in MNT as a result of painful states such as pelvic limb lameness. For example, dairy cows affected by mild and moderate mastitis showed an increased sensitivity to pain in the limbs on the same side of the mastitis quarter (Fitzpatrick *et al.,* 1998); in another study, limb MNTs were bilaterally lower in mastitic than in sound cows (Kemp *et al.,* 2008).

Note on instrumentation and data reporting

Data collected from different studies should be easily compared, but variation in methodology makes comparison difficult. Probe tip profile and diameter clearly affect MNT (Greenspan & McGillis 1991; Fosse *et al.,* 2011b; Taylor *et al.,* 2016). The nociceptor is activated by pressure (Le Bars *et al.,* 2001), and as pressure = force/area, larger-diameter probe tips will require higher forces to produce the same pressure. Although MNT (measured as a force) increases with tip diameter, the relationship is not linear (Greenspan *et al.,* 1997; Taylor & Dixon 2012a,b), so simple extrapolation from force or pressure and tip area still does not allow comparison between studies using different probe tips. Probe size also affects the tissue depth at which nociceptor activation occurs (Treede *et al.,* 2002). Although an animal is unable to report the precise location of the noxious stimulus, this may lead to further variability between individuals. Large tip diameters produced more variability than smaller tips (Taylor & Dixon 2012a,b; Duan *et al.,* 2014). It also appears that forces above 30 N are difficult to apply consistently with a hand-held probe and it is questionable whether high forces actually elicit nociceptive responses or simply displace the limb or animal, depending on the relative size. The effect of tip size is evident in the reports by Fosse *et al.* (2011a,b) where only the smallest probe (0.2 cm^2, ~5 mm diameter) elicited responses below the end point of 25 N in juvenile pigs. It is essential, therefore, that probe tip size and geometrical shape be specified whenever MNT is measured.

Conclusions

Mechanical nociceptive thresholds of the limbs of healthy sows were affected by both the specific anatomical location tested and the measurement method. There was a habituation effect, with a plateau being reached in the last measurements: the first measurements should thus be discarded until such a plateau is reached. Variability within a session was lower with the actuator than with the hand-held probe, and depended on the anatomical region and side of the body. Tail MNTs were influenced by day of measurement and were highly variable. Testing interval did not influence either the MNT or variability, and thus shorter intervals can and should be used, whenever animal welfare considerations dictate so (e.g., for lame sows). Meticulous attention to detail is required for MNT testing, and further studies comparing MNT before and after analgesic treatment are recommended to validate this method for clinical and experimental use.

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Conflict of interest

P. Taylor is a director of Topcat Metrology Ltd.

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Chapter 6 - General discussion

This general discussion focuses on an analysis of the obtained results and on recommendations for future research. From an analysis of the literature, it is clear that there is still insufficient knowledge about the ways in which different degrees of lameness affect the welfare of sows (**Chapter 2**). Apart from the production aspects, more needs to be known about the effects of lameness on the ability of sows to satisfy their needs, which in turn may be influenced by altered nociception mechanisms. Before such aspects can be better understood, it is indispensable to have reliable gait scoring methods available. One scoring method that was not studied previously in pigs is the continuous visual analogue scale (VAS). In **Chapter 3**, we described the use of such a scale with verbal anchors or "tags" (tVAS) and compared it with a 5-point and a 2-point scale with the same verbal descriptors. In **Chapter 4,** a mechanical nociceptive threshold (MNT) test was used in two configurations to verify the effect of lameness, determined by visual observation on the tVAS, on the MNTs of the limbs of sows. **Chapter 5** examined in more detail the factors affecting MNTs in healthy sows as well as intra-test and intra-individual variability when taking repeated measurements at the same anatomical site. A schematic representation of the findings of this thesis is presented in **Fig. 6.1**.

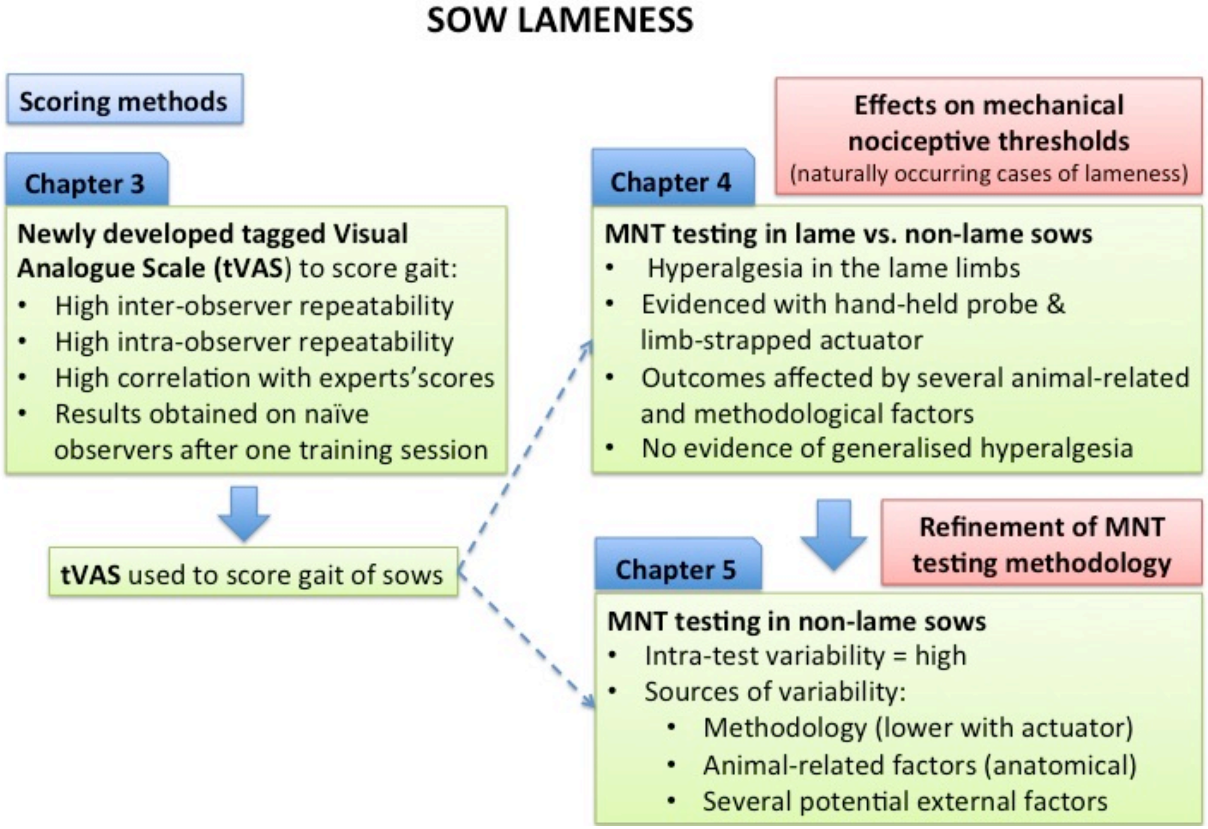

Figure 6.1. Schematic representation of the findings of this thesis. The dashed arrows indicate that the tVAS developed in Chapter 3 was used to score the gait of the sows selected for the experiments in Chapters 4 and 5. The findings of Chapter 4 indicated that the mechanical nociceptive threshold testing methodology was influenced by a number of factors, and that variability between repeated measureemnts was high. The study of Chapter 5 investigates factors affecting MNTs and variability in healthy sows, in an attempt to better understand to what extent this testing methodology can be refined in the future.

6.1. A tVAS as a repeatable instrument to assess sow gait

Visual scoring of sow lameness by a trained observer is perhaps the cheapest and the simplest methodology available, albeit with some limitations as described in **Chapter 1** of this thesis. **Chapter 3** describes a sow gait-scoring experiment in which the inter- and intra-observer repeatability of a tagged visual analogue scale (tVAS) were compared to that of a 2-point and 5-point categorical scale with the same verbal descriptors. Under the described experimental conditions, **third-year veterinary medicine students,** predominantly naïve to this type of scoring, **were able to identify the degree of severity of lameness of the sows on the tVAS and the 5-point ordinal scale with a high degree of correlation** with the experts. Additionally, the students **obtained good inter- (0.73) and intra-OR (0.80) with the tVAS** and after just one training session. Viñuela-Fernandez *et al.* (2011) also found that final year veterinary students attained a good (> 0.75) intra- and inter-OR when scoring the gait of horses affected by laminitis on a VAS without descriptors, even without a preliminary training session. Garcia *et al.* (2015) found that observers without prior experience in scoring lameness in dairy cattle achieved perfect within-observer agreement up to 60% of the time, after merely a 3-min introduction. **In our study, there was no statistically significant difference in inter- and intra-OR between the tVAS and the 5-point scale**. However, the **tVAS was superior to the 2-point scale for both inter- and intra-observer repeatability.** This indicates that lameness is not easily assessed as absent/present and that observers are more likely to disagree on whether a sow is lame *versus* non-lame compared to when they have the possibility to score more gradations in the severity of the condition. We found lower **inter- and intra-OR** when comparing the observers' performance **for gaits in the lower ranges of the tVAS** (≤ 45 mm) *versus* gaits > 45 mm. It should be noted that a gait score ≤ 45 mm indicates normal to very mild gait abnormalities, which potentially do not correspond to lameness associated with welfare impairment. The recent study of Bos *et al.* (2015) would seem to confirm this. The authors used a feeding motivation test to verify the extent to which various degrees of lameness as measured in mm on the tVAS impair the willingness of sows to walk for palatable rewards. They found that non-lame (0-30 mm on the tVAS) and mildly lame sows (30-60 mm) obtained more rewards than moderately lame (60-90 m) and severely lame sows (90-120 mm). This is new evidence that lame sows (moderately and severely affected) are impaired in their ability and willingness to reach palatable and presumably relevant resources. No differences were found between non-lame and mildly lame sows or between moderately and severely lame sows in the ability to walk in order to obtain food rewards (Bos *et al.,* 2015).

The study described in **Chapter 3** presented some limitations. There is no gold standard against which to benchmark visual gait scoring scales, as these are considered the "silver" standards against which all other methods are benchmarked. For this reason, it was impossible to verify the **validity** of our tVAS (i.e., if it measures what it is supposed to measure). Within our study, we did not calculate the **specificity** and **sensitivity** of the tVAS (i.e, the proportion of lame and non-lame sows correctly identified as such). This limitation can be overcome – at least in research settings – by combining different detection methods. As seen in **Chapter 1**, several instrumental methods to assess lameness are by now available, including force plates, accelerometers to record postural behaviour, etc. One such example is the SowSIS: this technology has recently been tested in combination with electronic feeding stations, and by measuring weight distribution while the sow is feeding it can automatically provide an indication of potential instances of lameness (Maselyne *et al.,* 2014). While sensor-based technologies could become the new gold standard in the future, their utilisation by farmers is limited by considerations of cost (van de Gught *et al.,* 2016). Visual inspection may remain as a silver standard in the majority of farms, and in this respect the tVAS offers several advantages over ordinal scales with few categories. For instance, the tVAS can be particularly useful when more detailed information is needed on the degree of lameness, rather than simply absence/presence. Even in the case of studies where the parameters of interest are the incidence and prevalence of lameness, it can be argued that having information on the severity as well as on the presence of lameness could help to fine-tune preventive and corrective measures. In turn, such an approach would require re-thinking the way in which we express the lameness status of a sow herd, no longer in terms of a binary variable, but rather in terms of mean locomotion score.

When thinking of specificity and sensitivity, the use of a continuous scale defies *per se* the concept that one can just categorise a condition as being "present" or "absent". Logically, if the tVAS is to be more widely adopted in research and practical settings, thresholds and ranges will need to be established to assess individual animals and herd prevalences. These thresholds and ranges have to be based on knowledge about the extent to which the welfare of sows with various degrees of lameness is compromised. Such work has already begun. Based on the findings of a previous study briefly described above (Bos *et al.,* 2015), Bos *et al.* (2016) used the tVAS to calculate the incidence of lameness in a group of sows and the effects of different floor types within one experimental herd. To determine the initial prevalence, expressed as the mean locomotion score, the authors classified as lame the sows with a gait score > 60 mm on the tVAS. In subsequent observations, to document how the lameness status changed over time, new cases were recorded if the gait score of individual sows had increased > 30 mm compared to a previous observation and if the previous observation was < 60 mm on the tVAS. Compared to the more common "lame-non lame" classification, this method has the advantage that it enables researchers/observers to closely monitor the evolution in time of the severity of the condition in individual sows and in an entire herd, as well as recording new cases. As previously discussed, at the herd level this methodology could prove very useful to monitor the effect of preventive or remedial measures. At the level of the individual animal, measuring lameness on a tVAS allows for a much more detailed analysis of the degree of improvement, for instance when comparing different treatments.

The **reliability** of the tVAS and categorical scales was only calculated within the experimental context. Experience with scoring may improve repeatability: if we consider inter-OR, this increases with successive locomotion scoring training (Brenninkmeyer *et al.,* 2007; D'Eath, 2012; March *et al.,* 2007). Additionally, our observers had a similar educational background (i.e., 3rd year veterinary medicine students). The observers' educational background, as well as their previous experience with the species they are scoring, can affect repeatability. In a recent experiment Garcia *et al.* (2015) compared the intra-OR of 102 observers from different backgrounds - and namely veterinary students, farmers, bovine veterinarians, researchers, and food sensory assessors - when scoring cow mobility on a 5-point scale. They found that fourth-year veterinary medicine students had a higher probability of perfect agreement compared to both first-year students and assessors with no previous experience of observing cows. Perfect agreement was highest in farmers, 4-year veterinary medicine students and veterinarians, i.e., the observers with the most experience in the field.

One final remark concerns the spacing of the different gradations of lameness severity on the tVAS, as indicated by colour coding and verbal descriptors. In our experiment, the spacing between increasing degrees of lameness was made visually equal, i.e., each spacing on the tVAS measured 30 mm. However, creating equidistant markings on the tVAS and aggregating different descriptors is not entirely correct, because 1) the categories of categorical scales may not correspond to equal spacings on a VAS (Boogaerts *et al*., 2000) and 2) observers can interpret certain descriptors (e.g., arched back, head tilt, shortened stride, etc.) as being more predictive of lameness than others, as found for instance by Vieira *et al.* (2015) in a recent study on lameness assessment in goats. Vieira *et al.* (2015) concluded that research efforts should be devoted to assess the exact location of thresholds along the continuum of the VAS, an aspect that we have already discussed in relation to the interpretation of prevalence and incidence of lameness when using continuous scales to score gait.

6.2. Evidence of hyperalgesia in lame sows, and factors affecting intra-test repeatability

The studies described in **Chapter 4** and **5** were the first to investigate mechanical nociceptive thresholds in cases of naturally occurring lameness in sows (Chapter 4), and factors affecting variability when measuring MNTs in healthy sows (Chapter 5). Our main findings in **Chapter 4** were the following: MNT testing with two different methods (a hand-held probe and a limb-strapped, remotely operated actuator) was able to identify hyperalgesia in the affected limbs of lame sows; gait score had no effect on MNTs; several factors influence MNT values. In **Chapter 5** we further investigated the methodology on healthy sows, and these were the main findings: variability in MNT measuring is high; several factors, only some of which can be at least partially controlled, affect variability. In our experimental setting, these factors included the instrument used, the time of day, the side of the body, thoracic vs. pelvic differences.

The results of the first study (**Chapter 4**) showed that **lame limbs had lower mechanical nociceptive thresholds (MNTs) compared to non-lame limbs**. This is an indication that sow lameness induces hyperalgesia to mechanical stimuli in the affected limb. To the best of our knowledge, this was the first study investigating MNTs in naturally occurring cases of sow lameness and constitutes an important contribution to a growing field of research. Other studies found evidence of hyperalgesia in experimental models of sow lameness (Mohling *et al.* 2014; Pairis-Garcia *et al.,* 2014; Tapper *et al.,* 2013). In these transient synovitis was induced by the intra-articular injection of Amphotericin B, a method described and validated by Karriker *et al.* (2013). Some of these studies successfully used mechanical and thermal nociceptive threshold tests to study the efficacy of certain analgesics and anti-inflammatories (Karriker *et al.,* 2013; Pairis-Garcia *et al.,* 2014; Tapper *et al.,* 2013). However, chemical synovitis models induce acute episodes of lameness with a predictable and reversible clinical course, and thus differ from spontaneous processes, which can have much more unpredictable durations (especially if not treated).

We found no effect of gait score on the sows' MNTs. As the effect of lameness at limb level was included in the statistical model, this result indicates that lameness in one limb does not determine generalised hyperalgesia, i.e., beyond the affected limb. Our threshold to initially classify sows as lame was prudent (> 30 mm on the tVAS), and the average gait score of the lame sows was low (68.1 ± 18 mm). It is possible that by choosing a higher threshold to classify sows as lame (> 60 mm), the results would have differed.

The studies described in Chapters 4 and 5 show that **several factors affect MNTs and intra-test variability**, none of which had previously been studied in relation to mechanical nociception threshold testing in sows. In **Chapter 5**, we concluded that some of these factors can be controlled to a certain extent, particularly in an experimental setting. For example, to establish normal values for MNTs in sows while trying to reduce sources of variability, the animals should be tested at the same time of day; ambient temperature should be controllable; the level of hunger of restrictively fed sows should not differ between testing sessions; there should be no drafts affecting one side of the body. Concerning the MNT testing instrumentation used, the stimulus should be given at a controlled rate; instruments that can be fixed to the animal are potentially preferable; tips with smaller diameters are preferable to tips with larger diameters. Other factors are related to the characteristics of the animals and to the anatomical locations tested, and thus can only be reported.

The methodological differences between MNT studies on pigs make it difficult to compare results. This becomes clear if we consider the range of MNT values reported in other recent studies on pigs (**Table 6.1**). As can be seen from the overview, the results from different studies are not easily comparable, even after conversion of the MNT results into units of pressure (kiloPascal). Not only are MNTs affected by size and age of the pigs (and probably also gender), but tip diameter, tip shape, and rate of application of the force also influence the outcomes. The most comparable MNT values to the ones we obtained are those of Di Giminiani *et al.* (2013), measured in the limbs of 60-kg pigs. Tapper *et al.* (2013) and Mohling *et al.* (2014) tested the MNT of sows before and after induction of lameness, but they used a probe with a flat, 1-cm^2 tip, which may have accounted for the wide differences in the recorded MNT (after conversion) between their studies and ours. The mean MNT obtained by Di Giminiani *et al.* (2016) in the dorsal aspect of undocked tails of 32 wk old pigs (2603.19 kPa after conversion) is much lower than what we measured in the ventral aspect of the (docked) tails of adult sows (11.7 N, equivalent to 14904 kPa). The type of instrumentation used, the anatomical location of the stimuli and the lower live weight and younger age of the pigs compared to the sows used in our experiment (average body weight, 233 kg; average parity, 5.6) may partially explain these differences.

Table 6.1. Examples of recent studies investigating mechanical nociceptive thresholds (MNT) in pigs by means of pressure algometry. Results obtained from studies using von Frey monofilaments were not included due to the different biomechanical characteristics (i.e., flexibility of filament) of the instrument. For comparison purposes, only results obtained in healthy, non-lame animals are reported.

*Units of measurements different from kPa were converted starting from the data provided in the study. Note that, whenever instrumentation and testing methodologies differ substantially between studies, especially regarding tip shape, diameter, and rate of application of the stimulus, conversions can only be considered as indicative and should be interpreted with caution.

N = Newton (unit of force); MNT = mechanical nociceptive threshold; gf = gram/force; g/s = grams/force*s-1; kgf = kilograms/force; kgf/s = kilograms/force*s-1; kPa = kilo Pascal (unit of pressure); n.s. = not specified.

Authors	***Pigs***	***Age***	***Live weight (kg)***	***Habituation***	***Instrument***	***Tip***	***Tip area***	***Units of force***	***Stimulus rate***	***Humane endpoint (cutoff)***	***Stimulus location***	***MNT***	***Conversion in units of pressure (kPa)***
Sandercock *et al.* (2009)	8 females	7-9 wk	8-10	2 wk	Custom made mechanical stimulator and force measurement system	Hemi-spherical	0.031 cm^2 (ø 2 mm)	N	2-17 mm/s	15 N	Volar aspect of feet	Mean 8.4 N	**2 709.7**
Fosse *et al.* (2011a)	12 piglets (9 males, 3 females)	13.5 (8-19) d	4.5 (3.4-6.0)	10 d	Purpose-built, hand-held, calibrated pressure algometer	n.s.	1 cm^2 0.5 cm^2 0.2 cm^2	N	5 N/s	24.6 N	Palmar surface of the left metacarpus between metacarpi II and V + the corresponding area of the right metacarpus and left metatarsus	With 1 cm^2 tip: mostly close or above 24.6 N With 0.5 and 0.2 cm^2 tip: not reported	Mostly close to or above **246**
Fosse *et al.* (2011b)	24 cross-bred piglets (11 males, 13 females)	11.7 (8-17) d	4.5 (2.9-6.2)	10 d	Purpose-built, hand-held, calibrated pressure algometer	n.s.	1 cm^2 0.5 cm^2 0.2 cm^2	N	5 N/s	24.6 N	Palmar surface of the left metacarpus + corresponding area of the right metacarpus and left metatarsus	With 0.2 cm^2 tip: mostly lower than 24.6 N With 0.5 and 1 cm^2 tips: mostly above 24.6 N	Mostly **lower than 1 230.8** Mostly **above 1 230.8**

Table 6.1. Examples of recent studies investigating mechanical nociceptive thresholds (MNT) in pigs by means of pressure algometry. Results obtained from studies using von Frey monofilaments were not included due to the different biomechanical characteristics (i.e., flexibility of filament) of the instrument. For comparison purposes, only results obtained in healthy, non-lame animals are reported.

*Units of measurements different from kPa were converted starting from the data provided in the study. Note that, whenever instrumentation and testing methodologies differ substantially between studies, especially regarding tip shape, diameter, and rate of application of the stimulus, conversions can only be considered as indicative and should be interpreted with caution.

N = Newton (unit of force); MNT = mechanical nociceptive threshold; gf = gram/force; g/s = grams/force*s-1; kgf = kilograms/force; kgf/s = kilograms/force*s-1; kPa = kilo Pascal (unit of pressure); n.s. = not specified.

Authors	*Pigs*	*Age*	*Live weight (kg)*	*Habituation*	*Instrument*	*Tip*	*Tip area*	*Units of force*	*Stimulus rate*	*Humane endpoint (cutoff)*	*Stimulus location*	*MNT*	*Conversion in units of pressure (kPa)*
Janczak *et al.*, (2012)	44 piglets (21 males, 23 females)	2 wk	4.6 ± 1.0	0 d	Commander Algometer, JTECH Medical, UT	Circular, flat	0.031 cm^2	N	n.d.	25 N	Supradigital palmar/plantar metacarpus/ metatarsus	Mean ± SD 16.8 ± 4.2 N	**5 419.4**
Di Giminiani *et al.* (2013)	48 castrated males	11 wk 16 wk	≈ 30 ≈ 60	3 d	Modified pressure application measurement device (Ugo Basile, Varese, Italy)	Blunt	0.002 cm^2 (ø 0.5 mm)	gf	100 gf/s	25 s or 1500 gf		Median + range	
											1) Flank	Large pigs 1118 gf (507-1454)	**54 819** (24 859-71 294)
												Small pigs 790 gf (443-1154)	**38 736** (21 771- 56 584)
											2) Upper hind limb, caudal aspect	Large pigs 444 gf (314-675)	**21 771** (15 396- 33 097)
												Small pigs 288 gf (206-387)	**14 121** (10 100-18 976)

Table 6.1. Examples of recent studies investigating mechanical nociceptive thresholds (MNT) in pigs by means of pressure algometry. Results obtained from studies using von Frey monofilaments were not included due to the different biomechanical characteristics (i.e., flexibility of filament) of the instrument. For comparison purposes, only results obtained in healthy, non-lame animals are reported.

*Units of measurements different from kPa were converted starting from the data provided in the study. Note that, whenever instrumentation and testing methodologies differ substantially between studies, especially regarding tip shape, diameter, and rate of application of the stimulus, conversions can only be considered as indicative and should be interpreted with caution.

N = Newton (unit of force); MNT = mechanical nociceptive threshold; gf = gram/force; g/s = grams/force*s-1; kgf = kilograms/force; kgf/s = kilograms/force*s-1; kPa = kilo Pascal (unit of pressure); n.s. = not specified.

Authors	***Pigs***	***Age***	***Live weight (kg)***	***Habitu-ation***	***Instrument***	***Tip***	***Tip area***	***Units of force***	***Stimulus rate***	***Humane endpoint (cutoff)***	***Stimulus location***	***MNT***	***Conversion in units of pressure (kPa)***
Chapter 4	14 sows	Mean parity 3.6 ±2	267± 33	7 d	ProD-Plus MNT1 digital algometer (TopCat Metrology Ltd., UK). Two configurations: hand-held probe & remotely operated actuator	Sharp, brass tip	0.785 mm^2 (ø 1 mm)	N	4 N/s	25 N	Dorsal aspect of the metatarsi and metacarpi (5 cm above coronary band)	Mean ± SEM 1) Probe 13.3± 0.53 N 2) Actuator 16.47± 0.54 N	 **16 955** **20 980**
Tapper *et al.* (2013)	12 mixed parity sows	Average parity 3.5 (2-5)	201± 30	7 d	Wagner Force Ten™ FDX 50 Compact Digital Force Gage, Wagner Instruments,CT, USA	Flat rubber tip	1 cm^2	Kgf	1kgf/s	10 kgf		Mean ± SEM	
											(1) Middle of metatarsus (Cannon)	(1) 6.93 ± 0.25 kgf (Cannon)	**679.6**
											(2) 1 cm above the coronary band on the lateral hind claw (Outer)	(2) 7.78 ± 0.28 kgf	**762.9**
											(3) 1 cm above the coronary band on the medial hind claw (Inner)	(3) 7.44 ± 0.30 kgf	**729.6**

Table 6.1. Examples of recent studies investigating mechanical nociceptive thresholds (MNT) in pigs by means of pressure algometry. Results obtained from studies using von Frey monofilaments were not included due to the different biomechanical characteristics (i.e., flexibility of filament) of the instrument. For comparison purposes, only results obtained in healthy, non-lame animals are reported.

*Units of measurements different from kPa were converted starting from the data provided in the study. Note that, whenever instrumentation and testing methodologies differ substantially between studies, especially regarding tip shape, diameter, and rate of application of the stimulus, conversions can only be considered as indicative and should be interpreted with caution.

N = Newton (unit of force); MNT = mechanical nociceptive threshold; gf = gram/force; g/s = grams/force*s-1; kgf = kilograms/force; kgf/s = kilograms/force*s-1; kPa = kilo Pascal (unit of pressure); n.s. = not specified.

Authors	***Pigs***	***Age***	***Live weight (kg)***	***Habitu-ation***	***Instrument***	***Tip***	***Tip area***	***Units of force***	***Stimulus rate***	***Humane endpoint (cutoff)***	***Stimulus location***	***MNT***	***Conversion in units of pressure (kPa)***
Mohling *et al.* (2014)	24 non-bred sows	n.s.	220.1± 21	10 d	Wagner Force Ten™ FDX 50 Compact Digital Force Gage, Wagner Instruments,CT, USA	Flat rubber tip	1 cm^2	kgf	1kgf/s	10 kgf for max 10 s	(1) Middle of metatarsus (Cannon)	Mean ± SEM (1) 6.58±0.30 kgf	**645.3**
											(2) 1 cm above the coronary band on the lateral hind claw (Outer)	(2) 5.51±0.30 kgf	**540.3**
											(3) 1 cm above coronary band on medial hind claw (Inner)	(3) 6.10 ± 0.30 kgf	**598.2**
Chapter 5	8 sows	Mean parity: 5.6 (2-11)	233 ±33 (176-269)	7 d	ProD-Plus MNT1 digital algometer (TopCat Metrology Ltd., UK) in two configurations: hand-held probe & remotely operated actuator	Sharp, brass tip	0.785 mm^2 (ø 1 mm)	N	4 N/s	30 N	(1) Lateral metatarsi and metacarpi	Mean ± SEM (1) 17.6 ± 1.2 N	**22 420**
											(2) Dorsal metatarsi and metacarpi	(2) 18.4± 1.2 N	**23 439**
											(3) Ventral aspect of tail	(3) 11.7 ± 1.8 N	**14 904**

Table 6.1. Examples of recent studies investigating mechanical nociceptive thresholds (MNT) in pigs by means of pressure algometry. Results obtained from studies using von Frey monofilaments were not included due to the different biomechanical characteristics (i.e., flexibility of filament) of the instrument. For comparison purposes, only results obtained in healthy, non-lame animals are reported.

*Units of measurements different from kPa were converted starting from the data provided in the study. Note that, whenever instrumentation and testing methodologies differ substantially between studies, especially regarding tip shape, diameter, and rate of application of the stimulus, conversions can only be considered as indicative and should be interpreted with caution.

N = Newton (unit of force); MNT = mechanical nociceptive threshold; gf = gram/force; g/s = grams/force*s-1; kgf = kilograms/force; kgf/s = kilograms/force*s-1; kPa = kilo Pascal (unit of pressure); n.s. = not specified.

Authors	***Pigs***	***Age***	***Live weight (kg)***	***Habitu-ation***	***Instrument***	***Tip***	***Tip area***	***Units of force***	***Stimulus rate***	***Humane endpoint (cutoff)***	***Stimulus location***	***MNT***	***Conversion in units of pressure (kPa)***
DI Giminiani *et al.* (2016)	123 gilts	9 wk (n=41)	22.3 ± 3.1	4 d	Modified pressure application measurement device (Ugo Basile, Varese, Italy)	Blunt tipped acrylic probe	3.14 mm^2 (ø 2 mm)	gf	120 gf/s	1500 gf (14.7 N)	3 locations on dorsal aspect of tail (proximal, intermediate, distal) Each region approx. 1/3 of entire tail length	Means ± SEM (across locations) 9 wk 464.0 ± 18.9 gf	**1467.83**
		17 wk (n=82)	58.5 ± 8.4									17 wk 642.1 ± 13.4 gf	**2031.24**
		24 wk (n=16)	114.2 ± 12									24 wk 751.6 ± 30.3 gf	**2377.63**
		32 wk (n=6)	152.3 ± 13									32 wk 822.9 ± 49.4 gf	**2603.19**

Variability between repeated MNT measurements had not previously been investigated in sows and is the specific topic of **Chapter 5**. Our study showed that this variability **was high and influenced by several factors. Our coefficient of variation across methods was 28.1%, and was highest in the tail (38.9%), and with the probe (33.4%).** In the literature, CV values beyond 30% are considered indicative of problems in the dataset or that the experiment "is out of control" (Brown, 1998). However, a high variability between repeated measurements at the same site has also been found in human medicine, where pressure algometry (another name for MNT testing) is already a well-established diagnostic tool for neuropathic pain. For instance, Cathcart and Pritchard (2006) found CVs ranging from 20.1% to 47.8% depending on the anatomical region tested in humans (on the hand vs. head). Another study examining the MNT in the neck muscles of female patients with chronic neck pain found CVs between 10% and 22% depending on the specific site (Ylinen *et al.,* 2007). If we consider animal studies, Raundal *et al.* (2014) tested the intra-site, inter-site and inter-observer repeatability of MNT testing with a pressure algometer and a von Frey monofilament in a large number (n=115) of healthy, unrestrained dairy cows. Depending on the instrument and the region, they found estimated mean CVs (± SE) ranging from 34% (±6%) to 52% (±6%; CVs and SE have been multiplied *100 to make the comparison). A methodological study by Taylor *et al.* (2016) investigated MNT testing in the metacarpi of eight adult horses using four probe configurations (sharp pin, blunt pin, 3-pin, spring-mounted). The respective CVs were calculated from 10 repeated stimuli, and the results ranged from 22.9% for the sharp pin to a staggering 72.3% for the blunt pin. In order to reduce variation, Taylor *et al.* (2016) suggested using pins with small tips. Pre-test familiarisation (Raundal *et al.,* 2014, 2015), use of small probe sizes, a controlled environment and instruments that allow a constant rate of application of the stimulus can all contribute to reduce intra-individual variation (Raundal *et al.,* 2014). Data on pigs are not abundant. Recently, Di Giminiani *et al.* (2016) tested the MNTs of the intact tails of pigs (proximal, intermediate and distal portions) with a thumb-adapted algometer. The authors found a similar degree of variability as in our study, namely 30.1-32.6 % across age groups and 30.9%- 32.8% across tail regions. Although the methodology and the ages and weights of the pigs in that study differed from ours, a high intra-test and intra-individual variability appears to be physiological, at least with hand-held instruments. Taking the average of more than five repeated measurements could reduce the CV, but while this can be done in experimental settings, the feasibility under practical conditions is much more problematic. It remains to be seen if intra-session variability can be reduced using a fixed actuator. Some authors have noted that environmental and procedural differences may create variability even within the same laboratory, where factors like ambient temperature, relative humidity, background noise can be controlled (Berge, 2014). While a highly standardised experimental setting can probably reduce the effects of these factors on variability, it is also true that a high degree of standardisation may increase the risk of overestimating spurious effects (Berge, 2014). Ylinen et al (2007) suggested that, whenever the within-subject CVs are too high, MNT testing would best be used in research settings to derive information at group level rather than at individual level. For the time being, our data indicate that this could be a prudent approach.

Some comments need to be made on the study in **Chapter 4. The pathogenesis and duration of lameness were unknown**, as no thorough diagnostic examinations (e.g., necropsy) were performed. The testing position was standardised (dorsal metacarpus and metatarsus), but potential causes of lameness are numerous (see Chapter 1), and the lesions could have occurred in any of the limb or foot joints, or even in the vertebral column. The clinical examination of the sows was only able to exclude the presence of ongoing painful claw lesions and systemic diseases. This means also that **we could not determine with certainty if the found hyperalgesia was of the primary or secondary type**. However, we can make some assumptions. The anatomical location tested was fixed, and, as seen in Chapter 1, the type of diseases that can cause lameness in sows are several and not limited to the metacarpal and metatarsal regions. Taking these factors into account, we can hypothesise that the found hyperalgesia could be of the **secondary type**, i.e., affecting a region adjacent to, or even anatomically remote from, the site of the primary injury, rather than limited to the exact region of the injury. Transient synovitis models lend themselves to a more refined investigation of how MNTs change in the region where the inflammatory challenge is started compared to more distant regions at different time points. While such models are not readily compared to spontaneously occurring cases of lameness, they hold a lot of potential for investigating primary and secondary hyperalgesia.

As the duration of the underlying pathology causing lameness was unknown, **we could not determine if the hyperalgesia we found** in Chapter 4 **was the result of an ongoing pathological process** or rather the persisting effect of a resolved injury or infection. When considering natural occurring cases of lameness, longitudinal observations of the animals after the administration of anti-inflammatories and/or antibiotics can be helpful for determining how MNTs change in response to therapies, if hypearalgesia persists after treatment, and for how long (Laven *et al.,* 2008; Whay *et al.,* 1998).

6.3. Perspectives for future research

The tVAS

- Future potential applications of the tVAS could be to determine herd/group prevalence and incidence of sow lameness based on average tVAS score in prospective large-scale studies. As already seen in the study of Bos *et al.* (2016), the tVAS lends itself to studies on lameness at herd level, with the added advantage that it provides a more refined way to express increases or decreases in the severity of the condition over time than the "classical" incidence. Additionally, the tVAS could be used to derive detailed information on the evolution of gait score in individual cases of sow lameness after treatment.
- The tVAS should be tested in combination with other methods, such as MNT measurement and behavioural observations (Bos *et al.,* 2015; Parsons *et al.,* 2015; Bos *et al.,* 2016) to clarify the overall cost of different degrees of lameness to the sow. This information will be helpful to determine "thresholds" for rest in a hospital pen, treatment with anti-inflammatories, culling, etc. As more data become available from various disciplines (pain physiology, kinematics and kinetics, ethology, etc.), it will be easier to classify individual cases and entire herds based on the proportion of sows into each gait range.
- Future research should examine the performance of the tVAS at different time points, and with different types of observers. Repeatability should be tested live as well as on video, and over a longer time-span to understand what degree of retraining is required to maintain good results.
- An interesting question that wasn't addressed in the current study concerns the effect of using ordinal versus continuous gait scoring scales on the statistical power for detecting treatment effects. For example, it is relevant to enquire if the number of animals used in this type of experiment could be reduced by scoring lameness on a continuous instead of ordinal scale.

Mechanical nociceptive threshold testing in sows

- More research on MNTs in experimentally induced and naturally occurring cases of lameness is required to clarify the relationship between increasing levels of gait abnormality as measured on the tVAS and changes in nociception.
- In future research studies, the techniques described in Chapter 1 (e.g., kinematics, static force plates, accelerometers, etc.) should be used to verify if other parameters than gait

score can be predictably associated with lower-than-normal MNT to diagnose lameness before patent clinical signs appear.

- When considering naturally occurring lameness cases, MNT testing could be followed by post-mortem examination of the claws, joints and vertebral column. This could clarify the relationship between various lameness-causing pathologies and the corresponding changes in MNT at different anatomical locations, both adjacent and non-adjacent to the site of primary injury. Post mortem examinations could be carried out, for instance, in culled sows that are also lame.
- The results described in Chapter 5 on the variability of MNT measurements in healthy sows need to be confirmed on a larger sample of animals to better understand if factors such as parity, live weight, gestation stage, can influence the outcomes. For instance, in the small sample considered, we found a tendency (P= 0.09) for a positive association between live weight and variability. Additionally, inter-rater repeatability needs to be tested, as it is an important component to establish the overall reliability of a measuring method.
- When trying to establish reference values for adult sows, the effect of familiarisation on MNT will have to be further investigated. Di Giminiani *et al.* (2015) found that pigs previously familiarised with the testing procedure had lower mechanical nociceptive thresholds (measured with a von Frey monofilament) than naïve pigs. All the sows used in our experiments had been habituated to the procedures, and therefore we cannot say to what extent and in which direction this may have influenced the outcomes.
- Further research is required into ways to reduce intra-test variability. One promising adaptation to the methodology is the application of a pre-load pressure, especially when using limb-mounted instruments. Having a baseline stimulus may reduce variability by eliminating spurious responses due to the sudden contact of the probe tip with the skin (Musk *et al.,* 2014; Raundal *et al.,* 2014).
- Further studies should examine if reducing variability when measuring MNTs in the pig's tail is feasible, for instance by using a fixed actuator instead of a hand-held instrument. A sow's tail could be an interesting sentinel location to verify the presence of organism-wide secondary hyperalgesia due to painful conditions such as lameness and verify the presence of more localized changes in nociceptive perception due to the practice of tail docking (Di Giminiani *et al.,* 2016).

The multidimensional nature of pain

- In the future, gait scoring and measures of nociception will have to be associated with methods to assess the full range of ethological, physiological, affective and motivational components of animal pain. The motivational components of pain can be studied by means of “operant” pain assays, whereby the animal can choose between tolerating a painful stimulus to receive a reward, or avoiding the stimulus but obtain no reward (reward-conflict tests). In the case of lameness, the painful stimulus will not be external, but will consist in having to stand up, or walk, to obtain a reward (see Bos *et al.,* 2015). Studies on the self-administration of analgesics, or on changes in feeding or social behaviours in the course of painful conditions, and following pain alleviation, can also be used. The tests described by Grégoire *et al.* (2013), Bos *et al.* (2015) and Pairis-Garcia *et al.* (2015) and discussed in Chapter 1 are relevant examples, as they showed that certain types of lameness can alter sow postural time budgets and normal behaviours, thus preventing or hindering access to important resources. The emotional components of pain can be studied by means of pain grimace scales: first developed for laboratory mice and rats (Langford *et al.,* 2010; Sotocinal *et al.,* 2011), these scales codify distinct features of facial expressions (e.g., position of the whiskers and ears, orbital tightening, nostril dilation, etc.) in relation to the painfulness of the disease/stimulus that the animal is experiencing. Pain grimace scales have been developed for several species, such as horses (Dalla Costa *et al.,* 2016; Gleerup et a. 2015a), dairy cattle (Gleerup *et al.,* 2015b), rabbits (Keating *et al.,* 2012), and more have been proposed for small ruminants (McLennan *et al.,* 2016) and piglets (Lonardi *et al.,* 2013; Di Giminiani *et al*., 2016b). For the moment, no such scale has been described in the literature to assess pain in adult pigs and in sows. In rats, peak grimace scales coincide with the development of chemically or surgically induced paw hyperalgesia. However, mechanical hyperalgesia persists after the grimace scale returns to baseline (De Rantere *et al.,* 2016). This reinforces the conclusion that the investigation of pain and hyperalgesia in animals requires a multidimensional approach, of which MNT testing can be one element.

Chapter 7 - General conclusions and implications

The full extent to which lameness adversely affects sow health, welfare and productivity is not yet clear and requires a multimodal investigation, which should include several components (neurophysiological, affective and behavioural). This thesis examined the inter- and intra-observer repeatability of a "tagged" visual analogue scale for visual gait scoring in sows, and investigated factors associated with the mechanical nociceptive thresholds in lame and non-lame sows, while also providing a detailed description of the methodological and animal-related factors affecting MNTs and variability.

Based on our results, the following conclusions can be put forward:

1) The tVAS is an easy-to-learn instrument that can substitute ordinal scales with few categories when scoring lameness in sows, with a high inter- and intra-observer repeatability. Such a scale can be used whenever it is desirable to produce continuous data that can be analysed with parametric statistics, and whenever it is considered important to have a more nuanced representation of the degree of lameness of an animal (or a herd) than an ordinal scale can offer. The implications of using a tVAS *versus* an ordinal lameness scale on the statistical power of the obtained data need to be further investigated.

2) Mechanical nociceptive threshold testing in sows evidenced the presence of hyperalgesia, i.e., a hypersensitivity to noxious stimuli, in lame compared to non-lame limbs. The outcomes were affected by several methodological and animal-related factors, and there was considerable variability in repeated measurements. It is possible that variability can be partially reduced by fine-tuning the instrumentation and the methodology, for instance by using fixed instrumentation as opposed to hand-held probes, and by applying a low pre-load pressure. Comparison of the findings of different studies will be greatly facilitated by accurate reporting of experimental conditions, and by the standardisation of the units of measurement. MNT testing is a well-established diagnostic tool in several fields of human medicine, such as neurology, pain therapy/medicine and physiotherapy. Its potential to become one objective method to measure the nociceptive effects of lameness in sows and the effectiveness of different treatments should continue to be investigated. MNT testing concerns only one dimension of lameness; other types of tests should be used in combination with MNT testing to investigate the affective and behavioural consequences of this condition.

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Samenvatting

Kreupelheid treft een aanzienlijk deel van de fokzeugen in de Europese Unie. In de afgelopen jaren, hebben de epidemiologie en pathogenese van kreupelheid steeds meer wetenschappelijke aandacht gekregen vanwege de ernstige negatieve gevolgen voor het welzijn, de levensduur en de vruchtbaarheid van zeugen. De eerste noodzakelijke stap om kreupelheid van zeugen beter aan te pakken is om betrouwbare en valide detectiemethoden te ontwikkelen. Tegelijkertijd is het belangrijk om de effecten van verschillende niveaus van kreupelheid op het welzijn en gedrag van zeugen beter te begrijpen.

De huidige stand van zaken met betrekking tot deze aspecten wordt gepresenteerd in **Hoofdstuk 1**, waarin de beschikbare technieken voor het detecteren van kreupelheid aan bod komen, evenals de technieken die momenteel worden gevalideerd. Het hoofdstuk gaat ook in op een aantal gedrags- en neurofysiologische effecten van kreupelheid, omdat dit een groeiend gebied van kennis is. Van bijzonder belang is hoe kreupelheid de bereidheid en mogelijkheid tot het uitvoeren van belangrijke biologische functies (zoals toegang bekomen tot voedsel of rustplaatsen) beïnvloedt, en of en hoe kreupelheid de normale perceptie van pijnlijke stimuli kan veranderen (veranderingen in nociceptie). De multimodale studie van de effecten van kreupelheid op het gedrag en de pijnperceptie van zeugen is belangrijk om verschillende redenen. Ten eerste is het onmisbaar om beter te begrijpen op welke manier en in welke mate kreupelheid het welzijn van zeugen aantast. Ten tweede, draagt het bij tot het actualiseren van besluitvormingsprocessen (vanaf welk stadium vereist kreupelheid aandacht? Vanaf welke niveaus van kreupelheid kunnen zeugen baat hebben bij opname in een ziekenboeg, of bij een behandeling met anti-inflammatoire middelen? Wanneer is het raadzamer om een zeug te ruimen ?).

Het algemene doel van dit proefschrift (**Hoofdstuk 2**) was om meer inzicht te krijgen in de verschillende elementen die het welzijn van kreupele zeugen aantasten. Meer specifiek, hebben we onderzocht hoe kreupelheid de mechanische nociceptie drempelwaarde (MNT) beïnvloedt en hebben we geprobeerd om de methode voor het bepalen van deze drempelwaarde te verfijnen.

De eerste specifieke doelstelling van dit proefschrift was om een gelabelde (“tagged”) visuele analoge schaal (tVAS) – t.t.z. een visuele analoge schaal met verbale beschrijvingen om de locomotie van zeugen te scoren – te ontwikkelen en vervolgens te testen op inter- en intra-waarnemer betrouwbaarheid. In **Hoofdstuk 3** presenteren we de resultaten van een studie met 108 nieuw opgeleide waarnemers waarin de inter- en intra-waarnemer betrouwbarheid van deze tVAS werd vergeleken met die van een 5-punts en een 2-punts ordinale schaal met dezelfde verbale beschrijvingen. De inter-waarnemer betrouwbaarheid van de tVAS bleek vergelijkbaar met die van de overeenkomstige 5-puntsschaal, maar de intra-waarnemer betrouwbaarheid van de tVAS was beter. Vergeleken met de 2-punts schaal, hadden zowel de tVAS als de 5-puntsschaal een superieure betrouwbaarheid. In het algemeen beschouwden waarnemers de tVAS als de beste methode voor het beoordelen van individuele dieren te beoordelen (in tegenstelling tot de gehele varkensstapel). Hoewel de tVAS beoordeeld werd als meer tijdrovend en moeilijker te leren dan de ordinale schalen, waren de prestaties beter met de tVAS dan met de 2-punts ordinale schaal.

In **Hoofdstuk 4** werd de nieuw ontwikkelde tVAS gebruikt om een groep van 12 gezonde zeugen en een groep van 12 zeugen met verschillende niveaus van kreupelheid te selecteren. Deze zeugen werden gebruikt in een studie met de volgende doelstellingen: (a) verifiëren of ledematen van kreupele zeugen lagere mechanische nociceptie dremplewaardes (MNTs) hadden dan die van niet-kreupele ledematen; (b) systematische verschillen in MNT onderzoeken, afhankelijk van de instrumentconfiguratie; (c) andere factoren onderzoeken die een invloed zouden kunnen hebben op MNT in de ledematen van zeugen. MNTs werden gemeten in de metatarsi en metacarpi van alle ledematen met een digitale algometer uitgerust met een koperen punt en in twee configuraties, een draagbare (*probe*) en de andere met een band bevestigd aan de poten en bestuurd van op afstand (*actuator*). We stelden hyperalgesie (t.t.z. lagere MNTs) vast bij de kreupele in vergelijking met de niet-kreupele ledematen. *Probe* metingen waren systematisch lager dan *actuator* metingen, en metingen aan de voorpoten waren hoger dan metingen aan de achterpoten. Andere factoren die de MNTs in deze studie beïnvloedden, waren de tijd van de dag (voormiddag t.o.v. na de middag) en de dag waarop werd gemeten.

De resultaten van de studie beschreven in Hoofdstuk 4 toonden aan dat er verdere methodologische testen noodzakelijk zijn, in het bijzonder met betrekking tot de herhaalbaarheid van MNT metingen. **Hoofdstuk 5** presenteert de resultaten van een studie uitgevoerd op 8 gezonde zeugen, waarin de MNTs en de variabiliteit tussen herhaalde metingen (intra-test variabiliteit) werden gemeten op 9 verschillende anatomische gebieden, met twee types configuratie van de algometer (een draagbare *probe* en een op afstand bediende *actuator*), en met 1- versus 3 minuten intervallen tussen vijf opeenvolgende metingen. De variabiliteit tussen opeenvolgende metingen was hoog en varieerde afhankelijk van het specifieke anatomisch gebied en van de algometerconfiguratie, maar bleek niet afhankelijk van het interval tussen opeenvolgende metingen. Zoals ook gevonden in Hoofdstuk 4, MNTs waren lager met de *probe* dan met de *actuator*, en voor de achterpoten vergeleken met de voorpoten. In deze studie vonden we ook een effect van de orde van de meting op MNTs (toename van MNT van de eerste tot de vijfde meting), en de laterale metatarsi en metacarpi hadden lagere MNTs dan de dorsale, gerelateerde posities. Bovendien was er een effect van het tijdstip van de dag op MNTs, mogelijk veroorzaakt door een verschil in de mate van honger in de zeugen getest voor de middag ten opzichte van zeugen die werden getest na de middag.

Hoofdstuk 6 presenteert de algemene conclusies en implicaties van onze bevindingen, de beperkingen van de studies en de mogelijke richtingen voor toekomstig onderzoek in dit vakgebied. De veel hogere intra- en inter-waarnemer herhaalbaarheid die we vonden met de tVAS in vergelijking met de 2-punts ordinale schaal geeft aan dat waarnemers meer kans hebben om het oneens te zijn wanneer ze kreupelheid moeten scoren als aanwezig of afwezig dan wanneer ze de mogelijkheid hebben om te scoren op een continue schaal. Toekomstige studies zouden de herhaalbaarheid van de tVAS moeten beoordelen in longitudinale studies, en met verschillende soorten waarnemers. De mogelijkheid om de tVAS te gebruiken om de gemiddelde locomotiescore van varkensstapels te meten om de effectiviteit te meten van corrigerende of preventieve maatregelen tegen kreupelheid, zou ook onderzocht moeten worden. Wat de mechanische nociceptie drempelwaarde test betreft, hebben we gevonden dat deze methode hyperalgesie in de aangetaste ledematen van kreupele zeugen (spontane gevallen) kan opsporen. In de toekomst zal het belangrijk zijn om een beter inzicht te krijgen in de effecten van kreupelheid en hyperalgesie op dierenwelzijn. Idealiter wordt dit gedaan door het combineren van gedragsgegevens (zoals motivatie om te eten, activiteitspatronen, gelaatsuitdrukkingen, etc.) met fysiologische parameters die het meest nauwkeurig kunnen worden gemeten door gebruik te maken van verschillende methoden (visuele locomotiescore, krachtenplatform analyse, kinematica, nociceptieve drempelwaarde metingen, anatomopathologie, etc.). Het zou ook interessant zijn om het effect van verschillende behandelingen op hyperalgesie in spontane gevallen van kreupelheid te onderzoeken. Vanuit een methodologisch oogpunt, vonden we dat een aantal factoren van invloed zijn op zowel MNTs als de variabiliteit tussen herhaalde metingen op dezelfde anatomische gebieden. Dit maakt het moeilijk om de resultaten van verschillende studies te vergelijken. Voor zover die gegevens worden vermeld, is de variabiliteit in MNT studies in het algemeen hoog. Daarom wordt het aanbevolen om het gemiddelde van verschillende herhaalde metingen op hetzelfde anatomische gebied te rapporteren. Het toepassen van een lage “pre-load" druk voor het uitvoeren van de MNT metingen zou kunnen helpen om variabiliteit te verminderen maar is nog niet getest bij varkens.

Summary

Lameness affects a significant proportion of breeding sows in the European Union. In recent years, its epidemiology and pathogenesis have received increasing scientific attention due to the serious negative consequences on sow welfare, longevity, and prolificacy. The first necessary step to better tackle sow lameness is to develop reliable and valid detection methods. At the same time, it will be important to better understand the effects of different degrees of lameness on sow wellbeing and behaviour.

The current state of affairs concerning these aspects is presented in **Chapter 1**, where the available lameness detection techniques are discussed, as well as the ones currently being validated. The chapter also touches upon some behavioural and neurophysiological effects of lameness, as this is a growing field of knowledge. Of particular interest is how lameness can influence the willingness or capability of an animal to perform biologically important functions (such as accessing food or resting places), and if and how it can alter the normal perception of noxious stimuli (i.e., changes in nociception). A multimodal approach to the evaluation of the effects of lameness on behaviour and pain perception is important for several reasons: first of all, it will be instrumental in understanding in what ways and to what extent lameness affects sow welfare; secondly, it will contribute to inform decision making processes (at what stage does sow lameness require attention? What degrees of lameness can benefit from hospitalisation in a hospital pen, or treatment with anti-inflammatories? When is it more advisable to cull?).

The general aim of this thesis (**Chapter 2**) was to gain more knowledge on the various components of welfare impairment sows experience when affected by lameness by investigating how lameness affects mechanical nociception and by trying to refine the mechanical nociceptive threshold testing methodology.

The first specific objective of this thesis was to develop a “tagged” visual analogue scale (tVAS) − i.e., a visual analogue scale with verbal descriptors to reliably score gait in sows along a continuum, and to subsequently test its inter- and intra-observer repeatability in newly trained observers, as well as the performance of the observers compared to the trainers. In **Chapter 3** we present the results of a study involving 108 observers in which the inter- and intra-observer repeatability of this tVAS was compared with that of a 5-point and a 2-point ordinal scale with the same verbal descriptors. The tVAS was shown to be as repeatable as the corresponding 5-point scale, but with a higher intra-observer repeatability. Compared to the 2-point scale, both the tVAS and the 5-point scale had a superior

repeatability. In general, observers considered the tVAS as the best methodology to assess individual animals (rather than entire herds), and although they judged the tVAS as being more time-consuming and difficult to learn than the ordinal scales, their performances were better with the tVAS than with the 2-point ordinal scale.

In **Chapter 4** we used the newly developed tVAS to select 12 sound sows (3 groups of 4) and 12 sows (3 groups of 4) with various degrees of lameness. The sows were used in a study with the following aims: (a) to verify if lame limbs had lower mechanical nociceptive thresholds (MNTs) than non-lame limbs; (b) to detect systematic differences in MNT depending on the instrument configuration, (c) to determine other factors affecting MNT in the limbs of sows. MNTs were measured in the metatarsi and metacarpi of all limbs with a digital algometer equipped with a brass pin, and in two configurations, one hand-held (probe) and the other fixed to the sow's limbs with cuffs and remotely operated (actuator). The results of our study showed that lame limbs were hyperalgesic, i.e., they had lower MNTs than non-lame limbs. Probe measurements were systematically lower than actuator measurements, and thoracic limbs had higher MNTs than pelvic limbs. Other factors affecting MNTs in this study were time of day and day of testing.

The results of the study described in Chapter 4 highlighted the need for further methodological testing, in particular concerning the repeatability of MNT measurements. **Chapter 5** presents the result of a study carried out in 8 healthy sows, in which the MNTs and the variability between repeated measurements (intra-test variability) were measured at 9 different anatomical locations, with two instrument configurations (hand held probe and remotely-operated actuator), and with 1- versus 3-minute intervals between five consecutive measurements. The variability between consecutive measurements at the same site was high and varied depending on anatomical location and instrument used, but did not depend on the interval between repeated measurements. As found in Chapter 4, MNTs were lower with the probe than with the actuator, and in the pelvic limbs than in the thoracic limbs. In this study, we also found an effect of order of measurement (increase in MNT from 1st to 5th measurement), and the lateral metatarsi and metacarpi had lower MNTs than the dorsal corresponding positions. Additionally, there was an effect of time of day on MNTs, potentially caused by the different degree of hunger in the sows tested in the morning compared with sows tested in the afternoon.

Chapter 6 presents the general conclusions and implications of our findings, the limitations of the studies and the potential directions for future research in this field. The much higher degree of intra- and inter- observer repeatability that we found with the tVAS compared to the 2-point categorical scale indicates that observers are more likely to disagree when they have to score lameness as being present or absent than when they have the possibility to score along a continuum. Future studies should address the repeatability of the tVAS in longitudinal studies, and with different types of observers. Use of the tVAS to determine the average gait score at herd level as a measure of effectiveness of remedial or preventive measures for lameness should also be investigated. Concerning mechanical nociceptive threshold testing, we found that this methodology is capable of detecting hyperalgesia in the affected limbs of lame sows (naturally occurring cases). In the future, it will be important to better understand the effects of lameness and hyperalgesia on animal welfare. Ideally, this should be done by combining behavioural data (feeding motivation, activity budgets, grimaces, etc.) with physiological parameters that can be measured most accurately by using different methods (visual gait scoring, force plate analysis, kinematics, nociceptive threshold measurement, anatomopathology, etc.). It will also be interesting to investigate the effect of different types of treatments on hyperalgesia in naturally occurring cases of lameness. From a methodological point of view, we found that several factors influence both the MNTs and the variability between repeated measurements at the same anatomical site. This makes it difficult to compare the results of different studies. When it is reported, variability is generally high in MNT studies. Therefore, taking the average of several repeated measurements at the same site is recommended. In the specific case of MNT testing in pigs, applying a low pre-load pressure before taking the measurements has not been done yet and may help to reduce variability.

Curriculum vitae

Elena Nalon was born in Venice (Italy) on 6 September 1973. She obtained her secondary school diploma in classical studies in 1992 and went on to get a B.A. (Hons.) with Masters' in Foreign Languages (English and Portuguese) from Venice University "Ca'Foscari", where she graduated *cum laude* in March 1998. She then followed vocational trainings in webdesign and web graphics, and was employed at the University of Padua as a multimedia technician from November 1998 to December 2010. In October 2003, Elena switched to part-time work and enrolled in the faculty of Veterinary Medicine of the University of Padua, where she obtained her qualification of Doctor of Veterinary Medicine in March 2010. In December 2010, she moved to Belgium and started her PhD at the ILVO (Institute for Agricultural and Fisheries Research) and Ghent University. In 2012 she won the poster prize of the Belgian Branch of the International Pig Veterinary Society (IPVS) with a poster entitled "Assessment of mechanical nociceptive thresholds in lame versus non-lame sows with two methods". From 2014 to 2015, she collaborated with Namur University and created an English online course in Animal Ethnography. In 2016, she became a diplomate of the European College of Animal Welfare and Behavioural Medicine (Animal Welfare, Ethics and Law subspecialty). She is also currently a member of the International Society for Applied Ethology (ISAE) and of the Animal Welfare Science, Ethics and Law Veterinary Association (AWSELVA). Since May 2014, she works as programme leader for farm animals at Eurogroup for Animals, an international federation of 51 animal welfare NGOs based in Brussels.

Publications

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