Biomechanical Studies of Locomotion in Pigs

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Abstract

Lameness is a major cause of lost productivity for the pig industry. The objective of this PhD was to develop an objective motion capture method for growing pigs and assess (1) the repeatability and sensitivity of the method (2) the gait characteristics of pigs housed on different floor types and (3) gait differences in pigs with conformational deficiencies, joint disease and/or clinical lameness. Infrared camera-based motion capture was applied to three different cohorts of pigs in three experiments, including an observational study following 84 gilts from grower- to second-parity stage. 3D coordinate data of reflective skin markers attached to head, neck, trunk and leg anatomical landmarks were collected. Temporal (time), linear (displacement) and angular (joint angles) kinematic gait parameters were calculated. Repeatability of the method varied with amount of overlying tissue and/or prominence of anatomical landmarks used for marker placement, but not necessarily with walking speed. Gait development of pigs reared on fully-slatted, partly-slatted or deep straw-bedded floors was not different. Lameness detection and evaluation was possible using relative linear and temporal kinematics. The within-stride trajectory of head and pelvic regions during walking differentiated pigs with front and multi-leg lameness from normal pigs, respectively. The ipsilateral swing-to-stance time ratio detected lameness in hind legs, but was not affected during multi-leg lameness. The frequency and magnitude of irregular steps was increased in lame pigs and in pigs with subclinical joint lesions of osteochondrosis diagnosed post slaughter. Step irregularity (as reflected in the step-tostride length ratio) was also predictive of impending lameness. The step-to-stride length ratio is a dimensionless and ideal parameter to monitor pigs of different age and size, moving at a self-chosen walking speed. Flexion asymmetry and joint flexion patterns were indicative of locomotor problems in some cases. Gait analysis therefore offers potential for automated prediction and early detection of lameness.

Dedication

I dedicate this PhD thesis to my parents, who infused me with love, support and a constant craving for education, knowledge, virtue and truth.

Indeed, philosophical inspiration is something I have acquired from my parents. They taught me modesty and endurance in life, the impermanence of matter and a sense that real satisfaction can only be achieved through the formation of the mind. Without their support many of the stages of my education would have been very difficult, if not impossible, to achieve. I owe my parents this major milestone of my focused labour. I hope their source of motivation, of optimism and admiration of the natural world continues to spring in me.

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Abbreviations

ANOVA Analysis of variance

BW Body weight

CI Confidence interval

cm Centimetre(s)

CoV Coefficient of variation

Deg Degrees

DF Duty factor**Exp** Experiment

h² Heritability estimate

Hz (Hertz) Unit of frequency, cycles per second

ICC Intraclass correlation coefficient

IQR Interquartile range

kg Kilogram(s)LW Live weight

m Meter(s)

mm Millimetre(s)

OC Osteochondrosis

OR Odds ratio

RoM Range of motion

s or sec Second

SD Standard deviation

SEM Standard error of the mean

Terminology

Anorexia Loss of appetite

Anterior Point/area to the front

Contralateral Body parts opposite in side in bilaterally symmetrical organisms

Crepitation Cracking sound

Diaphysis Middle region of a long bone

Discospondylitis Inflammation of the disks (intervertebral disks) between two

adjacent vertebrae (similar to spondylitis)

Distal Point/area in an extremity away from the body centre

Dyspnea Shortness of breath, difficulty to breath.

Endocarditis Inflammation of the inner lining of the heart

Epiphysis Proximal or distal end of a long bone

Exudate Fluid leaking from blood vessels into surrounding, usually inflamed

tissue

Gingivitis Inflammation of the gums

Growth plate Cartilage undergoing proliferation and ossification, responsible for

(physis) growth of long bones

Hyperemia Increase in blood perfusion of a tissue

Hypogalactia Insufficient production of milk in mammary gland

Hypoplasia Deficient formation of structures in the body. This is usually a

congenital condition, meaning that the structures are deficient from birth or do not develop during growth as they would be expected.

Atrophy, instead, refers to the disappearance or reduction in matter

of structures which were previously present

Ipsilateral Body parts of the same body side, left or right

LateralExterior point/areaMedialInterior point/area

Metaphysis Region between diaphysis and epiphysis in long bone

Osteomyelitis Inflammation of the bone marrow (content of the bone lumen in

blood-cell producing long bones or the spongy matter in short bones which produce blood cells), usually due to invasion of

microorganisms such as bacteria e.g in open fractures

Osteophyte Enlargement of normal bony structure (bone spurs)

Palpation Exploring forms, structures and textures by touching and exerting

pressure using the hands

Paresis Partial loss of movement or impaired movement. Paralysis, instead,

is the complete loss of movement

Periarthritis Inflammation of the tissues surrounding a joint

Polyarthritis Inflammation of more than one joint

Polyserositis Inflammation of the serous membranes surrounding viscera

(organs) in the thoracic cage and the abdominal cavity (e.g. pleura, pericardium and peritoneum), usually involving effusion and/or

fibrous thickening

Posterior Point/area to the back

Proximal Point/area in an extremity closer to the body centre

Pyogenic Usually bacteria (*Staphylococci*, *Streptococci*, *Arcanobacterium*,

microorganisms Bacillus spp.) causing the formation of pus in the inflammation

process

Pyrexia Fever

Septicemia The proliferation of bacteria in the blood with systemic

(generalized) symtoms involving damage of vascular endothelium (inner tissue) and diffuse endovascular clotting syndrome (DIC)

along with septicemic shock

Spondylitis Inflammation of the vertebrae, usually with osteolytic foci (local

dissolution of bone) and reactive formation of new bone, often caused by microorganisms travelling through the blood which are trapped locally in the vertebrae and form abscesses; e.g *Brucella*

spp are likely to cause this condition

Synovial fluid Fluid secreted by the synovial membranes to lubricate and nourish

the articular cartilage

Synovial Tissue layer between joint capsule and articular cavity

membranes

List of Scientific Papers Published from this Thesis

- Stavrakakis S, Guy JH, Warlow OME, Johnson GR, Edwards SA (2014). Intraoperator repeatability of skin marker derived segment measures and gait kinematics in pigs. *J Biosystems Engineering* 18, 1-6.
- **2.** Stavrakakis S, Guy JH, Warlow OME, Johnson GR, Edwards SA (2014). Longitudinal gait development and variability of growing pigs reared on three different floor types. *Animal* 8, 338-346.
- **3.** Stavrakakis S, Guy JH, Warlow OME, Johnson GR, Edwards SA (2014). Walking kinematics of growing pigs associated with differences in musculoskeletal conformation, subjective gait score and osteochondrosis. *Livestock Science* (in press).
- **4.** Stavrakakis S, Guy JH, Syranidis I., Johnson GR, Edwards SA (submitted, 2014). Preclinical and clinical walking kinematics in female breeding pigs with lameness a multiple case-control study. *The Veterinary Journal*.

List of Abstracts in Conference Proceedings

- 1. Predicting leg soundness through biomechanical assessment of gait in pigs.
- S. Stavrakakis, J. H. Guy, S. E. M. Lawson, S. A. Edwards
 Proceedings of the 2nd Hellenic Veterinary Congress for Productive Animals and Food
 Hygiene, Thessaloniki, Greece, 2011, p 195.
- Assessment of kinematic gait characteristicsof pigs reared on three different floor types with a single-plane stereophotogrammetric motion capture method.
 Stavrakakis S, Guy JH, Warlow O, Lawson SEM, Johnson GR, Edwards SA
 British Society of Animal Science Annual Meeting, Nottingham, UK, 2012, p 08.
- 3. Longitudinal gait development in pigs assessed with a single-plane stereophotogrammetric motion capture method and associations with clinical subjective scores of conformation, gait and osteochondrosis.

Stavrakakis S, Guy JH, Warlow O, Lawson SEM, Johnson GR, Edwards SA 22nd International Pig Veterinary Society Congress, Jeju, South Korea, 2012, p 314.

4. Seeking the most characteristic quantitative movement changes in lame pigs – potential for automatic herd lameness tracking on farms.

Stavrakakis S, Guy JH, Johnson GR, Edwards SA British Society of Animal Science Annual Meeting, Nottingham, UK, 2013, p 03.

5. Intra-operator reliability of skin marker placement in kinematic gait studies of the pig.

Stavrakakis S, Guy JH, Warlow OME, Johnson GR, Edwards SA Proceedings of the International Society of Biomechanics Congress, Natal, Brazil, 2013, p 250.

6. The pig as a quadruped model of modified body movement and inter-limb support coordination changes in the presence of lameness.

Stavrakakis S, Guy JH, Syranidis I, Johnson GR, Edwards SA

Proceedings of the International Society of Biomechanics Congress, Natal, Brazil, 2013, p 249.

Chapter 1. Introduction

1.1 Outline of the Problem

Lameness is a common cause of lost productivity for the pig industry worldwide and a significant threat to animal welfare (KilBride et al., 2009a). Lameness may arise from poor conformation, lesions in the claws or integument and other disorders in the musculoskeletal and nervous systems (Main et al., 2000). Common diagnostic methods require assessors with accurate diagnostic abilities and time for individual assessment, raising questions about the reliability of subjective gait assessment (D'Eath et al., 2012; Thorup et al., 2007; Main et al., 2000). Moreover, the trend for increasing average herd size in modern pig production has led to the need for novel techniques, which can monitor large numbers of livestock in an efficient, reliable, accurate and consistent way (Cornou et al., 2008; Spahr et al., 1993). Automated and objective biomechanical techniques could provide a valuable means to fulfil this task (Pluk et al., 2012; van Nuffel et al., 2009).

This PhD project investigated the potential of biomechanical techniques to contribute to the solution of locomotor problems encountered by pigs in commercial farming systems across the world. Such solutions could consist of detecting subclinical abnormalities and automating lameness detection, especially the detection of subtle lameness (Maertens et al., 2011). This in turn would improve the ability of breeders/farmers to indirectly select for longevity, both in nucleus and in replacement breeding animals (Serenius and Stalder, 2006). Furthermore, locomotor problems could to be treated before there is a progression in severity and ideally, such problems could even be prevented.

1.2 Experimental Approach

The goal of the present series of experiments was to parameterise normal and abnormal gait in pigs through the use of motion capture technology. Motion capture is one approach of biomechanical gait assessment, providing tools to analyse the kinematic characteristics of the animal's gait. Kinematic characteristics of gait are the changes in the position of body segments in space over time. Flower et al. (2005) studied the kinematics of cows with encouraging results, showing that kinematic gait analysis is a

promising approach in understanding how hoof pathologies affect gait. Motion capture in pigs has been rudimentary to date and there is a lack of description of kinematic gait patterns in unsound pigs (Thorup et al., 2008; Barczewski et al., 1990; Applegate et al., 1988).

Using motion capture, this PhD project aimed to capture movement parameters of a variety of pigs differing in musculoskeletal conformation and orthopaedic health status. Further objectives of the experiments were to evaluate the impact of growth and pen floor surface on the gait of pigs. Another objective was to determine whether and when gait analysis is capable to detect and predict lameness in pigs and which are the most sensitive and suitable movement parameters for each of these purposes. Finally, the aim was to identify simple gait parameters for the development of a farm-friendly method of objective gait assessment.

Infrared camera-based motion capture was applied to three different cohorts of pigs in three experiments. Experiment 1 was a longitudinal treatment study and evaluated the impact of three different pen floors (deep straw-bedded, fully-slatted and partly-slatted) on the gait characteristics of groups of eight pigs housed on each floor for a duration of 11 weeks (Chapter 4). Post-mortem assessment of leg joint lesions and the clinical assessment of leg conformation and subjective gait impression alongside repeated motion captures allowed for the investigation of associations between visually perceived characteristics and gait measures (Chapter 5). Experiment 2 was conducted to assess the repeatability of the methodology on three pigs, each pig giving 10 repeated measurements over a duration of five days (Chapter 3). Experiment 3 was a long-term observational study with repeated application of motion capture to subsets of 84 female breeding pigs over 1.5 years, expecting lameness in 10-20% of the individuals (KilBride et al., 2009, Ernster, 1994). Early (subclinical) and clinical gait characteristics of pigs with lameness were compared to control pigs without lameness (Chapter 6).

Movement parameters of interest in the present studies were step and stride lengths, limb support and swing phases, joint flexion and head and trunk movement in three-dimensional space. Analytical tools were developed to calculate these movement parameters based on raw data, in the form of coordinates, collected with the motion capture system. The calculated movement parameters were hypothesised to compile a gait profile of a pig, which allows for assessment of the soundness of its locomotor

system (Bockstahler et al, 2007; Back et al., 1994). It was assumed that the main goal of a gait in pigs is the coordination of the leg segments of all available legs, in such a way that an efficient anteroposterior displacement is achieved. Therefore one of the major hypotheses was that any problem in a limb be it proximal or distal will eventually impede anteroposterior displacement, especially in its linear component.

Before the experimental chapters, Chapter 2 provides a detailed review of the factors associated with lameness in pigs along with background to the development of kinematic methods of motion capture.

Chapter 2. Background to Lameness and Biomechanics in Pigs

2.1. General Introduction

The occurrence of lameness is a major welfare problem in commercial pigs, affecting both breeding and feeding herds (Nalon et al., 2013a; Jensen et al., 2009; Nielsen et al., 2001). Commonly, researchers use wider terms, such as foot and limb/leg problems or gait/locomotor disorders, to describe abnormalities in the leg and claw structures and movement, which can imply lameness, respectively. Leg weakness is also a widely used term to describe structural (conformational) abnormalities, but also abnormalities in locomotion. Wells (1984) defined lameness as impaired movement or deviation from normal gait. Smith (1988) added the notion that movement is brought about by the functional integration of the nervous system, muscles, tendons, joints, ligaments and the feet. Smiths (1988) further explained that pain is the most common cause of lameness, but any functional disorder of the musculoskeletal system will also impair or cause abnormal movement. For example, a neurological disorder might not be painful, but result in a similar reduced or even complete absence of weight-bearing during movement, as observed with arthritis (Boettger et al., 2009). Pain results from tissue destruction and inflammatory responses due to a variety of initiator pathways (Omoigui, 2007). Since the locomotor system is designed to receive and withstand large dynamic forces, it is especially sensitive during pathological processes (Whay et al., 1997 and 1998).

Causes of lameness in pigs are often not sufficiently identified, probably because pathogenesis of lameness is complex (Nalon et al., 2013a) and the diagnostic approach difficult (Kirk et al;., 2008; Dewey et al., 1993). Veterinary enlistment and medical treatment of lame pigs increase the cost of production (Jensen et al., 2007, 2012), while therapeutic attempts frequently do not lead to the rehabilitation of the affected animal (Kirk et al., 2008). There is little opportunity to inspect the feet and legs of pigs, since routine foot treatments common in other farm species, are not included in the management regime of most pigs farms (Jørgensen, 2000). In addition, the pig is not a cooperative patient and individual restraint of the animal for inspection is not always possible, especially when animals are penned as a group and individual feeding stalls are absent (Dewey et al., 1993). Lifting of a lower leg segment of a pig may be accompanied by a vehement reaction and agitation, especially in growing pigs, which

are not habituated to individual handling. As a consequence, mild lameness may pass unnoticed and severe and irremediable lameness in pigs often results in their culling.

2.1.1 Prevalence of lameness

Lameness has been identified as the second or third most important cause of culling in breeding pigs, accounting for 10-20% of all culled sows (Tarres et al., 2006; Lopes-Serrano et al., 2000; Webb et al., 1983; Dagorn and Aumaitre, 1979). Repeated breeding failure and reduced productivity due to old age are usually the first and second/third most frequent reasons for termination of use in the breeding herd, respectively (Stein et al., 1990). In a comparative study of a breeding population of Landrace sows, lameness caused the culling of 21% of a line selected for low-backfat, but only 14% of the high-backfat line (Grondalen and Vangen, 1974). The annual culling rate due to lameness ranged from 0%-38% in one breeding stock herd and the farms stocked by it (Dewey et al., 1993). KilBride et al. (2009b) reported a lameness prevalence of up to 16.9 and 14.6% of sows and gilts in England, respectively, defining lameness as any score above 0 using a 5-point visual lameness scale. Most reports of lameness in sows from other major pig producing countries quote a prevalence within this range. Lameness prevalence of sows was 13.1% in Norway (Gjein and Larssen, 1995), 8.8% in Finland (Heinonen et al., 2006), 15% in Denmark (Bonde et al., 2004) and 9.7% in Belgium (Pluym et al., 2011).

It was estimated that 20% to 50% of otherwise eligible boars at central testing stations were being eliminated from sales and culled because of leg weakness, a syndrome which is further explained in Section 2.2.3 of this thesis (Webb et al., 1983; Bereskin, 1979). Reiland (1975) previously reported that between 30% and 40% of boars at performance stations and approximately 24% of boars at artificial insemination (A.I.) studs were culled for leg weakness, a clinical syndrome which includes lameness. In the case of the A.I. studs, 75% of the boars culled were less than 18 month of age. These, and the previous values, highlight that lameness may be regarded as a major problem factor interfering with longevity in the breeding herd.

In finishing pigs, the prevalence of lameness can be as high as 19.7% (KilBride et al., 2009). However, other large-scale studies of finishing pigs estimate the prevalence of lameness to be much lower; typically 4% in Scotland (Smith and Morgan, 1997) and 1-2% in Switzerland (van den Berg et al., 2007). These major differences in the reported lameness prevalence are likely to be influenced by the assessment system

used for scoring lameness.

Between 5% and 20% of lameness might be attributable to foot lesions (Kirk et al., 2005; Dewey et al., 2003) and the prevalence of foot lesions is consistently reported to be quite high in pigs (KilBride et al., 2010, 2009b). Mouttoutou et al. (1999) found 94% of finishing pigs affected by foot lesions, which is a reason for major concern if this was reflected across the industry. In comparison, the prevalence of foot lesions in culled sows has been reported to be between 59% and 88% (Anil et al., 2007; Gjein and Larssen, 1995; Penny et al., 1963). However, it has not yet been clarified sufficiently whether foot lesions are a cause or a secondary implication of lameness and instability in the locomotor system (KilBride et al., 2009). Reiland (1975) examined 230 boars and sows culled for lameness and found few cases of foot rot and concluded that claw lesions were of secondary importance in leg problems after joint problems.

2.1.2. Cost and implications of lameness

The major problems associated with lameness are its impact on performance parameters and the welfare of the animal. Lameness may evoke one or more of the following outcomes:

- Lame animals usually prefer to be lying down instead of moving and standing (Chapinal et al., 2009; KilBride et al., 2009), spend less time at the feeder and are less able to compete for food. For growing animals, reduced feed intake naturally implies a reduced weight gain. Average daily weight gain was found to be reduced by 27-40 g in finisher boars with a history of lameness (Jensen et al., 2007). Recent research has also demonstrated that cytokines and chemokines, which are released during inflammatory processes, are responsible for the induction of lethargy and anorexia in animals, and may even block reproductive processes at tissue level (Tizard, 2008). During unfavourable nutritive conditions, the reproductive system suppresses its function in favour of other body systems (Bates et al., 1981). This implies inferior reproductive performance in lame breeding animals.
- Lame sows usually have difficulties accepting natural mating due to pain in the
 affected leg, and boars may show impotence due to inability to mount (Straw et al.,
 1999). Body condition of sows at farrowing and during lactation can be inadequate
 due to reduced feed intake (Anil et al., 2009).
- The risk of piglet mortality is increased in litters where the sow has lameness (Anil et al., 2009; Weary et al., 1996). This can be due to cumbersome and abrupt

- movements of the heavy body of the sow, while suffering from lameness.
- Swine carcasses with arthritic symptoms may be entirely or partly rejected at the abattoir (Bereskin, 1979). Clinically lame animals often have to be euthanized on farm on welfare grounds and are not permitted to be transported to slaughter (Grandin et al., 2010; Council Regulation (EC) No 1/2005).

Growth performance and reproduction can be adversely affected by lameness and in turn depress the productivity and profitability of the farm hosting lame animals (Willgert, 2011; Jensen et al., 2007 and 2012). As a result, there are economic losses for the pig producer and the cost of production may increase by between £19 to £266 per case of lameness (Willgert, 2011).

Simultaneously, lameness is a significant detriment to the animal's welfare. Gait is a fundamental function of animals and is involved in nearly all behaviours, such as foraging for food sources or escape from predators, movement to avoid a stressful environment or social challenge, or finding a mate (Biewener, 2003). Although, in modern production systems, the pig has no need to escape from predators or forage for food, these behavioural aspects and the desire to exhibit them should not be ignored. Reduced or absent locomotion can be a strong indicator that an animal is experiencing pain (KilBride et al., 2009; Telezhenko and Bergsten, 2005) which, as stated previously, is the most common cause of lameness (Smith, 1988). Voluntary abnegation from nutrient intake, in an attempt to avoid changing posture and standing, may be considered as an additional form of welfare impairment. If nutrient intake decreases below maintenance level requirements, normal body function of the animal is affected and subsequently, functional welfare of the animal may be compromised (Adelman and Martin, 2010; Kyriazakis, 2010). In effect, along with body and tail lesions, the presence of lameness serves as a very useful indicator in the assessment of animal welfare (Mullan et al., 2009; Dalmau et al., 2010, Webster, 2001). In a recent survey of expert opinions, lameness was considered to be the most important welfare indicator for pigs and dairy cattle (Whay et al., 2003). A project to improve and standardise animal welfare across the continent was funded by the European Union in 2004 and this underlines the importance of assessment of lameness (Welfare Quality®).

2.1.3 Definitions of lameness

Lameness may be described as a divergence from, or an impairment of, normal gait and posture patterns (Wells 1984). Thus animals showing abnormal gait can be regarded as lame. Normal gait could be defined as a fluent, harmonic, i.e. uniform and regular, and undisturbed sequence of limb and body movement, including the participation of all limbs and each limb exhibiting a cascade of stance and swing phases. Degrees of severity and aetiology of lameness vary (Main et al., 2000; Dewey et al., 1993). Different systems for the definition and scoring of lameness have been developed in order to determine whether lameness is present in an animal and, if so, which degree of severity it reaches. The vast majority of lameness scoring systems rely on methods using subjective judgement of trained observers (Main et al., 2000). Subjective methods allow for a readily implementable, inexpensive on-site assessment without the need for technical resources. These methods, however, are not unproblematic and require observers with experience, accurate diagnostic abilities and the time to invest in individual gait assessment (Main et al., 2000). Consequently, there are concerns about the reliability of subjective assessment methods owing to a lack of sensitivity and specificity and therefore reliability of visual gait assessment (Waxmann et al., 2008; Petersen et al., 2004). The potential of cognitive bias in visual gait assessment exists (Mullan et al., 2009). For example, if a group of pigs has a neglected appearance or comes from a farm with a lameness problem at previous visits, an observer could have a bias of 'expectation'. In contrast, the degree of abnormal gait of clean and well-kept pigs housed on straw, monitored by a friendly, professional and careful producer may be judged favourably (Mullan et al., 2009). The opinions of different observers are likely to disagree, particularly when lameness is only mild or moderate and therefore gait modifications are less obvious to the eye (D'Eath et al., 2012; Liu et al., 2009).

Severity of lameness may be described with the following terms: stiffness, lack of weight bearing or carrying the leg during ambulation and inability to stand and walk without assistance (Straw et al., 1999). A system for the scoring of lameness in cows was developed by Sprecher et al. (1996) that took into consideration the posture and gait of the animal on a 5-point scale. Characteristics, such as short-stride gait, the favouring of one or more limbs and extreme reluctance to bear weight on one or more limbs/feet are listed as being characteristic of 'moderate', 'literal' and 'severe' lameness, respectively. In addition to the aforementioned characteristics, 'arching of the back' is

an important feature in the Sprecher-system, and is expected to become more prominent as lameness severity increases. Sprecher et al. (1996) observed that cows with lameness changed their posture and, in particular, adopted a more crouched position as lameness severity increased. This modification of posture and gait during lameness is also known in horses (Weishaupt, 2008) and has been shown to alter the biomechanics in a way that loading of affected body parts can be reduced. Although the Sprecher-system is a simple, practical and therefore widely employed one (Poursaberi et al., 2010), some researchers argue that it is unreliable and of dubious use in clinical practice (personal communication, K. Nuss, Professor of Large Animal Surgery, University of Zurich). Arching of the back may not be a specific lameness indicator, but a general indicator for pain in the thorax and the abdominal cavity (Gordon, 2012). A lameness assessment system for sheep developed by Kaler et al. (2009) also accounted for head movement during gait cycles and anticipated that head movement, i.e. head nodding/flicking, increased with lameness severity. It is likely that movement alterations of the head and trunk/back during loaded phases of the gait cycles are as important in lame pigs as in the other three species mentioned above.

Main et al. (2000) appear to have been the first authors to publish and validate a lameness scoring system for pigs. Their scoring system was developed by observing the behaviour, standing posture and gait of pigs. Briefly, their subjective gait scoring scale ranges from [0] to [5], where [0]=normal, [1]=stiffness, [2]=lameness detected, [3]=minimal weight bearing on affected limb(s), [4]=pig may not place affected limb on the floor while moving and [5] =animal does not move (full description in Table 8.5, Appendices). However, according to Main et al. (2000) the difficulty in observing lameness in pigs is likely to contribute to problems in attributing consistent scores. They argued that compared with other species, pigs have a stilted locomotion and their natural response to disturbance is a short rapid locomotion rather than steady walk or trott. In addition, Main et al. (2000) stated that the relatively short neck of the pig limits the potential vertical movement of the head, whereas this is an important indicator of lameness in other species (Kaler et al., 2007; Weishaupt, 2008).

2.1.4 Aetiology of lameness

The aetiology of lameness is complex (Nalon et al., 2013a) and often it is not possible to thoroughly investigate the causes and record the symptoms and related data on a daily basis on farms (Kirk et al., 2008). This means that the actual facts about

lameness may not be available in reality. Lameness, which is usually recorded, is a symptom but does not provide information about the degree, the site, nor the cause of origin and likely persistence of the condition (Weishaupt, 2008). Several causes of lameness in pigs can be identified and these can be broadly classified into two major groups: namely maturation-dependent or developmental and maturation-independent lameness (Barneveld and van Weeren, 1999). Forriol and Shapiro (2005) reported that in children and adolescent humans, diminished muscle function due to various pathological conditions causing immobility during musculoskeletal maturation, leads to shortened bones, osteopenia (low bone density) and characteristic deformation. In the pig, the closure of the growth plates which signals the end of skeletal growth, takes place at approximately 18 months of age (Straw et al. 1999; Reiland, 1978). However, the aetiology of lameness varies according to the age of the pig before and after termination of musculoskeletal maturation, i.e. closure of growth plates. Some causes are limited to certain ages only and some causes potentially occur at any age and stage of growth. Specific lameness causes will be discussed in more detail in Section 2.2.1.

Research has shown that environment, management, genotype and nutrition can all influence lameness prevalence among pigs during maturation. Thus, floor type and condition in the pen, space allowance and stocking density, breed/trait selection and growth rate potentially exert an effect on musculoskeletal development (Nakano et al., 1987; Reiland, 1978; Grondalen, 1974). Osteochondrosis (OC) is a non-infectious, degenerative condition that is most commonly found in the young growing pig, in dogs, horses, cows and humans, and may be described as a chronic process of abnormal cartilage development and ossification failure (Straw et al., 1999). In pigs, OC has been associated with selective breeding for lean tissue growth rate and rapid weight gain (Stern et al., 1995; Woltmann et al., 1995; Lundeheim, 1987; Reiland, 1978) and may lead to abnormal skeletal growth and change in shape of various bones and joints (Reiland, 1978). Lameness occurs when OC changes cause pain and/or interfere with normal skeletal function. OC is seen as the major cause of a so-called leg weakness syndrome and lameness, particularly in growing pigs (van Grevenhof et al., 2011, Kirk et al., 2008; Dewey et al., 1993; Smith 1988).

It must be emphasised, however, that most of the previous research on lameness in pigs has used subjective methods to evaluate the presence of abnormal gait. It can be argued that post mortem scoring of joints for lesions as an outcome measure may be largely free of the bias associated with subjective conformation and lameness

assessment on a moving animal (van Grevenhof et al., 2012; Busch et al., 2011). Indeed, post-mortem joint lesions can be quantified and are non-dynamic, i.e. in contrast to subjectively perceived conformation and gait, lesions do not change with posture, muscle activation, leg stance or walking speed. However, the implications of joint lesions for locomotive behaviour and welfare of pigs still need to be investigated and therefore meaningful gait parameters for pigs need be identified. For example, to diagnose OC in live pigs, the only current alternative involves expensive imaging techniques, such as magnetic resonance imaging (MRI), or X-RAY and computer-aided tomography (CT) (Dingemanse et al., 2013; Jørgensen et al., 1995). Recently, a test for OC biomarkers in the blood has been developed, but the drawbacks of this screening test are that it is not yet validated across different populations or breeds (Frantz et al., 2010).

Pathological changes of an anatomical and functional nature, which entail various degrees of limb dysfunction in animals, may potentially arise from any tissue constituting the locomotory system of the organism: bone, cartilage, dense and loose connective tissues (tendons, ligaments, joint capsules), muscle and neural tissue (central nervous system and peripheral nerves) and vascular tissue along with blood supply (Carlson et al., 1988; Ytrehus et al. 2004). All of these tissues are functionally integrated to permit, coordinate and sustain locomotion (Smith, 1988). Therefore any abnormality in any of these tissues may induce a cascade of abnormalities in the other tissues. Furthermore, all tissue structures are dynamic and allow for changes over time. Even tissues such as bone, once formed, can be degraded again (Nigg and Herzog, 1999). This is a situation occurring for example in dietary mineral deficiency and chronic renal failure, conditions which induce secondary hyperparathyroidism. In hyperparathyroidism, homeostatic mechanisms balance calcium deficiency in the blood by supplying the element from deposits in the bones (Jamal and Miller, 2013). Radioopacity (i.e. the level of whiteness of dense structures on x-rays) of bone structures on radiographs is characteristically reduced in hyperparathyroidism. Joint stability, a different example, is ensured by the presence of all designated local anatomical structures, such as muscles, tendons, ligaments and joint capsules. Any deviation, destruction, atrophy (disappearance of structures previously present) or hypoplasy (deficient formation of structures in the body) of these structures can lead to joint instability and the onset of pathological changes, soon reflected in the adjacent tissues. For example, osteoarthritis (see Section 2.2.1) was seen to develop in dogs with an unstable knee joint after experimental trans-section of the anterior cruciate ligament

(Vilensky et al., 1994). Specific causes of lameness are reviewed in the following Section.

2.2 Causes of Lameness in Pigs

2.2.1 General pathology and risk factors

The causes of lameness in pigs vary according to the age of the animal, with different conditions affecting piglets pre- or post-weaning, growers/finishers and adult sows/boars. However, some conditions can potentially occur in any age group, with only the level of incidence varying between age groups. For example, **injury of the leg** (**traumatic damage**), **fractures**, and compression or injury to nerves and subsequent immobility can arise at any age, but may be more likely to occur in heavy sows on slippery floors and in suckling piglets due to crushing by the sow (Anil et al., 2005 and 2009; Barnett et al., 2001). A comprehensive review of the causes of lameness in pigs can be found in the chapter "Diseases of the Nervous and Locomotor Systems" in "Diseases of Swine" by Straw et al. (1999). Much of the following information on the causes of lameness is based on that review.

2.2.1.1 Lameness in pre-weaning pigs

In pre-weaning pigs, three main conditions are most commonly observed as the cause of lameness: Myofibrillar Hypoplasia (Splayleg), Polyarthritis, and Skin Abrasions. Other less common causes of lameness will be referred to briefly.

Myofibrillar Hypoplasia (Splayleg) is a form of muscular weakness that causes paresis in newborn pigs due to inability of leg adduction. It is a common problem that appears in many piglet rearing units, and usually only a few piglets per litter (typically 1-4) and a few litters at a time are affected. Unless extra support is provided to affected animals, mortality may actually be high due to lack of competitiveness, immobility and consequently, starvation or overlying. The condition has a genetic basis within breed and has been associated with short gestation length as well as Large White and Landrace breeds, low birth weight, choline or methionine deficiency in sow diets, Fusarium toxicity and slippery floors (Straw et al. 1999). Taping the affected legs together in order to keep them under the piglets's body, cross-fostering to decrease

competition and supplementary colostrum, milk and heat are the common treatment. If piglets survive to one week of age, muscle strength can resolve spontaneously.

Polyarthritis is a common problem affecting approximately 18% of litters and 3.3% of pigs after 4 days of age (Straw et al. 1999). Some 65% of the cases are caused by haemolytic streptococci, with fewer cases being attributed to staphylococci or Escherichia coli (Nielsen et al., 1975). Mortality due to polyarthritis is usually low (1.4%), but is higher in winter. Affected pigs often die by three weeks of age, however, about one third of animals can survive until the end of the lactation period. Incidence of polyarthritis has been found to be lower in female pigs, pigs from multiparous sows, small litters, closed herds, and herds where teeth clipping and taildocking is not applied (Smith, 1988; Nielsen et al., 1975). Joint lesions include increased synovial fluid, hyperemia of synovial membranes, fibrinous periarthritis, joint swelling due to exudate and abscesses. The carpal, elbow, hock and hip joints are most frequently affected. Often the meninges and brain are congested and concurrent pneumonia, endocarditits, and gingivitis may be observed (Nielsen et al., 1975). Pathogenesis is influenced by the individual pig's ability to eliminate pyogenic (pus-generating) microorganisms before they multiply in the joints. Early treatment with appropriate antibiotics will reduce the duration of illness and mortality. It is crucial to examine pigs at 10 and 18 days of age for signs of lameness (Nielsen et al., 1975).

Skin abrasions occurring bilaterally on hindlegs and forelegs may be evident within a few hours of birth. Piglets may show lameness and/or swollen joints due to abraded skin at the carpal joints and coronary band proximal to the claw, or show abraded horn at the toe. Rough floor surfaces increase the chance of skin abrasions and therefore the opportunity for the invasion of microorganisms (Barnett et al., 2001). In commercial systems, most piglets in the first few days after birth develop skin abrasions, yet only a few are heavily affected and usually the lesions heal within the first three weeks of life. Hard floor surfaces like cement are responsible for higher incidences of skin abrasions, whereas plastic-coated surfaces usually account for less. Similarly, incidence of skin abrasion is higher in litters of sows with hypogalactia and in cases where slatted floors have slots which are large compared to the piglet's foot (Straw et al., 1999).

There are miscellaneous other conditions which potentially entail lameness in the pre-weaning pig: systemic infestation with *Actinobacillus suis* causes septicemia, listlessness, dyspnea and lameness (purulent arthritis) when the condition becomes chronic. However, pigs may be found dead without prior symptoms. Piglets may be

affected by arthritis due to other than the aforementioned microorganisms. A piglet with arthritis is usually reluctant to stand or move, has one or more hot and painful joints and shows poor growth. In rare cases congenital hyperostosis (hyperplastic bones) might be responsible for lameness (Doize and Martineau, 1984). This condition is usually manifested in thick distal legs, which are firm, red, and the overlying skin appears taut. The piglet's gait is stilted and often this condition is fatal. Necropsy reveals thickened leg bones, fibrous tissue deposition and subcutaneous edema.

2.2.1.2 Lameness in post-weaning pigs

Several new microorganisms may be added to the differential diagnosis of lameness in post-weaning pigs. Losses in the abattoir, due to condemnations of either whole carcasses or parts, can be high due to infectious arthritis and other specific conditions, such as Swine Erysipelas, Polyserositis and Glasser's disease. A major reason for occurrence of these three diseases at the post-weaning stage is the loss of maternal, passive immunity at 6-8 weeks of age (Straw et al., 1999).

Erysipelas arthritis is the chronic form of Swine Erysipelas which, in its acute form, causes septicemia and characteristic diamond-shaped, elevated red skin lesions. Pigs surviving the acute form or those pigs with less severe infection may develop chronic Erysipelas, manifested as either endocarditis along with sudden deaths, and/or chronic arthritis. *Erysipelothrix rhusiopathiae* is the infectious agent (Brooke and Riley, 1999) and treatment and control is achieved by the use of antibiotics, such as penicillin, and vaccination, respectively (Amass and Scholz, 1998).

Glässer's disease causes severe, peracute or acute lameness, depression, fever, dyspnea, hot swollen joints, reluctance to stand or move, tremor, paralysis, and death (Straw et al., 1999; Nielsen, 1975). Recovering pigs may develop chronic arthritis. This disease is caused by *Haemophilus parasuis*, a bacterium which typically causes fibrinous polyserositis, meningitis, and arthritis in naïve animals, such as replacement gilts, exposed to a herd where the bacterium is endemic. Outbreaks occur, for example, due to regrouping pigs, at 1-2 weeks post-weaning, and with naïve breeding stock. In nurseries affected with Porcine Reproductive and Respiratory Syndrome (PRRS) virus, a disease associated with immuno-supression, pigs often exhibit polyarthritis as well as clinical signs involving other organ systems. In these herds, lameness can affect as many as 80% of the pigs and typically is the result of multiple etiologies, including H. parasuis, *S. suis*, *M. hyorhinis* and E. *rhusiopathiae* (Kern, 1994).

Polyserositis is usually caused by *Haemophilus parasuis*, although *Mycoplasma hyorhinis*, *Streptococcus suis* and *Pasteurella multocida* are often isolated as well (Straw et al., 1999). Polyserositis occurs mainly in autumn and winter. It is commonly expressed as peracute death or lameness, inability to rise, swollen joints and respiratory distress. Clinically affected pigs carry affected legs because of acute and severe pain (Straw et al., 1999). Inflammation of the serous membranes causes abdominal breathing and reluctance to move.

Mycoplasma hyosynoviae is an infective agent causing arthritis in pigs commonly after 10 weeks of age. Pigs infected with this agent may exhibit soft, fluctuating joint swellings and non-suppurative (purulent) arthritis (Nielsen et al., 2001). In order to distinguish between the various causative factors of bacterial arthritis, samples have to be sent to a laboratory for microbiological examination. However, the microorganism has also been present in joints without lameness (Nielsen et al., 2001). A clinical diagnosis can be made based on symptoms and response to treatment with either Lincomycin or Tiamulin (Jensen et al., 2007). In the Nielsen et al. (2001) study, the mean daily incidence of treatments due to lameness in nine Danish herds was 5.4 per 1000 pigs. Joint disease implied 30-90 min extra labour every day per 1000 pigs for surveillance and treatment, and 5% of the affected individuals were euthanized due to lameness. However, the average daily weight gain until slaughter of the affected pigs seemed unaffected by the lameness.

From the postweaning age onwards, various other conditions, which may also affect older pigs, can cause lameness. These include apophysiolysis, epiphysiolysis, rickets, the asymmetrical hindquarter syndrome, backmuscle-necrosis (part of porcine stress syndrome), foot-and-mouth-disease, osteodystrophia fibrosa, melioidosis, trauma, and finally, the PDS-syndrome in gilts and sows (causing laminitis). These conditions are usually presented with other accompanying symptoms or with a certain history and this can direct the person in charge of the diagnosis towards the right suspicion. Some of these causes of lameness, such as the hindquarter syndrome, back-muscle necrosis, osteodystrophia fibrosa and melioidosis are very uncommon. The remaining causes are further analysed in Sections 2.2.2 and 2.2.5, with more detail on lameness due to genetic or nutritional causes.

In addition to **arthritis** caused by micro-organisms, as discussed previously, the differential diagnosis of lameness in growing and breeding animals includes **foot rot**, **leg injuries**, **epiphysiolysis**, **apophysiolysis**, **osteochondrosis**, **arthrosis**, **osteomalacia** and **fractures** (Wells, 1984; Penny, 1963). In several studies, osteochondrosis (see

Section 2.2.2.) was found to be the most common cause of lameness in growing and breeding-age animals (Dewey et al., 1993; Hill et al., 1990; Reiland, 1978). The second most important cause of lameness was foot lesions, either foot rot, overgrown claws, or torn dewclaws (Dewey et al., 1993). However, outbreaks of foot problems can occur in breeding herds where up to 100% of sows are affected (Gjein and Larssen, 1995; Penny, 1980), although not all of these animals exhibit lameness. Therefore, the relationship between foot lesions and lameness needs further clarification. Osteochondrosis, epiphysiolysis and apophysiolysis will be discussed in greater detail in Section 2.2.2.

2.2.1.3 Lameness in breeding animals

In sows, the culling rate due to lameness varies from farm to farm and this suggests that there is a major problem on certain farms. A study conducted by Dewey et al. (1992) reported that the culling rate due to lameness in start-up herds was higher than in established herds, $26\% \pm 13\%$ compared to $8\% \pm 6\%$, respectively. Herds that were repopulating had a larger proportion of young breeding-age animals and a higher level of culling for lameness (Dewey et al., 1992). A possible explanation for this is that young pigs can be affected by developmental joint disease in addition to the common physical and infectious causes of lameness that affect all age groups (Jensen et al., 2009). This means that the prevalence of lameness among younger pigs may be higher compared to older pigs, which survived risk periods during their development.

Associations between the culling rate due to lameness in sows and various housing factors involving growing gilts indicate that the environment of the young growing animal may have an effect on the skeletal system that only becomes apparent later in life. Housing factors associated with high levels of culling due to lameness were slatted floors for gilt rearing or sows, the use of individual sow stalls, and a high density of pigs in the rearing area. Slatted floors are believed to exert a negative impact due to trapping of claws causing injuries and due to hardness and adverse impact absorption properties (van Grevenhof et al., 2011; Gillman et al., 2009; Scott et al., 2006). On the other hand, stalls and high stocking density are thought to exert an adverse effect through the impediment of exercise and therefore induction of muscular weakness (Harris et al., 2006; Marchant and Broom, 1994). This would suggest that managers of sow herds with higher than acceptable levels of lameness should examine the flooring and housing systems used for the young replacement animals, in addition to the adult sow housing environment (Straw et al., 1999).

Clinical symptoms in sows culled for lameness are usually caused by more than one problem (Kirk et al., 2005; Dewey et al., 1993). The primary cause of lameness is likely to be associated with genetics, predominant feed ingredients, housing type (specifically intensive vs. extensive), floor type, and drainage (Straw et al., 1999). Degenerative joint problems are among the most prevalent conditions, particularly in young growing and breeding animals, with infectious causes becoming more prevalent in older animals. Some of the specific conditions which may cause lameness in breeding animals are now explored in detail.

Infectious Arthritis is one of the most prevalent causes of lameness in sows according to Dewey et al. (1993) and Kirk et al. (2005). Animals developing this type of arthritis are usually older than 18 months of age. Infectious arthritis can often be associated with spondylitis, osteomyelitis and/or arthritis of the hock joint caused by *E. rhusiopathiae, streptococci*, or *Trueperella pyogenes*. Chronic proliferative arthritis and discospondylitis of the vertebral column may be secondary to lesions of osteochondrosis. In suppurative (purulent) infectious arthritis, if the bacteria enter the joint by direct penetration, only one joint is involved. However, if the bacteria are spread from a septic focus such as infected claw lesions, fight wounds, skin abrasions or uterine infections, then polyarthrits ensues. Clinical signs of arthritis are heat, swelling and pain of the affected joint, refusal to bear weight on the leg, pyrexia and anorexia (Hill et al., 1990).

Degenerative joint disease may be the most useful comprehensive term for conditions not implying primary infection, but describing lesions associated with osteoarthrosis, osteochondrosis and osteoarthritis (as described below). Osteochondrosis is discussed in Section 2.2.2.

Arthrosis (arthropathy, osteoarthrosis, or osteoarthritis) is a non-specific, degenerative condition of cartilage that develops in chronic joint disease. Lesions of osteoarthritis include fibrillation of joint cartilage, ulceration of the articular surface, osteophyte production, and thickening of the synovial membrane and joint capsule (see Figure 2.1). Lesions of osteochondrosis that affect the joint surface fill with fibrocartilaginous tissue, which is then replaced by osseous repair tissue (Nakano et al., 1987). The incidence and severity of arthrosis increases with age of the animal (Reiland, 1978). In the study of culled sows by Reiland (1975b, cited by Straw et al., 1999), the incidence arthrosis in sows less than 18 months of age was 7%, whilst in sows older than 18 months it was 82%. Arthrosis is usually a secondary complication of osteochondrosis (Reiland, 1978).

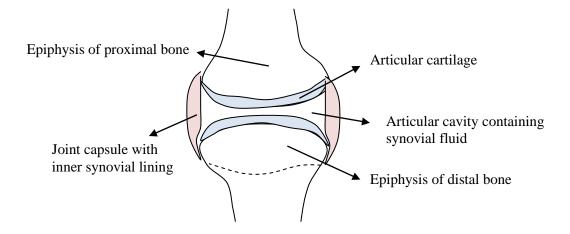


Figure 2.1: Schematic diagram of a synovial joint with related structures. For illustrative purposes, the otherwise closed joint capsule is presented in a frontal cross-section. Dashed lines indicate the distal borders of the joint capsule along the proximal epiphysis of the distal bone.

Torn dewclaws, overgrown lateral digits on the hindfeet and foot rot. These conditions collectively represent either the first or second most important cause of lameness in sows after infections (Dewey et al., 1993). Dry sows housed on partly-slatted concrete floors which become slippery may have their dewclaws torn as their feet slide outward in an attempt to stand. Overgrown lateral digits are seen in animals without exercise, especially those kept on nonabrasive floors such as plastic or steel slats. These sows should have their claws or dewclaws trimmed on a regular basis (Straw et al., 1999). Floors with rough edges or sharp prominences that cause abrasions and floors with poor drainage increase the incidence of foot rot (Straw et al., 1999).

Foot Rot begins as a crack in the wall of the claw which starts at the volar (lower) surface and extends two-thirds of the way to the coronary band (Vaughan, 1969). Secondary infection of the crack by *Fusobacterium necrophorum*, *Trueperella pyogenes*, or spirochetes leads to a deep necrotic ulcer of the laminae and coronary band or a necrotic track which may reach the coronary band and form an ulcer (bush foot) or an infection of the deep flexor tendon or phalangeal bones and joints (Vaughan, 1969). If the crack does not become infected, it is termed a false sand-crack and does not cause lameness. Clinically, foot rot causes a unilateral lameness in which the animal is reluctant to bear weight on the affected limb (Straw et al., 1999). The prevalence of foot rot and associated foot lesions (heel, toe, and sole erosions, white line lesions, and false sand-crack) have been reported to be as much as 64% in slaughter weight pigs (Straw et

al., 1999). Backstrom et al. (1980) found that almost 50% of slaughter pigs had moderate to severe foot lesions, particularly on the pads, sole, and lateral digits. Lesions of foot rot are commonly seen on the lateral claws of the hind legs. Claw injuries often occur in the lateral claw of animals with uneven claws where the lateral claw is larger than the medial claw (Vaughan, 1969; Grondalen, 1974). The uneven claw may also cause the animal to stand and walk with an abnormal gait. Animals housed in crates have fewer claw lesions than those kept in loose pens (Straw et al., 1999). Dewey et al., (1993) found a positive, linear relationship between severity of post-mortem scores for foot lesions and the clinical grade of lameness and parity of the sow. Older sows were more affected by foot problems than younger sows (Dewey et al., 1993), possibly due to age and wear phenomena.

Fractures may occur when an animal struggles to free a limb which has become trapped between floor slats or under a feed trough or pen rail or when it falls on slippery concrete or during transport (Vaughan, 1969). Clinically there is a sudden onset of severe lameness in one leg, the animal carries the leg and there is crepitation (crackling or rattling sound) and pain on palpation. The condition is widespread, but rare (Straw et al., 1999).

Osteoporosis is caused by excess resorption of bone resulting in endosteal thinning of the trabeculae (Figure 2.2, Section 2.2.5.2), i.e. small supporting beams of bony tissue found within bone, due to an increased demineralisation rate (Straw et al., 1999). In sows during mid- to late lactation or the early post-weaning period, osteoporosis can occur as the bones decalcify to mobilize calcium for milk production. These sows may develop fractures of their vertebrae, femurs and phalanges as a result of weakened bones. Clinically this is expressed as lameness, or the 'downer' sow syndrome, seen late in lactation or immediately after weaning when the sow is mounted by the boar or another sow. Typically this syndrome occurs late in the first or second lactation or during the post-weaning period (Straw et al., 1999).

Laminitis is the inflammation of the cutaneous tissue, i.e. layer between horn and deeper structures, of the claws, which appear hot and soft at palpation (Laursen et al., 2009). Affected animals are reluctant to move and generally prefer to lie down and there may be disturbances in locomotion, such as the animal walking on the dorsal (anterior) surface of the carpus. There are three main conditions involved in the aetiology of laminits: excessive consumption of feedstuff, the postpartum PDS-Syndrome (Periparturient Dysgalactia Syndrome) and intoxications. Any one of the three conditions can cause laminitis independently. Laminitis most frequently affects

pregnant animals or animals that develop fever after parturition. Boars can also develop laminitis. It is possible that all four claws of the animal are affected, yet laminitis is mostly seen in the forelegs (Straw et al., 1999). However, in recent years this condition appears less important (Zimmermann et al., 2012).

2.2.1.4 Lameness as part of systemic diseases, multi-symptomatic diseases, and non-age specific lameness

Some of the conditions causing lameness that have previously been mentioned may be multi-symptomatic, systemic diseases. For example, Polyserositis, Glaesser's disease, Swine Erysipelas, Rickets and Osteoporosis may all take a multisymptomatic course and affect other body systems apart from the musculoskeletal system. There are a number of vesicular diseases of pigs which are also multi-symptomatic and may result in lameness.

The symptoms evoked by the four important diseases which comprise the **vesicular disease complex** of the pig are clinically indistinguishable and only laboratory tests can confirm the diagnosis (Straw et al., 1999). All four diseases can cause lameness and represent the vesicular diseases of the pig, namely:

- Swine Vesicular Disease (SVD)
- Vesicular Exanthem of Swine (VES)
- Porcine Vesicular Stomatitis (VS)
- Foot-and-Mouth disease (FMD)

2.2.2 Osteochondrosis – a degenerative failure of cartilage development

Osteochondrosis (OC) is a noninfectious, degenerative (aseptic, ischemic necrosis; Carlson et al., 1991) and often generalised disease of cartilage, occurring during development. It is manifested as an abnormal differentiation of both physeal and epiphyseal cartilage with secondary bony changes, known as osteoarthritis (Hill et al., 1990; Reiland, 1975). Osteochondrosis is considered to be the major cause of leg weakness (see Section 2.2.3) in growing boars and sows (Nakano et al., 1987; Grondalen, 1974, 1981; Reiland, 1975). In a study conducted by Dewey et al. (1993), OC was the most important cause of culling due to lameness among the lame sows examined. The average parity of sows that were culled with OC (1.3 ± 2.9) was lower than the average parity for sows culled with lameness other than OC (3.4 ± 2.5) . This

relationship would be expected, because clinical signs of OC are typically seen in animals between 4 and 18 months of age. This culling of gilts and young sows is particularly costly, because they are removed from the herd before they have reached their peak level of performance (Dewey et al., 1993).

The incidence and severity of joint lesions due to OC in growing pigs were found to increase from 10 to 20 weeks of age (Nakano et al., 1987). Affected animals are typically 4-18 months of age and lesions are frequently seen in the weight-bearing joints (Straw et al., 1999). However, OC has also been detected in younger pigs and may also be found in non-weight bearing joints, such as the vertebrae (Ytrehus et al., 2004). The prevalence of OC can vary widely depending on the examined joints and the severity, but generally 50-90 % of pigs may be found with at least mild lesions in one or more joints (Ryan et al., 2010; Ytrehus, 2004; Jørgensen, 1995; Carlson et al., 1988). It has been reported that 20 to 80 % or more of growing pigs are affected by OC, causing economic losses potentially exceeding \$200 million in the United States alone (Johnson Boric Acid Patent Application, 2010; Ryan, 2010).

OC lesions are often bilaterally symmetrical, several joints may be affected in the same animal and the medial part of joints is frequently more affected (Ytrehus et al., 2007; Dewey et al., 1993; Nakano et al., 1987). Lesions most often occur in the articular-epiphyseal cartilage in the stifle and elbow joint, although the lumbar joints, hock, shoulder and hip may also be affected. Lesions in the growth plates of the femoral and humeral heads, in the distal femur, the distal ulna, the ischiatic tuberosity, thoracolumbar vertebrae and costochondral junctions may also be found. The growth plates that close last are more susceptible to OC (Straw et al., 1999). In a study conducted by Ytrehus et al. (2004), osteochondrosis manifesta (defined below) was most prevalent in the trochlea and the sagittal ridge of the humerus (i.e. both located within the elbow joint), the medial condyle and the medial sulcus obliquus of the femur (i.e. regions within the knee joint). According to the classification proposed by Ytrehus et al. (2004), Osteochondrosis manifesta is an intermediate in severity type of OC characterised by macroscopically and radiographically detectable lesions. This type follows after osteochondrosis latens, a microscopically detectable focal necrosis in cartilage, while osteochondrosis dissecans is signaled by a cartilage flap or loose body within a joint and represents the severest type of OC. Severity was highest in the two sites of the humerus (Ytrehus et al., 2004). OC may also be expressed as epiphysiolysis and apophysiolysis. Epiphysiolysis refers to the separation and displacement of the entire or part of the epiphysis. Apophysiolysis stands for the separation and

displacement of the pelvic processes (Straw et al., 1999). OC lesions may heal spontaneously, but detailed mechanisms of how and when healing can occur, have not yet been fully understood (Ytrehus et al., 2007; Barneveld and van Weeren, 1999; Straw et al., 1999).

2.2.2.1 Clinical signs of osteochondrosis

Clinical OC is a chronic, progressive, shifting lameness affecting one or more limbs in growing pigs (Reiland, 1978). Affected animals will prefer to spend time lying down, will not bear weight on the affected leg(s), and will favor different legs at different times. By 18 months, the incidence of clinical OC decreases, because either the animals have been culled or their lesions have healed (Reiland, 1978). The articular cartilage is devoid of nerves, but pain can be caused by an increased production of joint fluid and swelling of the joint capsule that occur secondary to the lesions of OC (Brennan and Aherne, 1987). Animals with severe OC or arthrosis of the elbow are often clinically sound unless the lesion is a displaced anconeal process (Nakano et al., 1987; Grondalen, 1974). OC of the knee joint causes a severe lameness and is seen in animals less than one year of age (Grondalen, 1974). Young sows with a separation of the tuber ischii (a process of the pelvic bones) will be observed to adopt a dog-sitting position, with their hindlegs directed forward, and if forced to rise, they stand for only a short time. Surprisingly, severe secondary arthrosis of the hock joint, mild arthrosis of the medial condyle of the femur, and repaired lesions of separated tuber ischii appear to cause little discomfort in sows (Grondalen, 1974). OC of the vertebrae, with or without spondylosis, results in kyphosis (humped back). Proximal femoral epiphysiolysis causes an acute severe lameness, and if the lesion is bilateral, the animal is unable to rise. Boars that are lame due to OC spend most of the time lying down, show stiffness when moving, and are unable to mount and copulate (Nakano et al., 1987).

2.2.2.2 Pathogenesis

OC occurs when the mechanism of endochondral bone formation is disturbed in various predilection sites, but its pathogenesis is not entirely clear yet (Ytrehus, 2007; Barneveld and van Weeren, 1999; Grondalen, 1974). While most researchers agree that probably a multi-factorial process is involved in the generation of lesions, it is uncertain which tissue component is abnormal and initiates the onset of the lesions: the

chondrocytes, the cartilage matrix, or the blood vessels. Recently, abnormalities in the regional blood supply are assumed to be crucial in the pathogenesis of OC (Ytrehus et al., 2004, 2007). Failure of endochondral ossification is associated with cell necrosis and reduced amounts of proteoglycans and collagen in the tissue (Carlson et al., 1991). The matrix of cartilage is mainly composed of collagen fibres, water, and proteoglycans, which are protein-glycosamino-glycan (GAG) complexes that exist in either a free form or in aggregates bound by hyaluronic acid (Ytrehus et al., 2007; Straw et al., 1999). Osteochondrosis dissecans and superficial fractures of the cartilage are associated with clusters of chondrocytes and a smaller than normal concentration of proteoglycans (Nakano et al., 1987). Osteochondrosis dissecans develops when a fissure in the subarticular cartilage extends to the articular surface, creating a flap of cartilage (Palmer, 1993). Advanced degrees of OC are expressed as osteochondrosis dissecans or secondary osteoarthrosis and frequently cause lameness in affected animals (Ytrehus, 2007; Nakano et al., 1987). Factors associated with the pathogenesis of OC are mechanical stress, high growth rate, low back-fat, genotype of the animal, conformation and nutrition (Frantz et al., 2008).

Mechanical stress plays a part in the etiology of various skeletal disorders, including OC, arthrosis, intervertebral disk (the cartilage cushion between vertebrae) degeneration, spondylosis and epiphysiolysis (Heijink et al., 2012; Nakano et al. 1987; Grondalen, 1974). Mechanical stress on the joints leading to OC lesions may be caused by local overloading of cartilage or bone tissue, rapid growth rate or weight gain, pregnancy, poor joint stability due to a variety of causes, and weak cartilage or bone tissue (Straw et al., 1999; Grondalen, 1974). Mechanical demands that exceed the ability of a joint to repair and maintain itself, may predispose the articular cartilage to premature degeneration. There is evidence regarding such unfavourable biomechanical conditions about the knee in humans, such as malalignment, loss of meniscal tissue, cartilage defects and joint instability or laxity (Heijink et al., 2012). Joint stability is a function of musculature, ligaments and joint geometry, therefore any problem associated with these structures can imply joint instability. In normal animals, the articular cartilage of the force-bearing areas of the knee joint is thickened and contains more chondroitin sulphate and less collagen than in the non-force-bearing areas, indicating that chondroitin sulphate is used for shock absorption (Nakano et al., 1987). More than 98% of the total protein-glycosamino-glycan (GAG) content of the pig's distal femoral articular cartilage is chondroitin sulphate and its concentration increases

with increasing age from 3 days to 30 weeks (Nakano et al., 1987). In affected pigs, the joint cartilage does not mature properly as they grow, which leaves the joint surfaces prone to damage from mechanical stress. Grondalen (1974) suggested that lesions may be due to a disturbance of the metaphyseal blood flow caused by a local overloading of one part of the joint. In heavy pigs, mechanical stress of large bodyweight on immature cartilage may cause a circulatory disturbance at the bone-cartilage junction, which contributes to the OC lesions (Nakano et al., 1987). Epiphysiolysis of the femoral head, fractures of bone trabeculae (supporting micro-beams in bone) and worn humeral head cartilage seen in boars may be due to overloading caused by weakened muscles, ligaments, cartilage or bone, or by poor conformation (Grondalen, 1974; Grondalen and Vangen 1974). This mechanical stress may cause the joint cartilage to be torn or eroded, leaving exposed bone.

2.2.2.3 The role of growth rate and fatness

It has often been suggested that rapid weight gain results in increased prevalence of OC, but there is inconsistency in the experimental results reported. The modern commercial pig is the product of genetic selection for rapid growth, low feed consumption, long carcass length, low backfat, and high carcass yield of lean meat (Rauw et al., 1998; Reiland, 1978). Bone growth and closure of growth plates is determined by age rather than weight of the pig or energy content of the diet (Grondalen, 1974). Pigs reach sexual maturity typically at 5-6 months of age but do not have a mature skeleton until 18 months of age. Adolescence, the time period between 6 and 18 months of age, is when the clinical signs of OC are frequently seen in the pig. According to Grondalen (1974) and Reiland (1978), the prevalence of OC is related to the pig's rate of gain and backfat thickness, which in turn are functions of both genetics and management practices. Rapid weight gain may increase the mechanical stress on the weight-bearing regions of immature cartilage. Several authors showed that when the growth rate of pigs was slowed by feeding only 50-60% of the feed recommended for their weight range, the clinical signs and severity of the lesions of OC were decreased (Nakano et al., 1987; Reiland, 1975; Grondalen, 1974). Simonsen (1993) added that the injection of Porcine Somatotropin (Growth Hormone) had a negative effect on bone physiology and leg health, including the hazard of leg weakness and OC. Simonsen (1993) assumed that modern pigs are indirectly selected for high levels of growth hormone, since the levels of this hormone are significant for growth performance.

Jørgensen and Andersen (2000) reported that in Danish Landrace and Yorkshire boars, OC traits were unfavorably genetically correlated with daily gain, as were leg weakness traits with lean meat percentage. OC, in the same study, was correlated with leg weakness traits. Hence, according to these findings, any attempts to breed from OC–free pigs could lead to a reduction in desired productive traits.

In 2004, Ytrehus et al. (2004a) published a paper on OC and the vascularisation of the epiphyseal growth cartilage of the distal femur in pigs. They considered the development of this particular growth cartilage with age, growth rate, weight and joint shape. Ytrehus et al. (2004a) concluded that osteochondrosis latens may not be caused by a general failure of blood supply or general factors such as growth rate, but rather is a consequence of local conditions affecting a limited number of vessels. Local compression is what they believed was a factor fitting this description (Ytrehus et al., 2004b). In the same year, Ytrehus et al. (2004c) investigated the effect of parentage and gender on prevalence, severity and location of OC in pigs and claimed that growth rate and weight at slaughter did not influence the presence of OC. In the latter study, there were significant effects of sire and dam on the location of the OC lesions and castrates had significantly higher lesion scores than sows. Previously, Stern and coauthors (1995) had studied OC in elbow and knee joints together with leg weakness traits in female and male pigs selected for lean tissue growth rate, fed either a low or a high protein content diet. Boars had higher lesion scores, even the boars on a low protein diet which grew slower than the females on a high protein diet. Stern et al. (1995) agreed with Nakano et al. (1987), in that the negative effects of selection for lean tissue growth rate on leg weakness and OC were limited in their study. Nakano et al. (1987) had already concluded that no simple association between growth rate and the incidence and severity of OC has been consistently demonstrated. Ytrehus et al. (2007) subsequently reviewed the etiology and pathogenesis of OC and stated that there is no consistent indication that rapid growth aggravates OC.

In conclusion, there seems to be a disagreement between various authors and their experimental findings about the role of growth rate in development of OC. The more recent findings (e.g. Ytrehus et al., 2007) tended to suggest a smaller involvement of growth rate and weight gain in the pathogenesis of OC. However, Busch et al. (2011) and van Grevenhof et al. (2011 and 2012) repeated their own experiments and again reported a negative impact of high growth rate on presence of OC in pigs. Therefore the contribution of rapid weight gain remains a controversial topic to date.

As will be discussed in Section 2.2.3, leg weakness is a clinical syndrome, including conformational deficiencies and poor locomotion, which is seen as the main clinical expression of underlying OC (Jørgensen, 2000). Jørgensen and Andersen (2000) subdivided leg weakness into different symptoms and OC into different localities and found that mainly buck-kneed forelegs, legs turned out, stiff locomotion and swaying hindquarters (Figure 3.1, Section 3.1.1) were correlated with OC in humeral and femoral condyles. Conversely, the conformation of the pig's body, feet and legs may affect the incidence of OC (Brennan and Aherne, 1987). Exterior conformation traits that were associated with an increased incidence of OC and resulting poor locomotion are a long back, narrow lumbar region, broad hams, short hindlegs, foreleg weakness, and small medial claws on the hindlegs (Grondalen, 1974). Thus, selecting replacement stock without these exterior conformation traits may reduce culling due to leg weakness and therefore underlying OC.

Heredity was found to play a role both in the leg weakness complex and OC (Ytrehus et al., 2004; Jørgensen and Andersen, 2000; Barneveld and van Weeren, 1999; Grondalen 1974). Heritability of OC was reported to range from 0.1-0.5 (Jørgensen and Andersen, 2000; Stern et al., 1995). Other studies also supported heritability estimates for OC that vary from low to high (h²=between 0.09-0.49) (Ytrehus et al., 2004; Yazdi et al., 2000). However, several authors consider osteochondrotic lesions to be unfavorably correlated with desirable production variables, such as low backfat, rapid growth and high lean tissue deposition (Webb et al., 1983; Reiland, 1978; Grondalen, 1974). OC in the humeral and femoral condyles were the most important osteochondral traits to select against in order to improve leg soundness, especially in the Yorkshire breed (Ytrehus et al., 2004). Among the four commonly used breeds in commercial production systems, i.e. Duroc, Pietrain, Landrace and Yorkshire, van der Wal et al. (1987) detected significant differences both between breeds and within breeds for the two sexes. In Large White and Meishan hybrid pigs, Lee et al. (2003) detected quantitative trait loci for osteochondrosis-related traits on various chromosomes. These findings suggest that there is a promising potential to identify gene-trait relationships, especially when the traits of interest can be accurately measured.

2.2.2.5 The role of nutrition.

There is some evidence that OC can be influenced by nutritional factors. For example, Frantz et al. (2008) found that the inclusion of several amino acids, such as proline, glycine, methionine and threonine and microminerals, such as copper and manganese, could reduce the presence and severity of joint lesions. The inclusion of fish oil however was not protective (Frantz et al., 2008).

2.2.3 The leg weakness syndrome

Body conformation and gait are two important features to consider, when the soundness and fitness of an organism is evaluated (Webb et al., 1983). In livestock, body conformation and gait are two interrelated features which are crucial for survival (Serenius and Stalder, 2006; Calabotta et al. 1982a). Any productive process is dependent on good body conformation and the ability to move at least over small distances (Jensen et al., 2007; Harris et al., 2006). Poor musculoskeletal conformation, but also abnormalities or lesions in integument and other internal organs, may exert an impact on movement due to discomfort, pain or functional constraint (Jensen et al., 2010; Barnett et al., 1984) Conversely, impaired movement can have an effect on body conformation due to potentially weakened bones, muscle atrophy and decreased ability of the animal to cope with the environment and potentially imposed challenges (Forriol and Shapiro, 2005). Hence, poor conformation can be both a cause and an effect of lameness, but more research is required to clarify how abnormal conformation and movement are related.

Economic implications of the leg weakness syndrome for the pig industry may be severe, given a report that 30 to 50% of animals in breeding herds are culled each year for reasons related to leg weakness (Calabotta et al. 1982a). Since this figure is higher than the commonly reported prevalence of lameness, which is 10-20%, there might be animals without lameness which display conformational abnormalities. Therefore judging by levels of leg weakness alone, the reported prevalence of leg problems might be higher. Farmers have a pronounced interest in keeping only animals with sound conformation and gait (Rothschild et al., 1988). Besides this interest in soundness and freedom from lameness, there is also keen interest in ascertaining the genetic components of soundness (Ytrehus et al., 2004). Animal breeding schemes, be they national or run by a private company, therefore wish to perpetuate musculoskeletal

soundness. This could be achieved either directly by including the phenotype in the selection index or indirectly, by selecting only from animals which have previously been shortlisted due to good conformation and gait (Kyriazakis and Whittemore, 2006).

2.2.3.1 Scoring of leg weakness

In this context, visual scoring systems for lameness and conformation assessment in pigs have been developed by various research groups and for different purposes (Guo et al. 2009; Fan et al. 2009; Main et al. 2000; Sprecher et al. 1997; Jørgensen, 1995; Van Steenbergen et al. 1989; Van der Wal et al., 1987; Drewry et al. 1979). Such purposes included the determination of lameness prevalence, the investigation of genetic correlations of beneficial traits with leg soundness and nutritional studies, examining the effect nutrients on musculoskeletal characteristics. Scoring systems of conformation have been developed on the basis of deviations from a conformational appearance considered normal and desirable (Van Steenbergen et al., 1989; Figure 3.1). These deviations have been named conformational deficiencies or structural unsoundness and are summarised in the so-called swine leg-weakness syndrome, which includes lameness (Nakano et al., 1987). Clinical leg weakness has been defined as the impairment of locomotor ability, structural unsoundness or lameness, anomalies in leg posture and the difficulty to rise and mount (Goedegebuure et al., 1988; Nakano et al., 1987, Van Steenbergen et al., 1989) and is moderately heritable (Bereskin, 1979; Webb et al., 1983). According to Bill Smith (1988), "leg weakness is a description given to a variety of abnormal gaits and locomotion and is not a specific disorder. In all cases the disorder should be either ascribed to very poor conformation or to one of the other recognised pathological conditions of the joints or bone". Jørgensen et al. (2000 and 1995) also included 'stiff locomotion' in their definition of leg weakness. Hence, conformational deficiencies of the typically standing leg(s) and lameness are characteristic traits of leg weakness which can be clinically observed and scored (Jørgensen, 1995; Van Steenbergen et al., 1989).

2.2.3.2 Causes of leg weakness

Complexity of the leg weakness syndrome is a major reason for the difficulty in identifying causes. Nutritional imbalances, high growth rate, lack of exercise, genetics, infectious agents and environmental factors were demonstrated to contribute to the

development of leg weakness (Jørgensen and Sørensen, 1998; Calabotta et al. 1982b). Calabotta et al. (1982b) analysed the side and rear view characteristics, i.e. leg weakness traits, of gilts allocated to different rearing intensities and found undesirable changes from 59 to 100kg. The hind pastern angle and the angle at the hock joint increased with time, suggesting development of post-leggedness (see Figure 3.1) as pigs increased in weight and age. Both left and right hock-joint deviation increased between the two time points. However, elevated phosphorus and calcium intake did not have an effect on this development of leg weakness. In a study by Barczewski et al. (1990), leg weakness scores were unaffected by previous dietary energy, calcium or phosphorus levels. Overall soundness scores were also not related to any quantitative feet or leg characteristics obtained (Barczewski et al., 1990).

Osteochondrosis has been suggested as the principal cause of leg weakness (KilBride et al., 2009; Jørgensen 1995, 1998; Smith, 1988; Nakano et al., 1987). Jørgensen and Andersen (2000) found that in Danish Landrace and Yorkshire boars the heritability of leg weakness traits ranged from 0.01 to 0.35. This is supported by data from Rothschild and Christian (1988) who reported that genetic control of front leg structure in Duroc swine was high (h² of up to 0.42). Similarly, Bereskin (1979) reported low to moderate rates of improvement of leg soundness in gilts by direct selection, and problems of unfavourable correlation with other traits, such as backfat and loin eye area. In the same study, Landrace pigs were found to be more severely affected than Yorkshire pigs regarding all leg weakness symptoms, except for front leg weakness traits. In studies conducted by McPhee and Laws (1976) and Jørgensen and Vestergaard (1990), the Yorkshire breed was found to have significantly more foreleg weakness, but better locomotion and overall leg score than the Landrace, hence confirming the previous results. The Duroc breed has also been associated with increased front leg weakness (Tarres et al., 2006; Jørgensen and Andersen, 2000; Draper et al., 1988). A recent study by Fan et al. (2009) provides a comprehensive list of candidate genes related to leg weakness traits that have been identified in the pig.

2.2.3.3 The need for new approaches in the assessment of leg weakness

To date, assessment of leg weakness and lameness is mostly based on visual appraisal of the respective, perceived characteristics of the animals (de Koning et al., 2012). This implies there is still a lack of non-visual, quantitative lameness and conformation assessment systems (Nalon et al., 2013a), and the repeatability of

subjective scoring is known to be deficient (Waxman et al., 2008; Petersen et al., 2004). A few researchers have begun to measure leg weakness using various approaches (Pluym et al., 2013a; Draper et al., 1988 and 1992). Nevertheless, the relationship between leg weakness and underlying disease causes, and the implications of the symptoms, are not yet fully understood. In horses, for instance, assessment of conformational deficiency is traditional practice and often believed to be associated with performance (Back and Clayton, 2001). However, only some relationships between conformational traits and performance characteristics could be scientifically confirmed when subjective and quantitative assessment techniques were combined (Back and Clayton, 2001). Therefore, the human perception of a positive or negative feature may not necessarily reflect functional aspects. It may be concluded that, if conformational deficiency is an effect or the cause of a leg problem, biomechanical implications are likely to exist. Quantifying these may help to better understand the role of conformational deficiency in the manifestation or generation of leg problems, such as OC, and more clearly identify its presence and specific pathogenesis.

2.2.4 Flooring and its biomechanical implications for lameness

Over the last three decades a considerable amount of research has been undertaken to examine the influence of floor surface on the gait of pigs (van Grevenhof et al., 2011; von Wachenfelt 2008 and 2009; Thorup et al., 2007 and 2008; Barnett et al., 2001; Applegate et al., 1988, Jørgensen 2003, KilBride et al., 2008, 2009a and 2009b, Mouttoutou et al., 1999). The research groups of Von Wachenfelt, Thorup and Applegate used biomechanical gait analysis techniques and demonstrated that pigs walked differently on floors with varying surface properties, ranging from dry to wet and greasy, and therefore representing soiled and slippery surfaces. Their findings suggested that demands on the musculoskeletal system, particularly on joints, vary with the floor surfaces on which pigs are housed. More quantitative research in this area is required, particularly to find out how the imposed demand is adjusted for by the musculoskeletal system and to clarify how floors may lead to leg problems in the long-term. Unless floors have an association with an increased microbial load, leading to higher infection rates (Scott et al., 2006), their involvement in the generation of lameness is likely to be of a biomechanical nature (Heijink et al., 2012).

Lameness is associated with many factors and cannot usually be blamed on only one factor (Nalon et al., 2013a; Straw et al., 1999). Housing appears to be an important

factor influencing the level of lameness that an individual herd experiences (Baxter et al., 1983). Floor types have been reported to affect the incidence of foot and limb disorders (Scott et al., 2006; Nakano et al., 1987). In the UK, a survey of Pig Health Scheme Herds showed that 44% of farms with slatted floors had pigs with injuries, whereas only 28% of farms with solid floors had pigs with injuries caused by flooring (MAFF, 1981). Good floors require a durable, low-cost, nonslip, nonabrasive surface and acceptable levels of cleanliness and ease of cleaning (Mouttotou, 1999). However, it can be difficult to obtain the correct balance between non-slipperiness and nonabrasiveness, particularly with solid concrete floors (Straw et al., 1999). Rough concrete floors are typically created by using sand of the wrong particle size during the manufacture of the cement, or by allowing the surface to be broken down by wear or organic acid in the urine. Poorly laid concrete floors can cause abrasions to areas such as the spine of the scapula, the claws, and the soles of claws (Bonde et al., 2004; Boyle et al., 2002). Slippery concrete can cause ataxia and tendon swelling, and newly laid concrete may cause lameness due to its slippery qualities as well as its surface chemicals. Similarly, wet concrete pens may cause cracked and bruised soles, which can lead to secondary infections (MAFF, 1981). Nonetheless, substandard concrete may be improved by using bedding, laying a new concrete floor, or coating the concrete with chlorinated rubber, epoxy resin, or polyurethane paints (Straw et al., 1999; Baxter et al., 1983).

Concrete floors were reported to cause more foot and limb disorders than earthen floors or deep straw bedding (KilBride et al., 2009; Gilman et al., 2009; Nakano et al., 1987). Perforated floors, such as partly-slatted or fully-slatted concrete or metal slatted floors were found to cause more lameness and injuries than solid concrete floors (Gillman et al., 2009; Newton et al., 1980). The edges of concrete slats can be excessively rough and the slats may be placed too far apart, causing abrasions of the coronary band and the accessory digits (Straw et al., 1999; Baxter et al., 1983). The dimensions of slats and slots are regulated by the European Council Directive 2001/88/EC and range from 11-20 mm depending on the age and therefore size of pigs. In farrowing crates, plastic and steel slats have been associated with a higher risk of lameness compared to solid floors (Bonde et al., 2004; Phillips et al., 1996). Plastic slats may be too slippery and cause the sow's feet to be overgrown, leading to secondary damage to the accessory digits (Newton et al., 1980). Foot pad lesions and claw cracks were positively correlated with claw and sole length, whilst claw length and sole length were found to be longest on plastic slats, followed by aluminium, steel, and concrete

(Newton et al., 1980). In their review of published articles, Straw et al. (1999) argued that the best floor type for the development of feet and legs is a solid floor with bedding.

The level of exercise can also be related to floor type and housing, thus pigs on straw-bedded floors were found to be more active compared to pigs on fully- or partlyslatted floors (Scott et al., 2006). Furthermore, pigs given exercise and pigs penned with other animals were observed with better gait scores and less lameness than pigs denied exercise or housed individually (Grondalen, 1974). Inactive, stall-housed animals have been diagnosed with muscular weakness and a reduction in cortical bone mass (Marchant and Broom, 1994). In contrast, pigs that were exercised had increased muscle strength and were more agile, allowing them surer movement on slippery floors (Grondalen, 1974). The duration of confinement was correlated positively to the degree of joint damage in the previous study (Grondalen, 1974). Perrin et al. (1977) found that boars given exercise had fewer conformational abnormalities such as bowlegs (Oshaped legs, Figure 3.1), flexion of the carpus, and sickle hocks than boars in confinement. Fredeen and Sather (1978) confirmed that confinement adversely affected the degree of joint damage. However, recent research raises concerns about the high lameness incidence experienced by group-housed sows (Pluym et al., 2011, 2013a). Compared to the previously widely used individual stall-housing system, group-housing was observed to increase foot and limb disorders (Pluym et al., 2013a; Karlen et al., 2007; Harris et al., 2006). These findings suggest that the challenges imposed on the musculoskeletal system by interaction between females in groups may outweigh the weakness induced by individual housing.

As stated previously, rough concrete floors, sharp edged concrete slats, and slats of other materials can result in severe foot injuries which can lead to changes in the way the pig walks (Straw et al., 1999). An altered gait may change the joint congruence and this can cause overloading in the joint, both by a change in joint force direction and force magnitude, and hence cause subsequent cartilage damage (Aherne and Brennan, 1985). Both insecure footing and foot lesions are believed to increase the mechanical stress to joints, which in turn may increase the incidence of leg weakness (Nakano et al., 1987), as was found on perforated floors (Nakano et al., 1987). Raising pigs on deep straw bedding rather than partly-slatted concrete floors was suggested to decrease the incidence of gait abnormalities, claw injuries and the incidence of clinical OC, i.e. leg weakness (Straw et al., 1999). Similarly, Fredeen and Sather (1978) stated that floor and housing type in the nursing period could increase the piglets' susceptibility to joint damage after weaning. Contrary to these reports, Perrin et al. (1978) and Brennan and

Aherne (1987) found that floor type did not influence the prevalence and severity of joint lesions in pigs. This finding was later confirmed by Scott et al. (2006), who additionally reported that toe lesions were worse on straw-bedded floors. In more recent research by van Grevenhof et al. (2011) and Jørgensen (2003), associations between fully/partly slatted floors and joints lesions were present and absent, respectively, compared to straw-bedded floors.

In conclusion, there is still conflicting evidence regarding the impact of floor type on the prevalence and severity of foot and limb disorders. Improving the methods of assessment and applying quantitative techniques is important, particularly for the understanding of the chronic pathogenic mechanisms of foot and limb disorders in relation to factors, such as floor type.

2.2.5 Musculoskeletal development and the role of nutrition

The development of the musculoskeletal system is a complex process beginning as early as embryonic cell differentiation (McGrath and Soltins, 1984). The musculoskeletal system originates from the mesoderm, one of the three embryonic tissue-generating membranes. Completion of the musculoskeletal system is achieved at maturity, i.e. closure of all growth plates, whereas its maintenance is dynamic throughout life. The different levels of this development are examined by disciplines such as biochemistry, histology, anatomy, physiology and mechanics. In mammals, growth is an allometric process, meaning that different tissues and organs grow at different rates at different times (Tanck et al., 2001). In growing mammals, nutrients are first directed towards bone, then muscle and finally fat tissue (Fortin et al., 1987), therefore the rate of change in the musculoskeletal system is rapid during the early postnatal period of life.

The skeletal system exerts two major functions: a physiological and a mechanical function. The physiological function includes the formation of blood cells (hematopoiesis) and the storage of calcium. The mechanical function includes the provision of support for the body against external forces (e.g. gravity) and the transfer of forces such as muscle-tendon forces by acting as a lever system. Provision of protection for vital internal organs, such as the brain, lung and heart is another mechanical function of the skeletal system (Hildebrand et al., 2001).

The muscular system has primary and secondary functions: The most important task of muscles is to create tension along the axis of muscle fibres, contained within the

muscles, and thereby to shorten their length (concentric contraction), thus moving a bone or constricting a space. Isometric contraction and eccentric contraction describe states, where muscles produce force at the same time as having constant or even increasing length, respectively (Nigg and Herzog, 1999). Isometric contraction may prevent motion by opposing gravity or the pull of other muscles, while eccentric contraction in the quadriceps extensor muscle at the knee joint, for example, controls the bending moment created after foot impact during locomotion (Whittle, 1996). Hence, with little or no motion, muscles may function to cause a part of the body to become more rigid or may offer controlled resistance to extrinsic forces that tend to stretch them out. As a secondary function, the muscular system makes an important contribution to the maintenance of the body temperature of endotherm organisms (mammals). Furthermore, because of its bulk, the musculoskeletal system distributes the weight of the body and influences the contours of the body, thereby offering protection to some of the viscera (Hildebrand et al., 2001).

Conformation may be defined as the physical appearance of an animal due to the arrangement of muscle, bone and other body tissue, such as fat. In other words, it is the sum of these body parts and how they blend together which determines the functionality and sustainability of the musculoskeletal system. Considering the musculoskeletal complex in its entirety, the major function is clearly a biomechanical one, i.e. to generate and coordinate movement. Therefore both the macroscopic (anatomical) and microscopic structure of the organs, forming part of this system, is very crucial. Although the form of muscles, bones and other features of the musculoskeletal system is far from incidental, consideration of microscopical (histological) features is important for the understanding of musculoskeletal development and pathophysiology (Palmer, 1993). Nigg and Herzog (1999) and Hildebrand et al. (2001) provide a thorough overview of the development of the musculoskeletal system, on which most of the following information is based.

The musculoskeletal system is comprised of bone, cartilage and muscles, along with tendons, ligaments and other connective tissues, all of which have different properties and/or purposes. The main differences between these tissues are the shape of their cells and the amount and characteristics of the extracellular matrix surrounding them. The presence or absence and the amount and orientation of fibres in the extracellular matrix play an important role in the mechanical properties of musculoskeletal tissues, particularly in tendons and ligaments. All these tissues fulfil different purposes and therefore have different histological characteristics, but

eventually all work together to generate forces (muscles), transfer these (tendons, bones) and produce motion (via joints bound together by ligaments) (Hildebrand et al., 2001).

Tendons and ligaments belong to a broader histological category which includes connective tissues (Leeson et al., 1985). Tendons and ligaments consist of dense connective tissue, rich in collagen fibres, which are organised and oriented mainly along the longitudinal axis of the organ. Tendons are tough, cord-like constructs joining muscles to bones. Ligaments join bone to bone, their collagenous fibres are somewhat less regular and they include elastic fibres (Nigg and Herzog, 1999). Joint capsules are built primarily of connective tissue and an inner layer of serous cells, which secrete synovial fluid to lubricate the joint cavity and nourish the avascular joint cartilage (Figure 2.1).

2.2.5.2 Muscle

Muscle tissue is a histological category of its own and comprises three types of muscular tissue, namely smooth, cardiac and skeletal muscle (Leeson et al., 1985). The most important properties of any of these muscular tissues are excitability, conductibility (especially relevant for cardiac muscle cells) and foremost, contractibility (Nigg and Herzog, 1999). Contractibility is implemented by contractile units of filaments, the sarcomeres, which are driven by specific biochemical reactions involving the binding of calcium ions.

The development and mass of skeletal muscular tissue depends on a variety of factors, including gender, age, nutrition, health, genetics and exercise. Development of muscle in terms of the number of muscular cells (muscle fibres) may be regarded as completed once the animal reaches terminal growth. In adult organisms there is a continuous protein turnover, but no new additional lean tissue deposited unless there is significant exercise, causing muscle cells to undergo hypertrophy (expansion of cells) rather than hyperplasia (proliferation of cells). Under normal nutritional conditions, and given a regular employment of muscles as occurs in daily routine, muscle mass is maintained by a protein turnover mechanism. Thus, once deposited, protein in the body is not permanent, but will be degraded and replaced by new equivalent protein, provided that the required amount and type of amino acids is supplied. However, in the case of protein imbalance, disease, hormonal disorders, circulatory issues, prolonged unemployment of muscles (due to immobility) or following motor neuron damage,

muscle atrophy occurs (Nigg and Herzog, 1999). This may be a reversible condition, yet depends on the cause and pathogenesis of the atrophy.

2.2.5.2 Bone

Structures in the body most subjected to the action of stresses and strains are the bones and associated cartilage. Stress is defined as force applied to a unit of area of a structure, therefore given the same force acting on a joint, a joint with greater cross section would experience less stress. Force is a vector, hence it is characterised by both magnitude and direction. Depending on the direction of application of force per area of joint, shear and direct stress can arise. Shear stress arises from a force vector component parallel to the cross-section on which it acts, while direct stress arises from the force vector component perpendicular to the cross-section. Strain refers to deformation in a structure in response to a force or stress applied to it (Nigg and Herzog, 1999).

There are two processes, which direct the creation and maintenance of the skeleton. On the one hand, there is bone modelling, a process during which trabecular (spongy) and cortical (compact) bone grows and develops and bone mass is added (Figure 2.2). Modelling is predominant during skeletal development, as the bone adapts to required loading conditions, and is less efficient in the adult skeleton. Bone remodelling, on the other hand, is responsible for the maintenance of the soundness of the skeleton throughout the lifespan of an organism (Nigg and Herzog, 1999).

During bone modelling, two basic independent mechanisms are responsible for osteogenesis (generation of bone), namely endochondral and endomembranous osteogenesis (Nigg and Herzog, 1999). Endochondral osteogenesis involves the generation of bone from previously present cartilage, both during and after embryonic development. This type of osteogenesis takes place within a cartilage scaffold, which was created during embryonic development, and this process permits growth while giving structure to weight-bearing structures at the same time (Mackie et al., 2008). Endomembranous osteogenesis involves a process in which bone tissue arises from a nucleus of bone formation located inside fibrous, connective tissue. Bones such as the flat skull bones are completely generated by endomembranous osteogenesis. The long bones of the extremities are modeled by the two osteogenic processes, working independently and simultaneously. There are several ossification centres present in each long bone after birth. The diaphysis and both epiphyses contain primary and secondary ossification centres, respectively. Between the primary and secondary ossification

centres lies the cartilaginous epiphyseal plate (or growth plate) at both ends of a long bone. The growth plate, together with the other ossification centres, controls the elongation of bones. Endomembranous osteogenesis directs the peripheral growth of bones, adding bone to the periphery of bones through the periosteum (Hildebrand and Goslow, 2001).

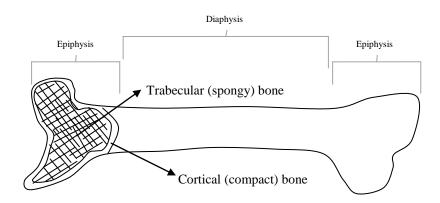


Figure 2.2: Schematic diagram of a long bone. One epiphysis is exposed to show the trabeculae, i.e. delicate bony beams, contained within.

Bone remodelling, also referred to as mechanical adaptation, occurs at the surface of bone and involves a dynamic process of morphological adaptation in both cortical and trabecular bone (Figure 2.2). The maintenance of bone is achieved through bone remodelling by the ongoing replacement of old bone by new, thus repairing microcracks that occur during normal activity. Bone remodelling provides the ability to adapt to environmental changes in loading, so that if mechanical load is increased through exercise, an increase in bone mass can occur. Conversely, decreased load in humans, as a result of space flight or prolonged bed rest can result in decreased bone mass. Not only can bone mass be changed but, particularly in the case of trabecular bone, the architecture may also adapt to the new mechanical environment. It is assumed that osteocytes (bone cells) may serve as mechanoceptors regulating this process (Nigg and Herzog, 1999).

Resorption and formation are processes taking place at cellular level in bones, which permit modelling and remodelling. Osteoblasts are a cell type within bone, which can form bone, while osteoclasts are cells which can resorb bone. Resorption and formation are coupled and, in the normal adult skeleton formation normally only occurs where there has been previous resorption. This process is carried out by a so-called fundamental functional unit, also named a basic multicellular unit (BMU). The basic

multicellular unit can be activated and, subsequently, osteoclasts resorb bone matrix while new bone matrix is formed by osteoblasts followed by mineralisation (Nigg and Herzog, 1999).

Bone modelling and remodelling is governed by the rules of physical laws (Nigg and Herzog, 1999). The configuration of mature bones is greatly influenced by mechanical interactions with the developing muscles. In the absence of normal muscles and muscle activity, the skeleton is abnormal (Hildebrand and Herzog, 2001). Currently, strain is considered to be the most important mechanical factor regarding bone remodelling. Strain can be expressed either in compression or tension, i.e. deformation along the long axis resulting in shortening or lengthening of the bone, both having osteogenic potential (Forriol and Shapiro, 2005). Within a normal range (or just above/below it), increases or decreases in compression both seem to accelerate cartilage growth (Forriol and Shapiro, 2005). This acceleration in cartilage growth is particularly true with intermittent dynamic loading. Dynamic loading describes loading with changes in magnitude, whereas static loading implies the application of a constant load (Nigg and Herzog, 1999). With compression well below physiologic limits, bone growth can be reduced or even halted, while massive increases in compression beyond the normal range severely retard growth. It was demonstrated that longitudinal growth suppression, resulting from the application of compressible loads to bone ends, was proportional to load magnitude (Forriol and Shapiro, 2005).

Forriol and Shapiro (2005) assessed endomembranous, periosteal bone formation in humans and demonstrated that dynamic loading increased osteogenesis, whereas static loading suppressed it. Growth and activity during childhood influence skeletal development in humans (Forriol and Shapiro, 2005). The development of the proximal femur reflects the changes in adaptation of the physeal and trabecular patterns to mechanical requirements. In humans at birth, the growth plate of the femoral head is transverse and the metaphyseal trabeculae are arranged longitudinally. During the first year of life, the neck of the femur develops and from then onward, the direction of the head and neck trabeculae reflect responses to compression and tension. The trabecular pattern is then well developed between the ages of five and seven years. Growth plate fracture separations account for about 15% of all childhood fractures and can, in some instances, damage the growth plates and lead to growth problems such as shortening, angular deformity, and joint surface irregularity predisposing patients to early osteoarthritis (Forriol and Shapiro, 2005). Previous research also supported that the misalignment of bones, as seen in varus (O-shaped) and valgus (X-shaped) leg

conformation, plays a role in the development of skeletal disease, such as osteoarthritis (Heijink et al., 2012; Yusuf et al., 2011; Lynn et al., 2007). Although such pathological adaptations are well known from human orthopaedic research, it is likely that similar adaption strategies exist in other species.

In conclusion, the development of the skeletal system represents a critical time interval during which conditions must be ideal to allow for optimum conformation. Any problems occurring in the skeletal system during this interval may induce an irreversible cascade of events, which ultimately could lead to permanent flaws in the system. These flaws in turn may imply chronic, latent tissue responses which attempt to compensate for instabilities in the musculoskeletal system, yet, may potentially entail increased weakness and therefore risk of lameness.

2.2.5.3 The role of nutrition

During the process of musculoskeletal growth (bone modelling) and musculoskeletal maintenance (bone remodelling), adequate nutrition is required to supply the process with the necessary structural and functional materials (van Riet et al., 2013). Whilst in the case of non-essential nutrients, any undersupply can be overcome using other nutrients in the diet, essential nutrients cannot be replaced and therefore have to be supplied directly from the diet at appropriate time points. Both over and under-supply of nutrients can be potentially harmful, depending on the nutrient of interest. During the period of fastest musculoskeletal growth, deficiencies can be particularly problematic and may show carry-over effects into adulthood (Varley et al., 2010; Tanck et al., 2001). The aim of this section is not to describe in detail the biochemistry of nutrition of the musculoskeletal system, but to discuss some of the most important and common nutrients required for soundness of the locomotor system, in particular the skeletal system and the claws of pigs.

Diets in modern pig production are typically made of grains supplemented with a protein source, vitamins, and minerals. Nutrient deficient soils, on which dietary ingredients are grown, or mixing errors in feed manufacture can result in nutritional deficiencies or toxicities (Straw et al., 1999). The minerals and trace elements which have a potential effect on the locomotory system and may be missing or excessive are copper, manganese, selenium, and sodium chloride (NaCl). Vitamins involved in the soundness of the locomotor system are Niacin (Vitamin B3), Pantothenic Acid (Vitamin B5), Vitamin A and Vitamin D (Straw et al., 1999). Problems arising in the locomotor

system due to nutrition can be caused directly, with under- or oversupply of minerals or vitamins involved in metabolic pathways in the musculoskeletal system. Problems can also occur indirectly, when the ingredients have an impact on the nervous system or the water and acid balance of the body.

For example, selenium and vitamin E deficiency causes white muscle disease in pigs, in which muscle fibre degeneration creates pale areas in muscles and causes locomotory issues and mulberry heart disease (a form of heart muscle failure). At the same time, the permeability of vessels is altered leading to exudative subcutaneous edema.

Vitamin D deficiency leads to enlarged joints and costo-chondral (rib cartilage) junctions in nursery piglets, average daily gain is decreased and the animal's gait is either stiff or there is lameness (Straw et al., 1999). The bone cortices are softened and thickened and epiphyseal cartilage may be uneven. Over-supplementation of Vitamin A (hypervitaminosis A) may lead to bowed legs, reluctance to stand, standing with legs under body and an arched back. Development of short legs and swollen non-painful joints may be observed due to premature closure of growth plates in this condition. Finally, bone cortices may be thin, growth can be slowed and affected animals may show hyperesthesia and red skin (Straw et al., 1999).

Lameness and neurological disorders may be caused by ingestion of poisons such as lead (contained in oil used to treat mange), mercury (contained in fungicides), nitrite (consumption of preformed nitrite by microbes from nitrates in whey), phosphorus (contained in rodent bait), and zinc (dairy products stored in galvanized containers). There are miscellaneous other rare neurological conditions which may include locomotory disturbances, acting via neuromotor control on the musculoskeletal system. Presence of concurrent neurological signs can guide the diagnosis (Straw et al., 1999).

Biotin deficiency, Cholin deficiency, and Pantothenic Acid (Vitamin B5) deficiency should be taken into consideration when foot and leg problems point toward a dietary-derived aetiology.

Biotin (also known as Vitamin H) is a B vitamin. The bioavailability of biotin in pig feed varies with the major ingredients of the feed (Brooks, 1982). Corn is a good natural source of biotin, but diets based on cereals, such as barley, that are low in available biotin may not provide sufficient biotin for horn integrity (Straw et al., 1999). This makes the claw wall prone to trauma on hard floor surfaces. The response of sows with foot lesions to the addition of biotin in the feed is variable because, although some

may be biotin deficient, there are many causes of foot lesions (Penny et al., 1980). Biotin deficiency causes foot lesions due to soft claws (Penny et al., 1980). Biotin increases the compressive strength and the hardness of the heel bulb (Webb et al., 1984). The heel bulb acts as a cushion, absorbing energy on contact and spreading the weight of the pig over the area of the foot. Biotin supplementation may reduce the likelihood of injury to the foot and allow an increase of 8% in the floor slot-to-slat ratio from 61% to 69% for 100kg pigs (Webb et al., 1984). Responsiveness to biotin supplementation in pigs was found to increase with age, with major effects occurring between 521 and 1090 days of age (Kornegay 1986). Whilst the effects of biotin can be preventative, reductive and also curative, overall however, structural soundness scores seem to remain unaffected by the addition of biotin to pig diets (Kornegay 1986).

Calcium and Phosphorus (Ca and P) are the major minerals of importance for the integrity of the skeletal system. Deficiency of these minerals can cause rickets in young and osteomalacia in adult animals (Straw et al., 1999). Maximum growth performance has been observed at mineral levels lower than required for maximum bone mineralisation and bone development (Cera and Mahan 1988; Nimmo et al., 1981). However, it remains unclear which calcium and phosphorus levels are commensurate with the development of an adequate skeleton for market pigs. With regard to breeding animals, maximum bone mineralisation is believed to be required during growth and development to safeguard structural soundness and good performance during subsequent breeding periods (Nimmo et al., 1981). Therefore, growing gilts and boars are typically fed a higher macro-mineral inclusion than finisher pigs (Kyriazakis and Whittemore, 2006). Due to environmental and economic concerns, there is pressure for feeding minimum amounts of digestible phosphorus and therefore feeding recommendations for inorganic P have decreased in the last decades while research has driven towards increasing the availability of phytate-bound P in the diets (Varley et al., 2010). However, since pigs require less dietary P for optimal growth than for optimal bone quality, finishing pigs are likely to be fed sub-optimally with respect to bone quality (Varley et al., 2011).

The influence of dietary calcium and phosphorus levels on serum minerals, soundness scores and bone development in barrows, gilts and boars was evaluated by various research teams (Brennan and Aherne, 1986; Kornegay et al.,1983; Calabotta et al.,1982a). Results showed that serum phosphorus and bone mineralisation of barrows, boars and gilts were reduced when less than NRC-suggested levels at that time (1979) of phosphorus were fed. Feet, leg and overall soundness scores were not influenced by

dietary calcium and phosphorus levels over the range 0.5-1.2% for Ca and 0.4-0.9% for P, for finishing and weaning, respectively. Barrows, boars and gilts responded in a similar manner to higher than suggested NRC levels of calcium and phosphorus. Maximisation of bone development occurred when pigs were fed 125% phosphorus (0.5-0.8%) in combination with 125% calcium (0.6-1%). These findings were consistent with other reports indicating that a deficiency of phosphorus will result in reduced bone development (Doige et al., 1975). In similar studies, feeding elevated calcium (same as above) and phosphorus (as above) levels during growth had no effect on incidence and severity of lesions on the toes and overall structural soundness of sows kept for three parities (Barczewski et al., 1990; Calabotta et al., 1982b). Cera and Mahan (1988) assessed the effect of dietary calcium and phosphorus level sequences on performance, structural soundness and bone characteristics of growing and finishing pigs. Maximum performance was achieved with intermediate calcium and phosphorus levels, both during the growing (Ca:P ratio=0.65:0.50) and the finishing (0.52:0.40) phase. Subjective leg soundness scores at the growing stage were unaffected by level of dietary calcium and phosphorus. Poorest leg soundness scores at the finisher stage, however, were given to those pigs fed the lowest dietary calcium and phosphorus (0.52:0.40) level during the grower phase. Percentage bone ash of the humerus, shaft thickness and bending moment of the femur increased as dietary calcium and phosphorus level increased both during the growing (0.2 P-0.28 Ca) and finishing (0.18 P-0.2 Ca) phase.

The effect of different energy and protein levels on leg weakness and osteochondrosis in pigs has been evaluated by various researchers (Jørgensen, 1995; Grondalen 1974). Many of the early researchers concluded that high energy-level feeding predisposed pigs to leg weakness. However, newer research introduced uncertainty to the early knowledge, as mentioned previously (Stern et al., 1995; Ytrehus, 2007). Jørgensen (1995) concluded that energy level affected three leg weakness traits, namely weak pastern, forelegs turned out and upright pastern on hing legs, and the total leg weakness score, but noted no effect on osteochondrosis. Protein level in the previous study influenced neither leg weakness nor osteochondrosis. Similarly, Reiland (1978) revealed that protein level did not affect the soundness of limbs in pigs. Slevin et al. (2001) re-evaluated the effect of protein on bone strength and osteochondrosis in gilts. Their findings indicated that higher protein content in the diet did not interfere with bone mineral absorption and there was no apparent effect on bone re-modelling rate. However, results of the Slevin-study suggested that interchanging periods of protein

restriction and abundance may be harmful to the soundness of cartilage. Nonetheless, there is evidence in humans that protein intake can have an effect on bone mass (Zhang et al., 2010).

2.3 Biomechanical Gait Analysis in Quadruped Organisms

2.3.1 Historical background

The development of gait analysis of quadrupeds in the past was mainly due to interest in the gait of athletic horses (Barrey, 1999). The first person to take an approach to the capture of fast equine locomotion was Edward Muybridge in the 19th Century. He used strings, stretched across a runway to trigger multiple successive cameras as the horse ran past. The trajectories of the joints and segments of the body in motion could be measured on the successive images taken at time intervals. During the second half of the 20th Century, human biomechanics started to develop. Several decades later, Clayton et al. (1990), Back et al. (1994), van Weeren and van den Bogert (1990) and numerous other researchers carried out biomechanical assessment of equine athletic performance and locomotor pathology. The horse is the quadruped about which research in locomotion has been greatest to date. Injuries of the locomotor apparatus of the horse can occur because of human management errors, such as in nutrition, training, shoeing or breeding. Bad environmental conditions on tracks and due to weather and an unfavourable constitution due to poor limb conformation and genetics may lead to lameness in the horse (Barrey, 1999). In Thoroughbred racehorses, about 53%-68% of the wastage is due to lameness. According to Barrey (1999), the extent of this problem justifies the great effort into equine locomotion research, including clinical applications and techniques for preventing lameness. Several other researchers, mainly biologists such as Biewener et al. (1983), used biomechanical approaches to derive knowledge on the evolutionary development of species, on comparative biology and structural adaptation of species. A third group of researchers developed interest in animal models for human research (Cake et al., 2013; Taylor et al., 2006; Prilutzky et al., 1996; Vilensky et al., 1994).

In the last decade, however, gait analysis has become more extensively used to evaluate the gait of dairy cows, sheep and pigs (Kim and Breur, 2007; Thorup et al., 2007; von Wachenfelt et al., 2008; Liu et al, 2009). Biomechanical studies in dairy

cows have made some advances in the detection and evaluation of lameness and focus largely on the automated, continuous monitoring of mobility scores (Maertens et al., 2011). A few earlier studies in the 1980s (Applegate et al., 1988; Calabotta et al., 1982b) contributed to an early characterisation of some gait characteristics of pigs. To date, most biomechanical studies in pigs have focused on adaptations of normal pigs to floor surface modifications (Wachenfelt et al., 2009; Thorup et al., 2008) or with nutritional interventions (Barszewsky et al., 1990). Biomechanical research into pigs with lameness has only just begun to emerge (Gregoire et al., 2013; Pluym et al., 2011). The great potential of biomechanical analysis lies in the possibility to replace and/or complement conventional subjective gait assessment by objective, more reliable and accurate and potentially automated procedures (Nalon et al., 2013a).

Principals of biomechanics have been well explained in studies concerning the horse and offer the possibility for application to other species (Barrey, 1999). The following section is based on the book by Nigg and Herzog (1999) on the Biomechanics of the Human Musculoskeletal System and a comprehensive review on biomechanical gait assessment techniques in the horse by Barrey (1999).

From a biological point of view, locomotion can be defined as the ultimate mechanical expression of exercise activity (Barrey, 1999). In order to sustain an exercise activity, the organism requires a synergy between several systems that are functionally linked and regulated by the nervous system. The cardiovascular and respiratory systems provide nutrients and oxygen to muscle which then transforms biochemical energy into mechanical work during muscle contraction. The complex organisation of the locomotor apparatus under neurosensorial control makes it possible to use all the individual muscle contractions for moving the limbs to support and propel the body. Biomechanically, locomotion involves moving all the body and limb segments in rhythmic and automatic patterns which define the various gaits. As with any other body system, movement can be explained by mechanical laws (Barrey, 1999).

A simple model of a quadruped skeleton can be defined as a set of segments articulated one to another. Consequently, the body of any animal or human follows exactly the same mechanical laws as a series of inanimate objects. However, these laws need to be applied carefully, because the mechanical equations that determine the motions of a set of articulated body segments are much more complicated than those that determine the motion of a rigid body system such as a bullet. This great difference is often the cause of theoretical errors. Since living organisms follow Newtonian

mechanics, there are two complementary approaches to studying the body in motion (Barrey, 1999), namely kinetics and kinematics:

- **Kinetics or dynamics** is the study of cause of the motion, which can be explained by the force applied to the body, its mass distribution and its dimensions. Kinetics is concerned with forces, accelerations, energy, and work which are also in relation to kinematic variables such as acceleration and velocity.
- **Kinematics** is the study of changes in the position of the body segments in space during a specified time. The motions are described quantitatively by linear and angular variables that relate time, displacement, velocity and acceleration. In kinematics, no reference is made to the cause of motion.

2.3.2 Gait terminology

The following section is mainly based on the books by Biewener (2003) on Animal Locomotion, Whittle (1996) on Human Gait and the book by Back and Clayton (2001) on Equine Locomotion.

A gait can be defined as a complex and strictly co-ordinated rhythmic and automatic movement of the limbs and the entire body of the animal, which result in the production of progressive movements (Barrey et al., 1999). Two types of gait can be distinguished by the symmetry or asymmetry of the limb movement sequence with respect to time and the median plane (Robilliard et al., 2007), namely symmetric and asymmetric gait. Within each gait continuous variations exist. The stride is defined as a full cycle of limb motion. Since the pattern is repeated, the beginning of the stride can be at any point in the pattern and the end of that stride at the same place in the beginning of the next pattern. A complete limb cycle includes a stance phase when the limb is in contact with the ground and a swing phase when the limb is not in contact with the ground. During the **suspension phase** at faster gaits (trot and gallop) there is no hoof contact with the ground (Robbiliard et al., 2007). The duration of the stride is equal to the sum of the stance and swing phase durations. The **stride frequency** corresponds to the number of strides performed per unit of time. The stride frequency is equal to the inverse of stride duration and is usually expressed in stride/s or in hertz (Hz). The **stride length** corresponds to the distance between two successive hoof placements of the same limb. The step length corresponds to the length displacement between two successive placements of two contralateral hooves within a pair, i.e. front and hind. The **step-to-stride length ratio** is the quotient between the step and stride

length and this relationship in a sound subject, when not walking around a curve, may be expected to be near 0.5 (Whittle, 1996).

2.3.2.1 Types of gait

The different types of gait are generally defined by the relative timing of support among the limbs of the animal during the stride and the sequence of footfalls. Changes in gait are associated with movement at different speeds and typically involve a discontinuous change in limb kinematics and/or the mechanics of support. Three general classes of gaits have been defined in animals: walking, running or trotting (also pacing and hopping) and galloping (Biewener, 2003). Walking and trotting represent symmetric gait, while galloping and the canter of the horse are asymmetric gaits (Robiliard et al., 2007).

Walking generally involves overlapping periods of support among the limbs. For quadruped animals, this means that walking typically incorporates periods during which three or even four limbs are in contact with the ground. This provides a statically stable base of support (Cartmill et al., 2002). Static equilibrium is achieved because the body's centre of gravity falls within the triangular area of support represented by the limbs. Stability is highest when the animal is standing on all four legs. Although static equilibrium enhances stability, it can only be achieved at very low speeds (Biewener, 2003). Walking gaits in four-legged mammals typically have a **lateral sequence** of footfalls (Back and Clayton, 2001), yet, primates and a few other animals also show diagonal sequence walks (Shapiro and Raichlen, 2005). In a lateral sequence walk, any hind leg footfall will be followed by the ipsilateral foreleg footfall and then the diagonal hind leg footfall (Gills, 2004). Ipsilateral legs are defined as the front and hind legs of the same side, i.e. left or right. Furthermore, walking is a **four-beat** gait meaning that one leg at a time hits the ground. Walking gait at higher speeds may involve phases with only two limbs in contact with the ground. However, the footfall sequence may still be a lateral sequence and not a diagonal one as in the trot described below. Also the timing of stride phases changes in the different gaits (Robilliard et al., 2007). During quadruped walking gaits, duty factor (the percentage of stance time within a stride) is equal to or above 50%.

During **trotting** and **running** gait, there are no overlapping support periods between alternating support limbs, hence left and right legs within a pair. In a quadruped trot, the diagonal forelimb and hindlimb move in unity, i.e. they are

considered to be 'in phase', contacting and leaving the ground at the same time. The diagonal legs leave the ground to begin their swing phase before the contralateral forelimb and hindlimb contact the ground to begin their respective support phases (Biewener, 2003). This "in phase" movement of one diagonal front and hind leg pair at a time generates a **two-beat** gait.

Pacing involves the use of two-limb support by the fore- and hindlimbs of the same side in quadrupeds, which move in unity. This is in contrast to the contralateral diagonal fore- and hindlimb support observed in trotting animals, however, pacing also has a **two-beat** rhythm. Contralateral legs are two legs, either both front or both hind or one front and one hind, but not of the same body side. Owing to its ipsilateral pattern of limb support, the pace is a less stable gait, which results in a greater rocking motion of the body compared to trotting. Therefore few quadrupeds pace under normal circumstances, yet, camels regularly employ this gait at faster speeds and present an exception (Biewener, 2003).

Running gaits, such as trotting and pacing, are similar in that they include a bouncing spring-like motion of the body on the supporting limbs. Quadruped trotters generally favour an increase in speed by increasing stride frequency. In all cases, the **duty factor** of the limb, i.e. the stance time of the limb as a percentage of total stride duration, decreases at faster speeds, requiring an increase in muscle forces (Biewener, 2003).

In addition to walking and trotting, quadruped animals have evolved an additional gait, **the gallop** (sometimes referred to as a canter when used at lower speeds), to increase speed beyond that which can be achieved by a trot. While pigs have been described to gallop (Main et al., 2000), it is unknown whether they can also use a canter gait. The transition from a trot to a gallop involves a relative shift in the support phases of the fore- and hindlimb, such that the two forelimbs move more or less in phase, followed by the two hindlimbs. By shifting the phase of limb support to allow the fore- and hindlimbs to act together as pairs, galloping animals are able to increase their stride length to a greater extent than is possible by the rotational movement of the limbs alone (Biewener, 2003). This is achieved by flexion and extension of the spinal column and rotation of the shoulder girdle and pelvis, which can increase stride length considerably. However, in larger ungulates, such as bovines, a rigid backbone is necessary for effective support of the trunk and spinal movement is modest or absent owing to scale constraints of size. Typically, increases in speed at a gallop principally involve increase in stride length with little increase in stride frequency.

At a slow gallop (or **canter**), one forelimb lands slightly ahead of the other forelimb, followed by a similar pattern of support by the two hindlimbs (Biewener, 2003). Most often the phase difference between the forelimbs is greater than that between the hindlimbs. At faster galloping speeds, the two forelimbs and two hindlimbs progressively land more in phase with one another and limb duty factor decreases to as low as 20% of the gait cycle (stride). Because of the reduced limb duty factor, galloping involves suspension phases which may intervene between one or both sets of limb support phase. These suspension phases are a necessary consequence of the increased stride length that the animals achieve to increase their speed at a gallop (Biewener, 2003).

2.3.3 Kinetic or dynamic gait analysis

External forces acting on the body can be measured using electronic force sensors which record the ground reaction forces when the hooves are in contact with the ground (Barrey, 1999). Ground reaction forces are reaction forces equal in magnitude and opposite in direction to the forces applied to the ground by the body during movement. These forces are defined by Newton's third law of motion (Nigg and Herzog, 1999). The sensors can be installed either in a force plate on the ground, or in a force shoe device, attached under the hoof. Force plates can provide the force amplitude and orientation (vector co-ordinates in three dimensions), the co-ordinates of the point of application of the force and the moment value at this point (Nigg and Herzog, 1999). The accuracy of this type of device is usually good, but the sensitive surface is rather small (about 0.5m^2) and a visual control of the hoof trajectory is required (Barrey, 1999).

Hoof force shoes are generally less accurate than force plates, and their main disadvantage is the additional weight and thickness of the special shoe. Another problem may be the knowledge of the orientation of the hoof without the inclusion of accurate kinematic data. The advantage of hoof force shoes is that they will measure the ground reaction forces during different types of exercise (Barrey, 1999).

Another indirect ambulatory technique of evaluating the ground reaction force was proposed using strain gauges glued onto the hoof wall (Barrey, 1999). After the training of the appropriate artificial neural networks, the ground reaction forces can be estimated from the hoof wall deformations (Barrey, 1999). Strain gauge techniques can

be applied to measure *in vivo* the loading of bone or tendon strains in relation to the ground reaction forces.

Body acceleration measurements are performed using small sensors (accelerometers) that should be firmly attached to the segment under study (Pfau et al., 2007). This type of sensor measures the rate of change of velocity of a body during a given interval, which corresponds to the acceleration applied to this body. The acceleration vector is proportional to the resultant force applied to the body where the sensor is attached (Nigg and Herzog, 1999). The ambulatory measurement provides a convenient way to study the kinetics of a body in various experimental conditions. In order to analyse locomotion, the accelerometer should be tightly fixed as near as possible to the body's centre of gravity. The acceleration signal can be treated by many signal analysis procedures in order to extract the dynamic and temporal stride variables. Calculating the double integral of the linear acceleration makes it possible to estimate the instantaneous linear or angular displacement, i.e. positional data (Barrey, 1999). The main advantage of using an accelerometric transducer is the simplicity of the measurement technique both in field or laboratory conditions. The main limitation of such a device is that the measurements are given for only one segment with respect to a set of body axes (Barrey, 1999).

2.3.4 Kinematic gait analysis

A more descriptive approach to locomotion study is to film the animals with one or more cameras in order to analyse the motion characteristics of each body segment (Back et al., 1995). The modern approach uses reflective markers, glued onto the body, which are filmed by cinematographic or digital video cameras. The successive images are then analysed to measure the parameters of interest. Markers consist of small white spots or half spheres glued onto the skin over standard anatomical locations (Barrey, 1999). The markers are intended to indicate the approximate instantaneous centre of rotation of the joint (Schamhardt et al., 1994). However, skin displacement over the skeleton during the locomotion generates some artefacts, especially in the proximal joints (van Weeren et al., 1990).

The processing of the film or digital motion capture data to collect the joint marker coordinates is undertaken manually using a computer. This can be a time-consuming task, but many temporo-spatial stride characteristics are obtained. With the improvement of the image sensors, many professional high-speed video cameras (100–

2000 images/s) and home video cameras (PAL or NTSC Standard: 25–30 images/s or 50–60 frames/s) can be simultaneously used for locomotion analysis. The video signal can be treated by a video interface in order to digitise the images, which are then analysed by the appropriate software to automatically or semi-automatically collect the marker co-ordinates in space and time (Barrey, 1999). A more sophisticated motion analysis system uses markers which consist of photodiodes (modified Cartesian Optoelectronic Dynamic Anthropometer CODA-3; van Weeren et al., 1990). The advantage of this system is its high resolution (0.2–2.6mm) in three dimensions, high recording frequency (300 Hz) and the possibility for automatic tracking of the active (light-emitting) markers (van Weeren et al., 1990). The main disadvantage is that the subject needs to be equipped with many photodiodes connected to wires.

Most quadruped locomotion studies show two-dimensional motion analysis, but some systems including four or more video cameras make it possible to reconstruct the motion in three dimensions (3D) and to analyse the limb motions on both sides (Barrey, 1999). One limit of these sophisticated gait analysis systems, however, may be the restricted field view which is only about 5 m, corresponding to a few walking or trotting strides in the horse.

After filming, the operator needs to track either manually, automatically or semi-automatically the co-ordinates of the markers on each image of the film (Barrey, 1999). In most systems, this tracking phase is the limiting factor because of the great number of images to analyse. Manual supervision is required, because the markers are not always easy to detect automatically, especially for distal segments and hidden markers, i.e. those markers which, for various reasons, may not be detected by the cameras. The use of specific algorithms, such as direct linear transform (DLT), is an efficient way of automatically detecting the location of the markers on the images. After collecting the co-ordinates of the markers, the linear and angular velocities can be obtained by computing the first derivative of the trajectories and angles with respect to time. If the filming image frequency is high, the second-order derivative of a trajectory, or angular variations with respect to time, can be made using appropriate smoothing and filtering techniques, thus providing linear and angular acceleration data (Barrey, 1999).

The Vicon T20 (Vicon, Oxford, UK) series of cameras currently represents one of the most sophisticated state-of-the-art motion analysis systems. The Vicon T20 motion analysis system is based on technology, i.e. soft- and hardware, which allows for reconstruction of 3D coordinates of markers reflecting infra-red light emitted by the cameras (Windolf et al., 2008). Application of such passive markers over stable,

anatomical landmarks allows for a time-series capture of the 3D movement of single markers or segments defined by two or more markers. The recent Vicon NEXUS motion capture software (v.1.7.1, Vicon, Oxford, UK) semi-automatically detects marker trajectories based on tracking algorithms and permits the export of coordinate data into compatible spreadsheet software, such as Microsoft Excel (Microsoft Corporation, Redmond, US) for further use in other software programmes.

The advantage of kinematic methods is that all the kinematic parameters (displacement, velocity, linear acceleration, angle of rotation, angular velocity and angular acceleration) of the identified segments can be obtained. Several methods have been described to estimate the location of the centre of gravity and the moment of inertia of each segment (Thorup et al., 2007; Barrey, 1999). Theoretically, if the centre of gravity and the moment of inertia of each segment can be determined by measuring their mass distribution and their dimensions, it is possible to estimate the kinetic parameters (forces and kinetic moment), which determine the motion of each segment, from the kinematic data. The kinetic energy can be estimated for each segment and for the whole body in motion (Barrey, 1999).

2.3.5 Applications of gait analysis

Because of the substantial economic losses due to lameness in different species, such as the horse, dairy cow and the pig and the difficulties of establishing a diagnosis, techniques for quantification of lameness have been a research priority (Thorup et al., 2008; Rajkondawar et al., 2006; Barrey, 1999). Both kinematic and kinetic methods are now available to measure gait irregularities (Gregoire et al., 2013). In order to be more easily applicable under practical conditions, gait measuring techniques should increasingly be simplified (Gregoire et al., 2013, Maertens et al., 2011). Kinematic and kinetic methods provide quantitative measurements of abnormal gait, but the results are generally not specific to a given injury (Back and Clayton, 2001). To diagnose the origin of lameness, the gait analysis system should be used as a complementary test to clinical and orthopaedic examination (Burton et al., 2009; Keegan, 2007). The calculation of the accelerations of the head and sacrum make it possible to estimate indexes of gait symmetry, which can distinguish between lame and sound horses (Pfau et al., 2007; Barrey, 1999). The degree of gait irregularity can be related to the degree of lameness established by a clinical examination (Pfau et al., 2007, Barrey, 1999). A force plate system embedded in a track can measure ground reaction forces indicative of

lameness (Khumsap et al., 2003; Weishaupt et al., 2001). One of the limitations of force plates, however, is the inability to control the location of the ground contact of the hooves. Kinematic methods provide many descriptive parameters of joint mobility, quantitatively describing clinical signs such as angle variations during the stride cycle. However, at present the high cost and technical maintenance of a gait analysis system limit this type of application to the laboratory environment (Barrey, 1999; Clayton, 1990).

Back et al. (1994) investigated the predictive value of foal kinematics for subsequent locomotor performance of adult horses. Horses were filmed at 4, 10, 18 and 26 months of age and kinematic parameters were calculated. Locomotion of the foals and the adults appeared to be closely related, when differences in segment length and joint angles due to growth were taken into consideration. Back et al. (1994) observed that joints were less flexed with age and hence, legs became straighter as the horses grew. The duration of stance phases and strides in the foals had to be linearly and dynamically scaled to the height of the withers to become predictive for the adult values. Duration of swing phase, the total range of protraction and retraction and the maximum tarsal flexion could be used as indicators of the quality of gait, as scored by professional horse performance assessors. Back et al. (1994) concluded that foal kinematics were able to predict the locomotor performance of adult horses.

Dow et al. (1991) investigated the possibility to identify subclinical tendon injury in horses from analysis of ground reaction forces. In retrospective analysis, they found that changes in the loading patterns became apparent before the horses exhibited clinical lameness. The method was proposed to serve as an objective means of detecting tendon injury at an early stage.

Bockstahler et al. (2007) found altered joint kinematics in dogs with radiographical, subclinical hip dysplasia. Differences in kinematic parameters in the subclinical dogs compared to healthy dogs were delayed time of maximal flexion at the hip joint, greater flexion and greater range of motion of the knee joint. Maximal angular velocity of the stifle and tarsal joints during the swing phase were significantly lower in healthy dogs compared to the subclinical dogs. Ground reaction forces, which were also measured, revealed no differences between subclinical and healthy dogs.

Hoffmann et al. (2010) demonstrated that gait alterations, such as a reduction in stance time and step sequence changes, preceded the pathological changes occurring during pristane-induced arthritis in rats.

These studies indicate that there is potential for predictive assessment of orthopaedic health in pigs using kinematic gait analysis.

In summary, lameness can be regarded as a non-functional condition of one or more limbs. As such, lameness is in opposition to limb soundness, which can be regarded as the ideal, physiological, functional and desirable condition of all limbs at the same time. If the features of gait in the pig which promote long-term limb functionality could be identified, this could lead to a better understanding of those gait patterns, which might be associated with lameness. New technologies are now available for the investigation of the locomotor apparatus. Gait analysis techniques have already revealed promising results in previous studies (Gregoire et al., 2013; Pluym et al., 2011; van Nuffel et al., 2009, Flower et al., 2005). In conclusion, the importance of research into sound locomotion at all possible levels is clearly illustrated. New methods may provide objective, more accurate and reliable measures of gait. This doctoral thesis presents an approach to biomechanical gait assessment in pigs with a focus on longitudinal gait development and detailed gait histories of lame and non-lame pigs.

Chapter 3. Methods for Gait Assessment and their Validation

3.1 Subjective Scoring Systems for Lameness and Leg soundness

3.1.1 Leg weakness traits

For details on the subjective scoring of leg weakness traits used in the present experiments, please refer to Chapter 5 Section 5.3 of this thesis. A summary and illustration of the scored traits is included in Table 3.1 and Figure 3.1.

Table 3.1: Scoring of leg weakness traits (adapted from Jørgensen et al., 1995 and Van Steenbergen et al., 1989)

Deficiency	Description
Buck-kneed	excessive anterior angulation of the carpal joint
Sickle-hocked	excessive posterior angulation of the tarsal joint
Post-legged	excessive straightness in hind legs
Cow-hocked	hocks turned in and facing each other
Standing under	hind legs reaching anterior underneath the abdomen
Varus	bow-legged or O-shaped in front or hind legs
Valgus	knock-legged or X-shaped in front or hind legs
Splay-footed	toes turned out
Pigeon-toed	toes turned in
Weak pastern	pastern (all phalanges) approaching the ground
Upright pastern	pastern at or near right angle to the ground
Buckle in pastern	pastern shows a plantar (anterior) buckle
Kyphosis	humped back
Lordosis	broken back

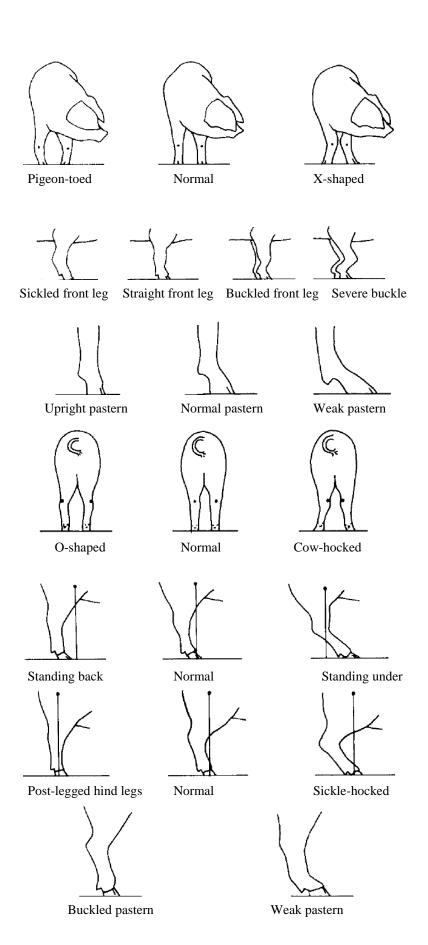


Figure 3.1: Leg weakness traits (Van Steenbergen, 1989)

3.1.2 Scoring of abnormal locomotion

For details on the subjective scoring of abnormal locomotion used in the present experiments, please refer to Sections 5.3 and 6.3 of this thesis. A summary is included in Table 3.2.

Table 3.2: Scoring of locomotion (adapted from Main et al., 2000).

Grades	Description
[0]	normal pigs with even strides and fluent walking
[1]	stiffness and an abnormal stride length
[2]	shortened stride and a detected lameness (reduced weight-bearing)
[3]	minimal weight bearing on affected leg(s),
[4]	no weight bearing on affected leg(s)
[5]	unable to move

3.1.3 Other diagnostic procedures

For the post mortem scoring of osteochondrosis lesions in the articular joint surfaces of the front and hind legs in Exp 1, a joint-level scale from 0 to 4 was used (adapted from Scott et al., 2006). Table 3.3 presents a summary. In preparation for the recognition of joint lesions, the 'Colour Atlas of Diseases and Disorders of the Pig' (Smith et al., 1990) was studied.

Table 3.3: Scoring of osteochondrosis joint lesions in Experiment 1 (adapted from Scott et al., 2006)

Grades	Description
[0]	no lesions, regular, even and opaque articular cartilage
[1]	thinning of cartilage
[2]	irregularity in cartilage (fissures, ridges, clefts and/or craters)
[3]	extensive or deep irregularities (fissures, ridges, clefts and/or craters)
[4]	extensive erosion, ulceration and/or flaps in articular cartilage.

For the inspection of the claws, claw lesion descriptions by KilBride et al. (2010) were enlisted as a general guideline. The walls and whenever possible lower surface of the claws were inspected for lesions, such as cracks, haemorrhages, erosions, flaps and abscesses. A general impression of claw health, shape, size and evenness was noted.

For the tentative diagnosis of lameness origin in affected pigs, careful observation of the pig while standing and walking as described further in Chapter 6, Section 6.3 was applied. Pressure tests including palpation on all joints in the affected leg(s) were made.

3.2 Development of a Quantitative Optoelectronic (Stereophotogrammetric) Motion Capture Method for Pigs

3.2.1 Hardware and software resources

The hardware used during the experimental procedures of all experiments was identical and is described in Sections 3.3.3, 4.4, 5.3 and 6.3 of this thesis. A depiction of the camera system can be viewed in Figure 8.7 in the appendices. In brief, the equipment comprised a PC, featuring Windows 7 operating software (Microsoft Corporation, Redmond, US), a MX Giganet box control unit (Vicon, Oxford, UK) for connection between the cameras and the PC, six Vicon T20 series cameras and six solid tripods (Manfrotto, Cassola, Italy). Furthermore, a 5-marker calibration wand (Vicon, Oxford, UK) was used for calibration of the motion capture volume before every data acquisition session. An image pixel error between 0.1 and 0.2 was accepted. Reflective markers were prepared using reflective tape (Megapixels Ltd, Hampshire, UK), a Pound Sterling coin and scissors. Double-sided sticky tape (SupaBrands, Worsley, Manchester, UK) was used for attachment of the reflective markers to the subjects of study.

Softwares used to collect, reconstruct, label, process and manipulate data were Vicon Nexus v. 1.7.1. (Vicon, Oxford, UK), Matlab R2010b (Mathworks©, Natick, USA), Microsoft office Excel (Microsoft Corporation, Redmond, USA), Minitab v.16 (Minitab Inc., State College, USA) and SPSS statistical softwares v.17.0 (IBM Corp., Armonk, New York, USA). Procedures of data processing are described in Sections 3.3.3, 4.3, 5.3, 6.3 and depicted in Figures 8.6 and 8.8 in the appendices.

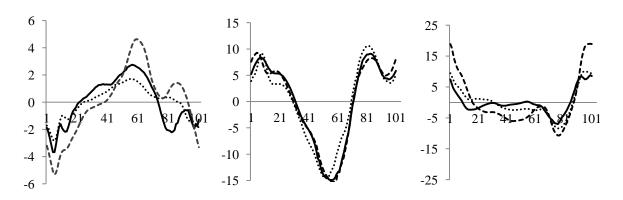
3.2.2 Confounding factors on gait and measures of control during experimental data acquisition

Five major factors with an impact on gait beyond the effects of interest were identified and controlled for during experimental and/or statistical procedures: walking speed, limb length, subject bodyweight, marker placement and skin movement (Lipfert

et al., 2012; Biewener, 2005; Vaughan and O'Malley, 2005; Gasc, 2001; Pierrynowski and Galea, 2001; McMahon, 1975).

Subject bodyweight was obtained usually on the day of motion captures. Front and hind **leg lengths** were calculated as the average stance-phase sum of the leg segments within a motion capture film (trial). Statistical comparisons of the effects of interest were only made across subject groups with similar bodyweight and leg length ranges. Using this approach, body weight and leg lengths were excluded as a cause of any observed differences.

It was not possible to quantify **skin movement** artefact in the present studies. Implantation of bone pins with mounted markers, fluoroscopy, X-ray or other control methods would have been required to evaluate skin movement artefact (Li et al., 2012; Baumann et al., 2010). Therefore, skin movement remains one of the sources of unquantified error in the measurements derived from markers over skin, especially the regions of the body with deep tissue over bone (Schwencke et al., 2012; van Weeren, 1992). An indirect control measure undertaken during the present studies was to compare only pigs of a similar age and weight range, assuming that skin movement was comparable between these pigs (Figure 3.2, a, b and c). There are two justifications for this approach: (1) in the current experiments, joint kinetics which would have required accurate bone alignment simulations were not among the calculated parameters; (2) unless an additional measurement directly relating to subject-specific skin movement is identified, applying general correction formulas will not remove bias from the data.



(a) Pelvic segment length

(b)Femoral segment length

(c)Radio-ulnar segment length

Figure 3.2 a-c: Examples of segment length changes (mm) due to skin elasticity during stance and swing phases of the gait cycle in three pigs. Solid line=41 kg pig; dashed line=47 kg pig; dotted line=51 kg pig. Lengths were normalised to within-pig mean and plotted over % stride time. Data are from Exp 2.

Subjects were allowed to walk at a preferred walking speed, while following a human operator, with the motivation of a reward upon successful completion of the walking task. Studies have shown that gait stability and adaptability are highest, hence variability lowest, when subjects walk with their comfortable and preferred walking speed (Jordan et al., 2007). This approach is taken in many current gait laboratory settings across the world (Menant et al., 2009). Various researchers have reported differences in stride characteristics of horses moving on a treadmill compared to normal overground movement (reviewed by Back and Clayton, 2001). Apple rewards were observed to be required to achieve consistent motivation for repeated walking samples in the present pigs. Within-subject (between-trial, within-session) variability of gait parameters was reasonably low, i.e. the mean coefficient of variation of all parameters ranged from 2-9% for motion captures of pigs at 63 and 87 kg bodyweight. Compared groups were routinely checked for absence of significant differences in walking speed, to exclude the latter as a factor other than the characteristics of interest potentially causing differences. Since preferred walking speed was relatively more variable than body weight or leg length among compared groups (Table 3.4), walking speed effects on gait parameters were quantified in Exp 1 and 3. A table showing the correlation of walking speed with the gait variables used in the present studies is presented in Table 3.5. Furthermore, a graph showing walking speed and a selection of gait parameters measured at different time points in female breeding pigs is in Figure 3.3 below.

Table 3.4: Mean total coefficients of variation for leg lengths (including front and hind), body weight, and walking speed within capture and between 24 pigs across five repetitions from 63-90 kg body weight. Data are from Exp 1.

Pig characteristic	CoV
Leg length	4%
Body weight	9%
Walking speed	13%

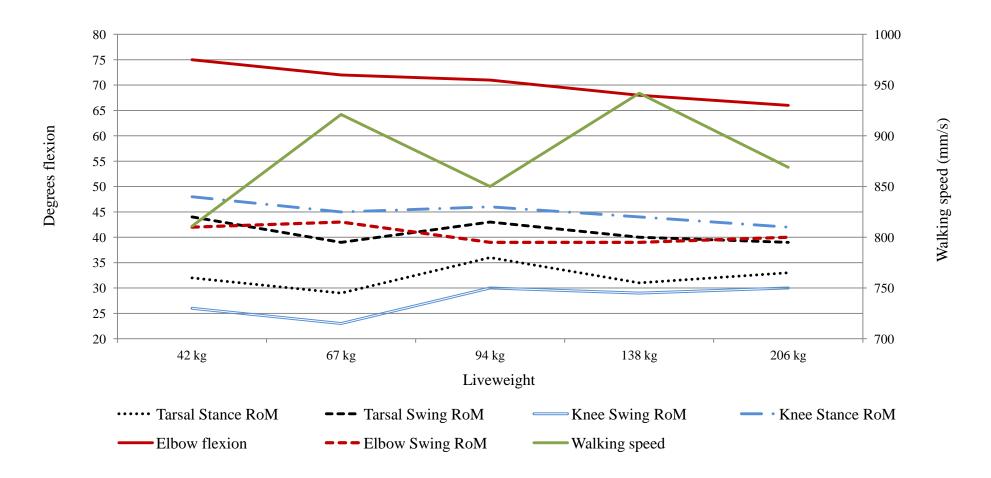


Figure 3.3: Changes in joint angles over time in female breeding pigs, partly explained by fluctuations in walking speed. Note that the tarsal joint angles follow the pattern in the walking speed consistently. Flexion values are an average value of flexion extremes during the stance and swing phases of gait cycles. Data are from Exp 3.

Table 3.5: Correlations between walking speed and selected gait parameters in 24 pigs across four repetitions of motion captures from 63-87 kg bodyweight. Walking speed in the last row is given for each motion capture. Angular parameters are measured in degrees flexion, temporal in seconds and spatial in mm. Walking speed is in m/s. Data are from Exp 1.

Gait parameter		63 kg	76 kg	81 kg	87 kg
Angular	Knee	RoM Stance -0.560; P≤0.007	RoM swing 0.573; P≤0.007	Max Swing 0.436; P≤0.038	Max Swing 0.445; P≤0.043 RoM Swing 0.521; P≤0.016
	Elbow				RoM Swing 0.469; P≤0.028
	Tarsal			Min Stance 0.418; P≤0.047 RoM Stance -0.716; P<0.001	RoM Stance -0.532; P<0.011
Temporal	Stance time Swing time	-0.818; P<0.001 H: -0.582; P=0.003	-0.828; P<0.001 H: -0.480; P=0.024	-0.882; P<0.001 H&F: -0.628; P=0.002	-0.909; P<0.001 H&F:-0.618; P=0.006
Spatial	Stride length	0.434; P=0.034	0.626; P=0.002		
Temporospatial	Walking speed	0.85 SEM (0.019)	0.94 (0.025)	1.06 (0.032)	0.95 (0.027)

3.3 Intra-Operator Repeatability of Skin Marker Derived Segment Measures and Gait Kinematics in Healthy Pigs

3.3.1 Abstract

There is a lack of biomechanical research into locomotor pathology in pigs despite orthopaedic problems being a major concern for the industry. This study evaluates the intra-operator repeatability of marker placement in pigs undergoing biomechanical investigation. Three pigs were fitted twice per day on five consecutive days with skin markers over anatomical landmarks; data were captured with a 3D optoelectronic system and 10 markers were used here for segment length and gait parameter calculation. There were significant differences between front and hind leg and proximal and distal segment length repeatability ($P \le 0.05$). Repeatability showed a similar extent and location variability to human and other quadruped studies. The source of the greatest segment differences was the femoral segment in the hind leg. Segmental differences at the shoulder and elbow joint were limited in this application. For all segments, except the femoral, differences above 0-10 mm were observed at or less than 7% of the marker applications, which may be an acceptable level of disagreement. Gait parameter repeatability generally confirmed the segment length findings and resembled intra-operator achievement in horses. Implications in pigs will depend on the relative size of any effect of clinical conditions with an impact on gait parameters. Future studies should determine such effects by recruiting subjects with known clinical conditions whilst controlling for other confounding factors.

3.3.2 Introduction

Leg disorders are a major problem afflicting 10-20% of pigs on farms (KilBride et al., 2009). Joint angles receive increased attention, as variations of these constitute a so-called leg weakness syndrome (Jørgensen et al., 2003). Kinematics could help to investigate joint movement (Holcombe, 2009), however, to our knowledge, no published study on marker placement reliability in pigs exists.

In camera-based kinematic studies markers are typically attached over bony landmarks near joint rotation centres, although newer and more complex methods use marker clusters to approach joint centres functionally (Della Croce et al., 2005). Depending on the choice of landmarks, palpation skills/anatomical understanding of the

operator, but also the docility, patience and anatomy of the subject, marker misplacement may entail misinterpretation of an individual's gait (Gilette and Angle, 2008).

This study estimates marker placement repeatability in pigs. The proposed marker set (adapted from Thorup et al., 2008) represents a uniplanar, linear kinematic model (Torres, 2011) and is deployable in studies involving multiple animals or measures. It was hypothesised that misplacement would be best detected in segment length measures, as (1) these may be less variable than gait parameters during dynamic conditions and (2) no choice of particular gait parameters is imposed on future researchers as kinematic research has yet to establish pig-relevant parameters.

For the assessment of marker misplacement magnitude, segment stance phase measures (least variable) were considered. However, since gait is of interest to our studies, a selection of gait parameters from the stance and swing phases during walking were also considered to provide a complete gait cycle analysis. The term *repeatability* here refers to the magnitude of the differences found across repeated measures (McGinley et al., 2009).

3.3.3 Materials and Methods

3.3.3.1 Experimental design and data collection

All procedures on animals were in accordance with institutional and UK animal welfare regulations (http://www.ncl.ac.uk/research/ethics/animal/animalpolicy.htm). Seven clinically healthy pigs (Hermitage Genetics, Kilkenny, Ireland) were randomly selected from the Newcastle University pig unit (34 kg liveweight, SD 2.8) and housed in a partly-slatted concrete pen (9 m²). Two weeks of habituation to human contact and short isolation from pen mates followed. The three most cooperative pigs were chosen for data collection thereafter.

Subsequently, in the morning and afternoon 17 retroreflective circular markers (Ø15 mm) were placed on each side of one unrestrained pig at a time over anatomical landmarks (Table 3.6). Author SS, a Veterinarian with three months of marker placement experience, including two necropsies with prior landmark marking in pigs, performed the marking. Markers were attached with double-sided, adhesive tape (Supa Brands, Worsley, Manchester) and completely removed after the capture of sufficient trials (2-4 trials containing usually 3 strides each; multiple trials compile a session). At least two

hours later markers were reattached and there were no residual signs of previous positions.

The current pig was moved into the motion capture area, a concrete walkway 3.5 m long and 2 m wide, framed by cameras to one side. The 3D optoelectronic system included six infrared cameras (Vicon T20, Oxford, UK) set up in an array and connected to a PC featuring Nexus software (v1.7.1, Vicon, Oxford, UK); frames were sampled at 125 Hz. Pigs were trained to follow a human at a regular and continuous walking pace along the walkway. The procedure was repeated on five consecutive days giving 10 sessions for each pig.

3.3.3.2 Data processing and analysis

Results were generated for markers (N=10 left side) requiring repeatable placement in ongoing studies (see Table 3.6). For stride event detection, parameter calculation and time normalisation, data were imported into Matlab (R2010b, Mathworks©, Natick, USA) and processed by a custom-written programme.

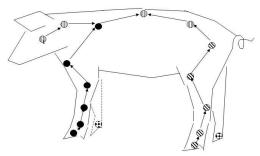
Due to poor precision of standing trials (pigs were easily distracted when not focusing on following the operator) and to avoid skin movement artefact (Leardini et al., 2005) interfering with the estimate of segment length, two approaches were taken: (1) variation in segment length was increased during the swing phases of the leg resulting in selection of average stance measures only; (2) the effect of walking speed (through skin movement artefact) on stance segment length distortion was investigated at trial-level.

Statistical analysis was performed in SPSS software (v17.0; SPSS Ltd, Portsmouth, UK). Within-subject session mean values for eight segments per pig (N=26 per segment in total for 3 pigs; some sessions were unusable, therefore N≠30) were used in the analysis, except for within-trial correlation of walking speed and segment length. Within-session variability of segment lengths was generally small and the mean considered representative of the individual marker positioning. For details on data dispersion, see table legends. Wilcoxon tests were used to test front vs. hind limb and lower vs. upper extremity differences. Significance was accepted at P≤0.05. The overall mean segment length within pig represented its 'reference' length and the difference from this reference length for every placement (session mean) defined the magnitude of misplacement. Initially, the differences of all segments and all pigs (with a mean of zero) were plotted against the corresponding segment length values to generate

a plot similar to the "difference-versus-mean" plot between two measurement methods and limits of agreement were derived (Bland and Altman, 1986). Next, the absolute values of the differences were taken and the distribution of these differences was summarised by descriptive statistics (kurtosis values are included). Differences were also grouped based on bands found in the literature: 0-5 mm; 5-10 mm; 10-15 mm; 15-20 mm and 20-30 mm (Weller et al., 2006; Della Croce et al., 2005) and relative frequencies within band calculated.

Table 3.6: Anatomical position of markers with schematic illustration of segments

mustrano	n or se	egments			
		Anatomical location of markers in rostro-caudal			
Body part		(body) and proximo-distal (legs) direction			
Head	lacksquare	Centre of Masseter muscle*			
Neck	\bigcirc	Lateral cervical midpoint*			
Trunk	lacksquare	Highest point of pig along lateral spine*			
	lacksquare	Tuber coxae (pelvic bone)*			
Front leg		Spinous tuber of scapula (shoulder blade)*			
		Caudal part of major tubercle (shoulder joint)			
		Lateral epicondyle of humerus (elbow joint)			
		Lateral styloid process (radio-carpal joint)			
		Lateral metacarpophalangeal joint (midpoint)			
		Caudolateral toe wall			
		Caudomedial toe wall of contralateral claws*			
Hind leg	\odot	Caudal part of greater trochanter (hip)			
		Lateral patella (knee joint)			
		Lateral malleolus (tibio-tarsal joint)			
		Lateral metatarsophalangeal joint (midpoint)			
		Caudolateral toe wall			
		Caudomedial toe wall of contralateral claws*			



^{*}These markers are used for tracking relative displacement to themselves in our studies, hence repeatability of their placement was not analysed here.

3.3.4 Results

The stance segment length measures: Within pig trial-based segment length was not correlated to corresponding walking speed; hence walking speed was excluded as a cause of the observed differences in segment lengths.

The mean session-based difference from the reference segment length ranged from 2.3-8.8 mm for all segments and pigs (Table 3.7). The absolute differences ranged from 6-34 mm and represent the difference between two extreme measures of a segment in the same pig during the total study time. The absolute differences were of similar magnitude for most segments across all pigs.

Differences between front and hind leg and proximal and distal segment length repeatability are analysed in Table 3.7.

Relative frequencies of extreme differences were 4% in the 20-30 mm range and 12% in the 15-20 mm range for the femoral segment only. Femur (12%), tibia/fibula (7%) and ulnar/radius (4%) generated some differences in the 10-15 mm range; all other segments appeared exclusively in the bands of 0-5 mm and 5-10 mm. For the femoral segment, the tibia/fibula and the metacarpal segment, 46%, 31% and 38% of the differences were observed in the 5-10 mm band, respectively. All other segments had 85-88% of the differences within 0-5 mm.

The limits of agreement (Bland and Altmann, 1986) include 95% of the observed differences, if the distribution of the differences is normal. The data presented here were either normal or close to normal (see kurtosis, Table 3.7). In 68% of cases, i.e. within one standard deviation of the mean, the difference would fall between -10.3 and +10.3 mm (-20.6 and +20.6 mm for two SDs) for the least repeatable femoral segment in this study.

The stance and swing gait parameters: Repeatability of elbow joint peak angular measures was higher compared to the same knee joint measures (Table 3.8). Joint range of motion (RoM) measures were less susceptible to variation between session means than peak flexion values (local extrema of angle curves), i.e. within/between marker application SD ratio >1. Linear parameters (step and stride length; Whittle, 1996) and walking speed varied more between trials within session than between session means.

Table 3.7: Descriptive statistics of segment length differences between marker placements*

	Mean	Kurtosis	Median	SD	95% CI**	Segment length (mm)
Segment	(mm)		(mm)	(mm)	(mm)	Mean (SD)
Humerus	$2.7^{a(d)}$	2.4	2.1	2.02	± 0.78	110 (6.4)
Ulnar/radius	3.3 ^{ab}	2.3	3.0	2.40	± 0.92	111 (3.5)
Metacarpal+carpus	4.7 ^b	2.2	4.5	2.97	$\pm \ 1.14$	199 (5.8)
Phalanges front	2.8 ^{ac}	2.6	2.0	2.00	± 0.82	64 (1.6)
Femur	8.8 ^e	1.7	8.0	5.06	± 1.94	157 (4.6)
Tibia/fibula	4.8 ^{bc(d)}	3.3	4.5	3.68	± 1.42	180 (3.5)
Metatarsal+tarsus	3.3 ^{ac}	3.2	3.0	2.08	± 0.80	104 (2.5)
Phalanges hind	2.3^{a}	3.1	2.0	1.74	± 0.67	68 (2.6)

^{*}Difference of session mean segment length from total mean segment length within-pig. Means and SDs here are parameters of

abc Within a column, means followed by a different superscript are significantly different at P \!\! \leq \!\! 0.05.

(abc) designate when P≥0.05 but <0.075.

the difference distribution with N=26 including all pigs and sessions.

^{**}CI, confidence interval for the mean difference.

Table 3.8: Gait parameter variability between (b) and within (w) sessions. Angles are in degrees flexion. RoM = Range of motion.

		Repeatability					Paramet	er descrip	tives			
•		mean diff*	SD diff	1	Mean Parameter	**	SD^{w}	SD^b	SD^{w}	SD^b	SD^{w}	SD^b
	Gait parameter	N=29	N=29	Pig A	Pig B	Pig C	Pig A	(N=10)	Pig B	(N=9)	Pig C	(N=10)
•	Swing minimum	4.2	2.73	40	40	42	1.6	6.3	2.4	5.9	1.8	3.5
	Swing maximum	4.2	3.30	88	85	89	2.9	5.9	2.5	5.5	2.8	5.2
Knee	Stance minimum	4.0	2.30	50	50	53	3.5	5.3	4.5	5.0	3.3	4.0
joint	Stance maximum	4.8	3.17	68	67	76	1.4	7.6	1.2	5.8	1.8	4.5
angle	Swing RoM	2.1	1.42	48	45	47	3.6	2.8	2.2	2.2	4.1	2.8
	Stance RoM	1.7	1.29	18	17	23	3.7	3.7	4.9	2.0	2.9	2.0
	Swing minimum	3.0	2.43	53	53	62	2.9	5.2	1.8	3.2	2.0	3.5
	Swing maximum	3.0	2.40	90	87	95	3.4	4.5	2.5	3.2	2.5	4.2
Elbow	Stance minimum	3.7	2.74	56	58	66	3.3	5.6	2.2	4.5	1.8	4.0
joint	Stance maximum	3.8	3.19	73	77	83	3.5	4.9	2.4	5.5	2.1	4.6
angle	Swing RoM	1.4	1.25	37	34	33	3.1	2.5	2.6	1.1	1.9	2.3
	Stance RoM	1.4	1.07	17	18	17	2.5	1.9	2.7	1.6	1.8	2.0
	Swing minimum	0.7	0.68	3	5	6	0.3	1.0	0.3	1.1	0.3	1.0
	Swing maximum	1.3	0.95	19	13	22	2.1	2.0	1.1	1.5	1.9	1.7
Carpal	Stance minimum	0.7	0.62	3	5	7	0.3	0.9	0.2	0.9	0.3	1.1
joint	Stance maximum	0.7	0.52	5	6	8	0.3	0.8	0.2	1.0	0.2	1.0
angle	Swing RoM	1.2	1.23	16	8	16	2.2	1.2	1.1	0.9	2.0	1.5
_	Stance RoM	0.3	0.22	1	1	2	0.3	0.3	0.2	0.2	0.3	0.3
	Swing minimum	2.9	1.80	6	6	10	1.6	3.1	1.2	2.9	2.0	4.6
	Swing maximum	3.9	2.66	42	41	52	1.5	6.6	0.9	4.2	1.8	4.0
Tarsal	Stance minimum	3.2	2.13	12	9	20	1.9	5.0	1.5	3.2	1.4	3.9
joint	Stance maximum	4.0	2.67	37	39	48	1.6	6.5	1.3	4.2	1.9	4.3
angle	Swing RoM	2.1	1.70	36	35	42	2.3	4.1	1.6	2.1	2.1	1.4
C	Stance RoM	2.0	1.06	25	29	29	2.2	2.6	2.1	2.6	1.5	1.9
	Step length hind (mm) ¹	7	6.9	298	303	326	23.6	11.0	21.1	10.8	16.5	8.8
	Step length front (mm)	6	5.2	295	301	323	19.7	10.6	24.1	3.5	22.2	10.2
Other	Stride length hind (mm)	13	8.3	597	598	653	19.5	13.2	25.9	18.2	26.5	13.0
variables	Stride length front (mm)	13	8.6	598	592	652	23.5	12.8	28.1	28.0	26.4	13.4
	Walking speed (mm s ⁻¹)	35	25.5	879	845	760	96.3	73.1	100.1	20.1	91.5	42.2
	Leg length hind (mm)	9	8.4	494	502	513						
	Leg length front (mm)	8	6.3	475	474	500						

^{*}Mean of the differences of the session means from the overall mean of parameter within-pig and the standard deviation.

^{**} Mean of session means with corresponding SDb.

w SD within marker application (session). SD was obtained from 2-4 trials within-session and averaged across 9-10 sessions per pig.

b SD between marker applications was gained from 9-10 different session means.

¹The step length is the length of a rectangle drawn using the same ground contact points of left and right claws of a pair as the diagonal of the rectangle.

²The stride length is the distance measured between the same ground contact points of two consecutive contacts of the same claw.

3.3.5 Discussion

Requests for repeatable, more accurate and objective techniques to investigate lameness in livestock are increasing (Grégoire et al., 2013). The current study assessed the repeatability of an objective motion capture method in pigs. Lack of repeatability due to skin movement artefact (van Weeren et al., 1992) was beyond the scope of this study and should be subject of future investigation. Also, since the three most cooperative pigs were chosen, repeatability may be reduced in less placid pigs.

Anatomical landmarks, such as the greater trochanter of the femur, are large and lie deep within muscles. This may be a source of greater variability, particularly in pigs selected for high muscularity around the femur (the ham), which was confirmed by this study. Segmental differences in the proximal front limb were small, indicating better placement repeatability at the shoulder and elbow landmarks in pigs. Angular gait parameters from the knee and elbow joint generally corroborated these segmental differences, although angle repeatability at the elbow joint was only marginally better compared to the knee joint.

Additional sources of variability may influence gait parameters. For example, skin movement artefact (Leardini et al., 2005), which was reduced for segmental measures due to stance phase averaging, may have differentially influenced instantaneous angle values (Baumann and Chang, 2010; Chau et al., 2005). Although repeatability was assessed within-pig and a similar skin movement might be expected each time, this may have varied slightly with variations in the preferred walking speed (Li et al., 2012). The latter may also have induced variability in the front leg angular parameters due to a balancing and supporting rather than a propulsive function of the front compared to the hind legs (Thorup et al., 2008).

Further, segment length repeatability was better in the distal hind limb compared to the distal front limb, likely due to prominence of the tibio-tarsal and radio-carpal anatomical landmarks. Relative repeatability of gait parameters normalised to their mean (derivable from data provided) is similar in carpal and tarsal joints. The magnitude of the coefficients of variation is reasonably consistent with recent findings in pigs by Grégoire et al. (2013). Differences between studies are likely to arise from selected landmarks near joints and the size of the animals (i.e. sows in the Canadian study).

The linear gait parameters are relative measures (i.e. the difference between two potentially biased by the same magnitude and direction values) and uncontaminated by

skin movement, as the markers used for their calculation are attached to horn on the claws. These parameters varied more within session than between session means. This probably reflected the true stride-to-stride fluctuations in the pigs, which were removed after within-session averaging. However, marker placement-induced variability is captured as the residual variability between sessions and this was illustrated by the peak joint flexion values here.

The overall mean intra-operator limits-of-agreement interval obtained from horse segments by Weller et al. (2006) was 38.1, SD 24.2, mm. This interval represents the distribution of the differences and includes 95% of the observed differences of all segments analysed. The same estimate here was 20.1 ± 9.57 mm suggesting that overall repeatability is higher in pigs. This may reflect the smaller size of landmarks, especially at the young age in the present study. However, it may be argued that pigs are more likely to fidget during marker placement and landmarks might be comparably less superficial due to deeper overlying tissue. Lastly, the above estimate in horses was derived from standing trials, while the present segmental measures were based on the least variable phase during walking.

Generally, a larger misplacement can only be tolerated if an effect of treatment/condition is greater than the bias in the gait measure. Fortunately, joint angle waveform patterns may remain largely uninfluenced by marker misplacement in contrast to absolute flexion values (Torres et al., 2011). This means that interval measures (ranges of motion (RoMs)) might be more reliable than local extrema, as confirmed by gait measures from the pigs here. However, in human gait clinics, average angular changes of five degrees are generally taken as the minimum detectable clinical difference (Wilken et al., 2012; Maynard et al., 2003). Based on the present study, this same threshold value of five degrees can be introduced for pigs. In subsequent studies of pig gait therefore, provided that an effect is greater than this threshold, absolute flexion values may still be successful in detecting significant differences.

In conclusion, detecting a true gait deviation may require repeated marker placements only for anatomical regions known to promote misplacement, such as the proximal hind limb. This could provide a within-subject average and a confidence interval, which should be better estimates of the true value. When asymmetry within animal is the variable of interest, this approach could help increase confidence in the measurements. Gait measurements with low repeatability should be interpreted with caution.

Chapter 4. Longitudinal Gait Development and Variability of Growing Pigs Reared on Three Different Floor Types

4.1 Abstract

Biomechanical investigation into locomotor pathology in commercial pigs is lacking despite this being a major concern for the industry. Different floor types are used in modern, intensive pig production systems at different stages of the pigs' production cycle. The general perception holds that slatted and/or hard solid concrete surfaces are inferior to soft straw-covered floors regarding healthy musculoskeletal development. Previous studies have compared pigs housed on different floor types using clinical, subjective assessment of leg weakness and lameness. However, reliability studies generally report a low repeatability of clinical lameness scoring. The objective of this study was to quantitatively assess the long-term effect of pen floors, reflected in the biomechanical gait characteristics and associated welfare of the pigs. A cohort of 24 pigs housed on one of three different floor types was followed from 37-90 kg average liveweight, with gait analysis (motion capture) starting at 63kg. The three floor types were fully-slatted concrete, partly-slatted concrete and deep straw-bedded surfaces, all located within the same building. Pigs underwent five repeated camera-based motion captures, 7-10 d apart, during which 3D coordinate data of reflective skin markers attached to leg anatomical landmarks were collected. Pigs walked on the same solid concrete walkway during captures. One-way ANOVA and repeated measures ANOVA were used to analyse the gait data. Results revealed changes over time in the spatiotemporal gait pattern which were similar in magnitude and direction for the pigs from different floor types. Significant increases in elbow joint flexion with age were observed in all pigs (P≤0.050; +6 degrees). There were few differences between floor groups, except for the step-to-stride ratio in the hind legs being more irregular in pigs housed on partly-slatted floors (P=0.012; 3.6 times higher SD) compared to those on 5-10 cm straw-bedding in all pen areas. As the level of clinical problems was generally low in this cohort, it may be that floors elicit problems only when there is a primary predisposing factor increasing weakness in susceptible tissues.

4.2 Implications

The introduction of quantitative locomotion research in pigs presents an objective and accurate alternative to traditional subjective assessment methods, decreasing the potential for bias. Gait analysis can detect subtle changes hidden to the human eye and, in contrast to x-ray and sensor placement into tissue, is a non-invasive means to examine the musculoskeletal system offering much potential for the pig industry to exploit. Factors believed to have an impact on musculoskeletal health need to be better understood and arguments for or against husbandry practises should be evidence-based and underpinned by data. This study provides a novel perspective to address the ongoing uncertainty about the role of floors and identifies important gait variables for further research.

4.3 Introduction

Despite the general pursuit of problem control in the modern swine industry, lameness remains an unresolved challenge and affects to some degree 10-20% of the pigs on commercial farms (KilBride et al., 2009). In the UK it has been estimated that lameness may account for £5 million per annum (extrapolating from BPEX, 2013 and Willgert, 2011), a figure which takes account of the cost of initial treatment, veterinary visits and replacement of breeding females. Reduced growth performance in lame growers and finishers can cause delayed market weight achievement and entail carcass downgrading (Correa et al., 2006). Persistent conditions may necessitate euthanasia due to European legislation prohibiting transport of lame animals. Furthermore, the welfare of lame animals is seriously compromised, which is unacceptable within the modern standards of pig production (Jensen et al., 2010).

The common causes for lameness are genetic predisposition to weakness of the musculoskeletal system (Jørgensen and Andersen, 2000), infections (Nielsen et al., 2001), injuries, nutrient deficiencies and systemic diseases which target musculoskeletal components (Straw et al., 2006). One of the predisposing factors to lameness in modern pig production is considered to be the type of floor on which the animals are housed (Mouttotou et al., 1999). In the last decade, there has been increasing interest in the effect of floor type on the prevalence of lameness and lesions of the legs and claws (KilBride et al., 2009; Scott et al., 2006). In addition, although some papers have shown that selection for rapid weight gain has lead to increased lameness problems (Busch et

al., 2011; Simonsen, 1993), other investigators could not confirm this growth rate association (Ytrehus, 2007; Stern et al., 1995).

Leg weakness describes clinical deficiencies of leg posture (conformation) and locomotion considered to arise from problems in the musculoskeletal system (Fan et al., 2009). Assessment protocols have been developed for the evaluation of leg weakness symptoms and lameness which rely on visual, clinical scoring of the animal (Jørgensen and Andersen, 2000; Main et al., 2000; Van Steenbergen et al., 1989). Studies have then reported elevated clinical levels of leg weakness on fully-slatted floors compared to straw-bedded floors (KilBride et al., 2009; Jørgensen, 2003). However, this was not the case for lesions of osteochondrosis, a degenerative joint disease, in the same pigs although this has been considered as the most important underlying factor of leg weakness (Van Grevenhof et al., 2012). KilBride et al. (2009) also found increased lameness in finishing pigs housed on solid concrete with sparse bedding and partly-slatted surfaces compared to pigs kept on deep straw-bedded floors.

One of the major shortcomings of most studies conducted on lameness and/or leg weakness is their reliance on subjective, qualitative assessment which implies a potential for bias (Dalmau et al., 2010). Several repeatability studies reported unfavourable results for subjective lameness assessment (Waxman et al., 2008; Quinn et al., 2007; Petersen et al., 2004). This may be expected to be worse in on-farm situations where individual pig assessment is impeded within a pen with other pigs. Previous quantitative studies have investigated the instantaneous gait adjustment of pigs on slippery and non-slippery surfaces by means of gait analysis with force plates and 2D video kinematics (Von Wachenfelt et al., 2009; Thorup et al., 2007). However, there appears to be no longitudinal study quantifying the gait and gait variability of pigs exposed to different commercial floor types for an extended part of their development.

The aim of this study was to investigate and present quantitative gait data of pigs followed through an important growth stage, spent on one of three commonly used floor types: fully-slatted concrete, partly-slatted/partly solid concrete or a deep straw-bedded floor. A multiple camera-based 3D motion capture technique with markers placed near joint centres was chosen. From the data obtained by the camera system, a comprehensive selection of gait parameters was calculated. Some of these gait parameters were previously successful in differentiating between normal/abnormal gait and changes in gait over time in dogs (Bockstahler et al, 2007), cows (Flower et al., 2005) and horses (Back et al., 1994).

There were two hypotheses. (1) Pigs housed on softer surfaces such as straw show fewer gait problems and less gait variation than pigs growing on harder surfaces, with a potential differentiation between partly-slatted and fully-slatted floors. To test this, it was assumed that gait problems would be reflected in at least one of the measured gait parameters, representing a variety of geometric features of movement. (2) Fast growing pigs have a more cumbersome gait and weak pasterns due to greater body mass acting on the passive tendon/ligament structures in the lower leg. To test this, the gait of fast, intermediate and slow growing pigs as present in this study was compared.

4.4 Materials and Methods

4.4.1 Animals, management and experimental housing

All procedures on animals were in accordance with institution guidelines and UK animal welfare regulations. The experimental population consisted of 12 entire male and 12 female clinically healthy growing pigs of a commercial crossbred genotype (Large White x Landrace damline and Pietrain x Duroc sireline; Hermitage Genetics, Kilkenny, Ireland), weighing 37 kg (SD 3 kg) at date of entry. The pigs were randomly selected from three double-litter groups previously housed on plastic slatted flooring at the Newcastle University pig unit. Only pigs with no signs of lameness were included, i.e. they showed no signs of stiffness or limping when moving along a walkway and had no visible body surface abnormalities. Initially all pigs scored 0 according to the scoring system described by Main et al. (2000). Lameness was visually assessed on all pigs during all motion captures.

The pigs were divided into groups comprising four males and four females, with groups then randomly assigned to one of three pens within the same room in an experimental building with a controlled climate. Pens were 3.0 m x 3.2 m and had one of the following floor types: fully-slatted concrete (20 mm gap width and 80 mm slat width), partly-slatted (3.0 m x 2.5 m solid concrete and 3.0 m x 0.7 m slatted, with 20 mm gaps and 80 mm wide slats) or deep straw-bedded. The straw-bedded pen was continuously covered with a regularly renewed layer of 5-10 cm straw bedding. Pigs had ad libitum access to concentrate feed and water.

Five weeks followed during which pigs were familiarised with separation from pen mates, close human contact, marker application and walking training. Pigs learned to follow a target led by a human operator to obtain a treat, in this case a small piece of apple, when movement was acceptable. Successful pigs walked with regular and continuous strides without distraction and without changes in walking speed.

4.4.2 Data collection

A neighboring building with a solid concrete floor contained the motion capture area comprising a motion capture walkway, measuring 3.5 m long x 2 m wide, together with preparation and waiting areas, each measuring 3.8 m x 2.27 m. Pigs were moved to the waiting area before the filming session. Starting at mean bodyweight 63 kg (SD 7kg), every 7 to 10 days pigs were subjected to the bilateral application of 34 circular, reflective markers over key anatomical landmarks as described in Table 3.6. Markers were attached with double-sided, adhesive tape (Supa Brands, Worsley, Manchester). A uniplanar, linear kinematic model was used (Torres et al., 2010; Thorup et al., 2008). The method was previously validated for gait parameter repeatability in a study by Stavrakakis et al. (2014a) with 1.4 to 3.8 and 1.7 to 4.8 degree mean differences between repeated marker placements for elbow and knee angle measures, respectively. Mean differences for step and stride lengths, as defined in Table 4.1, were 6 to 13 mm. One pig at a time was moved away from its group in the waiting area and prepared with markers while it could freely move in the preparation pen. Pigs became habituated to the procedure and allowed palpation of the landmarks without crate confinement. Once all markers were in position, which took on average 30 minutes, the current pig was moved into the motion capture area and filmed while following the operator at walking speed. The motion capture system comprised six infrared cameras (Vicon T20, Oxford, UK) set up in an array flanking the walkway at a 3 m distance to the centre. This system was connected to a computer and movements on the walkway were captured with motion capture software (Nexus v.1.7.1, Vicon, Oxford, UK); the sampling rate was 125 Hz. At least two acceptable sequences of three to four strides in each direction (i.e. 4 trials including both sides; multiple trials constitute a session) were captured by the cameras on each occasion.

When pigs achieved market weight of approximately 90 kg liveweight, they were slaughtered at a local abattoir. The motion capture period lasted six weeks allowing five captures for most pigs. Pigs that achieved market weight earlier (N=8) were not included in the fifth capture. Rarely, pigs could not be motivated to move along the walkway or their films had to be discarded later because of poor marker visibility, and therefore not all captures include all 24 pigs.

4.4.3 Data processing

For stride event detection, parameter calculation and time normalisation, data were imported into Matlab (R2010b, Mathworks©, Natick, USA). The timing of toe-on and toe-off stride events was automatically determined from the vertical velocity graph of each toe marker (vertical velocity close to 0 m/s) and confirmed through visual examination of the toe marker vertical position graph. The toe-on timing was used to define the beginning of a gait cycle of an individual claw, i.e. a gait cycle lasted from one toe-on to the next toe-on of the same claw. Toe-off points were used to define swing and stance time durations for individual claws. If at least one of the four claw displacement graphs was incomplete, for example due to marker occlusion, the complete trial was rejected. Only continuous sequences of strides were included, usually containing three consecutive strides. Pigs had already undertaken several strides when they entered the camera view field and continued to walk after leaving it. Therefore it was assumed that the strides filmed by the cameras were equal and not influenced by initial propulsion or terminal braking events.

Figure 4.1 shows five selected angles of interest, namely the carpal and elbow angle in the front leg, the hind pastern, knee and tarsal angle in the hind leg. Angle values were normalised for time and all angle graphs were plotted as a function of gait cycle time and visually examined for the presence of enough data points to compose a smooth curve. Both maximum and minimum values and the range of motion during stance and swing were obtained only from well-defined angle curves. In this analysis, a selection of angular, linear and temporal kinematics is presented. Angular kinematics included are the elbow joint flexion in the front leg and the knee joint flexion in the hind leg. Linear kinematics include the step lengths of front and hind leg pairs, the front and hind stride lengths and the step-to-stride length ratio (Whittle, 1996; see Table 4.2). Temporal gait parameters are represented by the swing-stance time ratio, while walking speed is presented as a spatiotemporal parameter. Additionally, the front and hind pastern angles were included in the analysis of the effect of weight gain on gait. To account for possible morphological scaling differences between pigs, leg length was calculated as the sum of the four segments constituting the legs. This length was then tested for differences across floor groups.

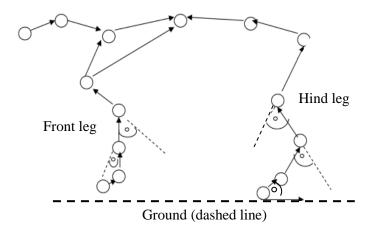


Figure 4.1: Vector skeleton model. Two markers defined each anatomically relevant segment with the vector between them. Vectors all pointed centrally allowing the angles between segments to be calculated in degrees of flexion. Note that floor vector points opposite to direction of travel. Angle examples in this figure are (from distal to proximal) in the hind leg: hind pastern angle, tarsal and knee angle. In the front leg: carpal and elbow angle.

4.4.4 Data analysis

Within-subject means and standard deviations were calculated for every gait parameter and capture, and were weighted for number of strides when originating from more than one trial. With the exception of the effect of weight gain on gait, in this analysis all results are based on measures obtained from the right side. Left side data were also collected for a follow-up paper on pathology-based asymmetry. An initial analysis, before within-subject averaging, was performed to test for stride-to-stride variability and between-pig variability by calculating the coefficients of variation (CoVs=SD/mean*100). The within-pig CoV was obtained by averaging the data of usually two different trials. The significance of the pig effect on gait, both within capture and between captures (1-5), was tested in a one-way ANOVA (Minitab v.16, USA) with pig as a fixed factor and within-pig gait parameter values as a response. To test whether growth rate had an effect on gait, pigs were classified into slow (600-700 g/d), intermediate (700-900 g/d) and fast growing animals (900-1090 g/d) and these categories used as fixed factors with gait parameters as a response in one-way ANOVAs for every capture. The floor effect on pig gait variability was investigated for every capture separately in a one-way ANOVA with floor as a fixed factor and individual pig CoVs as a response. Differences between floor groups in factors potentially affecting

gait were tested for every capture in a one-way ANOVA with floor as a fixed factor and leg length, walking speed and body weight as a response. For the first capture, the gait parameters of two pigs were excluded since their walking speed was substantially greater than all other pigs (see Section 4.5.3). This measure was taken to avoid walking speed causing differences in the walking pattern and compromising the detection of a potential floor effect (Walker et al., 2010). A repeated measures ANOVA (SPSS Statistics v.17.0) was used to test gait parameters for differences between captures and to investigate whether gait characteristics of pigs on different floors changed in a different way over time. Floor was a between-pig factor and capture was a within-pig factor in the latter analysis. Deviations from normality were assessed using an Anderson-Darling test in Minitab (v.16, USA), and where appropriate Kruskal-Wallis and Mann-Whitney tests replaced parametric statistics. P-values ≤0.050 were considered significant in all analyses. Tukey's tests were used for post-hoc analysis after significant overall effects.

4.5 Results

4.5.1 Gait variability

Within-pig and between-pig variability. Within all individual captures, the effect of pig on most measured parameters was significant (Table 4.1). Between the five different captures, a similar pattern was found and the pig effect was significant in all parameters, except the minimum elbow flexion both during the stance and swing phases. These findings demonstrate consistency in the measurements obtained from individual animals. Floor effect on gait variability. Floor type appeared to play no role in the variability of the pig's gait (all parameters measured in this study) during any of the five motion captures.

4.5.2 Weight gain effect on gait

The swing-to-stance ratio in front legs was smaller (P=0.045; 0.86 vs. 0.96) in fast growing pigs compared to slow growing pigs at the last motion capture before slaughter at approximately 90 kg liveweight. The minimum front pastern angle showed a trend (-7 degrees; P=0.06) to be smaller in fast growing pigs compared to slow growers at this same time point. However, it has to be noted that of 14 pigs left at this capture point,

only two were fast growing, ten were intermediate and two were slow growers according to the aforementioned definition. In this case only data from left side trials were analysed to reconfirm the finding and the same picture was obtained, with the minimal front pastern angle being significant for this data set (-7 degrees; P=0.011). Walking speed and leg length were not significantly different among the pigs in all cases.

4.5.3 Differences between floors and captures in factors known to affect gait

There were no significant differences in the front and hind leg length of the pigs in different groups at any of the five motion capture occasions. Total leg length of both front and hind legs significantly grew from the first to the second motion capture and then again from the second to the third. After the third motion capture the total leg length remained unchanged. Pig liveweight was significantly increased at all motion capture time points, but did not differ at any time point between flooring groups. There was a significant floor × time interaction between the first and second capture, when pigs on straw-bedded flooring grew significantly faster compared to the other groups (P<0.001; + 5 kg). Walking speed (Table 4.2) was not different between floor groups at any motion capture, except the first, when two outlier pigs from the fully-slatted floor group walked faster than all other pigs (+ 0.18 m/s, group mean 0.84 m/s SD 0.084 m/s). Data from these pigs were excluded.

4.5.4 Floor and time effect on gait parameters

Knee joint angle. No significant changes were detected at the knee joint for pigs from different floors or for different time points (Table 4.2 and 4.3).

Elbow joint angle. A significant increase was observed between the first and the second motion capture for all measured flexion values and all pigs (Table 4.2). Pig average liveweight during this period rose from 63 to 76 kg. P-values for the effect of time/growth on minimum and maximum elbow flexion during stance and swing phases were P<0.001, P=0.042, P<0.001 and P=0.010, respectively. The range of motion during the stance phase decreased over time, with a significant decrease from the first to the second capture (P=0.013). The swing phase range of motion at the elbow joint also decreased gradually over time, and was significant between the second and third capture (P=0.003). For floor group differentiation, see Table 4.3.

Linear parameters. The hind step length numerically showed a gradual increase over time, which was significant from the first to the second (P=0.009) and the second to the third capture (P=0.042). The front step length showed an identical pattern to this, with differences occurring between the first and second (P=0.034) and the second and third (P=0.043) capture. Pen floor had no influence on this development.

The stride length of the hind legs gradually increased, with significant differences occurring between first and second (P=0.035) and second and third captures (P=0.004), after which it remained similar. The stride length of the front legs showed a similar pattern to the hind legs with changes between the first and second (P=0.008) and the second and third capture (P=0.013). There was no effect of floor on this development nor was there a floor \times time interaction.

The ratios. Overall the step-to-stride length ratio in the front and hind legs did not differ between any of the captures. However, there was a significant floor effect on the within-pig standard deviation of the hind step-to-stride ratio, with pigs on straw showing less deviation from 0.5 (maximal symmetry) at capture four compared to pigs on partly-slatted concrete flooring (P=0.012) and, by tendency, also compared to pigs on fully-slatted flooring (P=0.061) (Table 4.3). The swing-to-stance ratio in the hind legs increased from the first to the second (P=0.05) capture, was similar during second and third and then decreased again from the third to the fourth capture (P=0.026). The swing-to-stance ratio in the front legs decreased between capture three and four (P=0.011). No floor differences or floor x time interactions were present.

Walking speed. Pig walking speed was increased at the second capture (P=0.009) and there was a further significant increase at the third capture (P=0.026). After the third capture, walking speed was reduced again at capture four (P=0.017), which did not differ from the walking speed during the second capture.

Table 4.1: Average coefficients of variation (CoVs) within- and between-pigs per capture. Columns represent motion captures 1-5, respectively. Pig weight is average liveweight at motion capture. W=within-pig, B=between-pig. RoM = range of motion.

Gait parameter variability (CoV)	Pigs at		Pigs at	_		at 81 kg =24)	Pigs at	_		t 90 kg =14)
	W	В	W	В	W	В	W	В	W	В
Swing minimum knee angle	5.2*	13.1	6.4 *	14.3	3.7 *	12.6	4.1 *	14.6	4.1 *	10.2
Swing maximum knee angle	2.6*	5.6	3.2*	7.6	4.5 *	5.4	2.2 *	7.8	2.4 *	6.1
Stance minimum knee angle	6.0*	11.4	5.4*	12.7	4.0 *	11.0	5.2 *	11.3	4.2*	11.0
Stance maximum knee angle	3.1 *	8.3	2.9*	9.8	3.7 *	8.0	2.1*	10.4	1.9*	8.2
Swing RoM knee	7.6*	8.7	8.8 *	11.5	12.0*	9.3	5.3*	8.0	6.5 *	15.0
Stance RoM knee	13.4 *	15.1	13.8*	16.2	12.3*	13.8	15.1 *	20.3	12*	15.2
Swing minimum elbow angle	5.0*	14.2	6.6*	11.0	4.2 *	12.6	5.4 *	11.7	6.2 *	10.2
Swing maximum elbow angle	2.6*	10.1	5.0*	7.0	3.7*	8.6	2.7 *	8.2	3.6*	6.4
Stance minimum elbow angle	4.5 *	13.7	5.4*	10.2	4.1 *	13.4	4.3 *	11.8	5.3 *	9.9
Stance maximum elbow angle	4.1*	12.5	5.2*	9.6	4.2 *	11.1	3.8*	9.7	5.1*	8.2
Swing RoM elbow	6.9*	15.4	9.6*	15.2	13.8 *	11.3	6.9 *	13.0	10.4 *	11.2
Stance RoM elbow	13.1 *	23.8	17.4 *	22.3	15.0 *	20.5	13.3 *	21.7	20.8 *	21.2
Step length hind**	7.1 *	5.1	9.2*	8.3	5.9*	7.3	6.0 *	6.6	7.0*	7.2
Step-to-stride length ratio hind	5.4	3.5	7.4	4.5	6.3*	4.6	5.2	3.6	5.1 *	5.1
Step length front	8.1 *	6.6	8.9 *	7.0	6.3 *	7.8	10.2	7.1	8.9 *	7.8
Step-to-stride length ratio front	5.9	3.9	7.2	3.3	9.3	4.0	9.1	3.3	5.7	4.3
Stride length hind***	5.0 *	5.9	5.7 *	6.8	3.7*	6.3	3.3 *	5.9	4.4 *	5.4
Stride length front	4.6*	6.2	5.8 *	7.0	5.5*	5.8	3.8*	6.7	4.4 *	5.7
Swing-stance time ratio hind	10.4 *	12.1	15.6*	11.5	14.0*	15.9	11.2*	13.2	12.9*	11.8
Swing-stance time ratio front	11.1*	19.6	15.6*	20.8	16.1*	19.3	13.0 *	18.2	15.2*	19.5
Walking speed	7.9*	10.9	12.2*	12.4	14.3 *	14.3	9.7*	12.7	12.0*	11.8

^{*}A star accompanies parameters for which pig effect within capture was significant at P≤0.050, i.e. within-pig variability typically smaller than between pig variability.

^{**}The step length is the length of a rectangle drawn using the same ground contact points of left and right claws of a pair as the diagonal of the rectangle (Whittle, 1996).

^{***}The stride length is the distance measured between the same ground contact points of two consecutive contacts of the same claw (Whittle, 1996).

Table 4.2: Changes in gait measured over time. Mean gait parameter values at different motion capture time points. Last capture values are reported without statistical comparisons (due to reduced number of participants left, this capture was excluded from the repeated measures analysis). Columns represent motion captures 1-5, respectively. Angles are in degrees flexion, linear parameters in mm, speed in m/s. Comparisons were made to the previous and following levels, but not across all captures, with the exception of walking speed.

		Pigs at 63kg LW	Pigs at 76kg LW	Pigs at 81kg LW	Pigs at 87kg LW	Pigs at 90kg LW
Gait parameter	Gait phase	(N=24)	(N=22)	(N=24)	(N=22)	(N=14)
		\overline{x} (SEM)	\overline{x} (SEM)	\overline{x} (SEM)	\overline{x} (SEM)	\overline{x} (SEM)
Minimum knee flexion	Stance	50 (1.2)	51 (1.4)	52 (1.3)	53 (1.3)	56 (1.6)
Maximum knee flexion	Stance	70 (1.2)	72 (1.5)	72 (1.4)	72 (1.6)	77 (1.7)
Minimum knee flexion	Swing	43 (1.2)	44 (1.4)	46 (1.4)	46 (1.4)	48 (1.3)
Maximum knee flexion	Swing	85 (1.0)	88 (1.5)	90 (1.0)	89 (1.5)	92 (1.5)
Minimum elbow flexion	Stance	51 (1.4) ^a	58 (1.3) ^b	59 (1.6) ^b	59 (1.5) ^b	62 (1.7)
Maximum elbow flexion	Stance	$72(1.8)^{a}$	77 (1.6) ^b	$77(1.7)^{b}$	77 (1.6) ^b	80 (1.7)
Minimum elbow flexion	Swing	$47(1.4)^{a}$	54 (1.3) ^b	56 (1.4) ^b	$56(1.5)^{b}$	60 (1.6)
Maximum elbow flexion	Swing	$88(1.9)^{a}$	93 (1.4) ^b	92 (1.6) ^b	92 (1.6) ^b	94 (1.6)
Range of motion knee	Stance	21 (0.7)	20 (0.7)	20 (0.6)	19 (0.8)	21 (0.8)
Range of motion knee	Swing	43 (0.8)	44 (1.1)	44 (0.9)	44 (0.9)	44 (1.8)
Range of motion elbow	Stance	$21(1.0)^{a}$	$19(0.9)^{b}$	$18(0.7)^{b}$	$18(0.9)^{b}$	18 (1.0)
Range of motion elbow	Swing	$40(1.3)^{a}$	$38(1.3)^{a}$	$36(0.8)^{b}$	$36(1.1)^{b}$	35 (1.0)
Step length hind**		327 (3.5) ^a	343 (6.1) ^b	350 (5.3) ^c	355 (4.8) ^c	371 (7.1)
Step length front		$329 (4.3)^{a}$	$343 (5.1)^{b}$	$351(5.4)^{c}$	361 (5.2) ^c	360 (7.6)
Stride length hind***		$659 (7.8)^{a}$	$685 (10.0)^{b}$	$704 (8.9)^{c}$	$710 (9.0)^{c}$	729 (15.0)
Stride length front		655 (8.2) ^a	686 (10.2) ^b	709 (8.3) ^c	709 (9.9) ^c	719 (14.0)
Step-to-stride length ratio hind (x10 ⁻¹)		4.98 (0.035)	4.97 (0.048)	4.96 (0.050)	5.01 (0.037)	5.07 (0.069)
Step-to-stride length ratio front (x10 ⁻¹)		5.00 (0.042)	5.00 (0.035)	4.95 (0.040)	5.05 (0.035)	4.92 (0.057)
Swing/stance time ratio hind (x10 ⁻¹)		$8.59 (0.219)^a$	$9.06 (0.222)^{b}$	$9.54 (0.312)^{b}$	$8.84 (0.276)^{c}$	8.82 (0.279)
Swing/stance time ratio front $(x10^{-1})$		8.84 (0.377) ^a	$8.68 (0.385)^{a}$	9.63 (0.431) ^a	$8.56 (0.320)^{b}$	8.97 (0.468)
Walking speed (SD x10 ⁻¹)		0.85 (1.93) ^a	0.95 (2.47) ^b	1.05 (3.18) ^c	0.96 (2.71) ^{bd}	0.97 (3.00)

^{a, b} Means within a row followed by a different superscript differ significantly at $P \le 0.050$.

⁻⁻⁻ These are unidimensional parameters, i.e. measured only once per gait cycle.

^{**}The step length is the length of a rectangle drawn using the same ground contact points of left and right claws of a pair as the diagonal of the rectangle (Whittle, 1996).

^{****} The stride length is the distance measured between the same ground contact points of two consecutive contacts of the same claw (Whittle, 1996).

Table 4.3: Differences in measured gait between pigs growing on different pen floors. Mean gait parameter values of all pigs within a group at time points when a significant difference was observed. Angles are in degrees flexion (deg).

				Pen floor	
Gait parameter	Gait phase	Significant capture	Fully-slatted \bar{x} (SEM)	Partly-slatted \overline{x} (SEM)	Straw-bedded \overline{x} (SEM)
Knee flexion	All	None	NS	NS	NS
Range of motion knee	All	None	NS	NS	NS
Maximum flexion elbow	Swing	Second	96° (2.4)	87 ^b (1.8)	95 ^a (1.9)
Range of motion elbow	Swing	First	45 ^a (1.9)	36 ^b (1.1)	40 ^{ab} (2.5)
Linear parameters		None	NS	NS	NS
Within-pig SD* of hind leg step to-stride length ratio (x10 ⁻²)		Fourth	1.1 ^{a(b)} (1.09)	1.8° (5.84)	0.5 ^b (0.55)
Swing/stance time ratios		None	NS	NS	NS

a, b Means (Medians for step-to-stride ratio SD) within a row followed by a different superscript differ significantly at $P \le 0.050$. (b) Indicates tendency, where P = 0.061. NS=not significant.

^{*}SD=Standard deviation; A Kruskal-Wallis test was applied, hence medians and interquartile ranges are reported.

4.6 Discussion

The purpose of this study was to investigate and present quantitative gait data of pigs followed through an important growth stage on one of three commercial floor types. Although fully-slatted and partly-slatted concrete floors are regarded as less beneficial to the healthy musculoskeletal development of pigs (Van Grevenhof et al., 2012; Jørgensen, 2003), results of this study showed no indication of detrimental effects on gait development over time. However, compared to previously conducted studies with similar objectives using subjective assessment, a compromise of the present detailed study is the smaller number of subjects.

In marker-based kinematic studies, there are some sources of error within the methodology, such as skin movement artefact and failure in anatomical landmark identification (Maynard et al., 2003; van Weeren et al., 1992). The repeatability of the gait variables included in this study has previously been determined in young growing pigs (Stavrakakis et al., 2014a). The range of mean differences between marker placements was comparable to achievement in horses (Weller et al., 2006) and humans (Maynard et al., 2003), including both stance and swing phases of the gait cycle. Although skin movement has been found to be increased during the swing phase (van Weeren et al., 1992), the joint angles during this phase undergo substantial changes, as confirmed in the current study where pigs showed a greater range of motion. Gait analysis in pigs is still at a relatively early stage (Gregoire et al., 2013) and therefore effect sizes of conditions and treatments have yet to be established. In the current study, a broad selection of gait parameters from front and hind legs, stance and swing phases of the gait cycle and from more and less biased sites were presented. For example, the ranges of motion at joints, all within-session CoVs and the linear parameters are relative measures and therefore less susceptible to marker misplacement error. The markers used for calculating linear and temporal gait parameters are attached to horn on the claws and therefore unaffected by skin movement. This increases the confidence in the results and conclusions from this cohort of 24 pigs, especially since there were five repeated measures.

Nonetheless, the differences in the gait of fast growing pigs at their heaviest capture point cannot reliably be interpreted from a mechanical point of view. These pigs were too few in number to draw any conclusion. However, if the dense connective

tissues of tendons and ligaments in the lower extremity receive increased loads and loosen over time, resulting in a "weak pastern", this could increase the likelihood of dewclaws being trapped between slats and increase soft heel-to-floor contact, potentially leading to injuries.

Gait variability may increase in the presence of neuromuscular disorders or other pathology in the musculoskeletal system (Hausdorff et al., 2005). Coefficients of variation (CoVs) based on the stride-to-stride variability of the pigs in this study were analysed across floor groups and revealed no differences. The CoVs between- and within-pigs are included here and may be useful in selecting gait parameters in future studies. Such studies may focus on the long-term effects on those gait parameters with a small within-subject value, indicating higher individual consistency, in a population displaying greater between-subject variety i.e. higher CoVs. Weller et al. (2006) describe the long-term effects of individual gait patterns on orthopaedic health in horses.

There were significant changes in the gait of the pigs over time, such as increases in linear parameters of stride and step length as well as walking speed. These changes are typical and may be ascribed to increases in leg length and to maturation of the locomotor system resulting in stabilisation of walking patterns in growing subjects (Sutherland, 2007; Clarke and Still, 2001). The changes in the swing-to-stance ratio may to some extent reflect changes in walking speed, as this ratio often increases at faster walking speeds (Robilliard et al., 2007). Growth changes occurred in all animals in this experiment, were symmetric as confirmed by results from the left side for the change in elbow flexion, and in an order of magnitude which was not possible to perceive or evaluate visually. This highlights the additional potential in detecting differences when using kinematic motion capture. One of the few published and validated subjective pig gait scoring systems (Main et al., 2000) uses the "length of stride" as one of its main criteria for lameness detection.

Noteworthy are the changes at the elbow joint, which were not accompanied by similar changes in the flexion pattern at the equivalent hind leg hinge joint, the knee. This could indicate differences in the development and function of the proximal front and hind legs. However, the repeatability of angular parameters from the knee joint is reduced compared to the elbow joint and therefore the detection of a true deviation at this joint was impeded.

Significant increases in the flexion extremes at the elbow joint were observed in this study. Yet the ranges of motion in both stance and swing phases decreased over time. Increased flexion during walking may be correlated to factors such as leg length and walking speed (Biewener, 2005; Back et al., 1994), but this was generally not the case for the pigs in this study. However, bodyweight showed a consistent trend for a negative correlation with the elbow swing range of motion within captures among all pigs. This could explain the total reduction over time in this gait parameter as all pigs grew heavier, and might suggest that heavier pigs experience increased mechanical constraints at the elbow joint. Nonetheless, increased general flexion at the elbow joint in pigs may equally represent a normal growth-related change in front leg conformation. The reduced range of motion could then be a secondary result of restricted space available for motion.

Generally, the step-to-stride length ratio did not depart substantially from 0.5, which is the most frequent observation in normal pigs and is altered in lame pigs (Stavrakakis et al., 2013). Nevertheless, there was an interesting difference between pigs housed on concrete (both fully-slatted and partly-slatted) and straw-bedded surfaces, with pigs on straw being closer to the expected normal (Whittle, 1996). This could indicate an increased irregularity in the displacement of left and right hind legs in the former pigs, which in turn would suggest some underlying pathology or disturbing factor affecting hind legs only. This finding probably deserves further investigation and it would be worthwhile to expand the follow-up period for motion capture of pigs exposed to different pen floors in the future. Thus, breeding animals could be monitored into gestation and lactation.

Visual lameness detection suffers from clear limitations when conditions are mild (Keegan, 2007; Quinn et al., 2007), therefore biomechanical methods could have a potential to mitigate against this. The step-to-stride length ratio in the current study was the only parameter unchanged over time. This means that the relationship between these lengths remains stable and is independent from age/growth and walking speed. If the ability of this parameter to detect subtle lameness can be confirmed, this would be a suitable parameter for automatic lameness detection in pigs, which would be a welcome tool for on-farm monitoring of lameness. Such an application would require gait parameters insensitive to heterogeneous groups of pigs walking at their preferred walking speed, even with variation in the latter.

In conclusion, the measured changes in gait patterns over time were largely unaffected by the floor surface on which the pigs were reared. Therefore the quantification of locomotion in this longitudinal observational study of pigs housed on different floors could not confirm differences observed in previous epidemiological studies using subjective gait assessment. Prevalence of perceivable problems in the present population was low, since only stiffness and no lameness were recorded in 21% of pigs on average. However, there were subtle changes which could be measured by the motion capture system but were invisible to the naked eye. The application of precise quantitative techniques to a large population of farm animals is challenging. Nevertheless this study has identified meaningful gait parameters, such as the step-to-stride length ratio and the elbow joint angle, which are worth exploring further.

Chapter 5. Walking Kinematics of Growing Pigs Associated with Differences in Musculoskeletal Conformation, Subjective Gait Score and Osteochondrosis

5.1 Abstract

Despite orthopaedic problems being a major concern for the pig industry, there is a lack of biomechanical, hence quantitative, investigation of locomotor pathology in pigs. The objective of this study was to determine whether there are detectable changes in joint and stride kinematics of clinically sound and unsound pigs, with or without post mortem joint lesions of osteochondrosis. A cohort of 24 pigs underwent five camera-based motion captures, 7-10 d apart and between 63-90 kg average liveweight, during which 3D coordinate data of reflective skin markers attached to leg anatomical landmarks were collected. Pigs walked on the same solid concrete walkway during captures. Statistical analysis was performed within captures across pigs which were categorised based on a series of deficiency characteristics. Results showed that pigs with clinical deficiencies and degenerative joint lesions had altered kinematics, although there were capturerelated fluctuations in the findings. For pigs with conformational and perceived gait abnormalities there were significant differences in joint flexion values and left and right flexion symmetry, mainly reflected in the swing ranges of motion, compared to sound animals. Buck-kneed front legs were found to cause flexion deficiencies during the stance phase of stride cycles (P \le 0.05; -9 degrees). Pigs with osteochondral joint lesions had mainly stance-related angular changes (P≤0.05) and asymmetry. Irregularity in the step-to-stride length relationship was elevated in both clinically and subclinically deficient pigs (respectively P=0.06, 1.5 times greater; P \leq 0.05; 1.5 times greater), but not the healthy pigs. These data provide basic kinematic values for clinically sound and affected pigs which could be used for further research into early and automated detection of leg disorders as well as for improved selection of breeding animals for longevity characteristics.

5.2 Introduction

Locomotor problems in modern pig herds are a major concern for animal welfare and production efficiency and, with the exception of antibiotic treatment in the case of infections, are often very difficult to treat (Willgert et al., 2014; Heinonen et al., 2013; Nielsen et al., 2001). Due to premature culling, lameness can be the second most important cause for significant economic losses in breeding animals, particularly in the case of young sows before they reach their third parity (Abiven et al., 1998). Genetic selection and prevention through control of on-farm risk factors are the methods of choice to reduce lameness problems (Pluym et al., 2013a; Fan et al., 2009). Since longevity is an important characteristic of future breeding animals, the focus on musculoskeletal conformation, mobility and freedom from disease is especially important (Serenius and Stalder, 2006).

Lameness is not a specific disease, but a common clinical symptom of various structural and functional conditions and is defined by observable changes in gait (Weishaupt, 2008). The common causes of lameness in pigs are genetic or acquired musculoskeletal weakness, infections, injuries, nutrient deficiencies and systemic diseases which target musculoskeletal components (Jensen et al., 2009). Osteochondrosis (OC), a non-infectious, degenerative failure of endochondral bone formation with a proven genetic component, is one of the most important underlying causes of lameness in growing pigs (Jørgensen and Andersen, 2000a; Nakano et al., 1987). Therefore focusing genetic selection on this condition, coupled with an improved ability to detect pigs carrying osteochondrosis could reduce the prevalence of lameness on farm. However, clinical distinction of osteochondrosis from other non-inheritable conditions in the live animal is not possible (Jørgensen, 2000b) unless using expensive imaging techniques, such as x-ray or MRI (magnetic resonance imaging) (Ytrehus et al., 2007; Jørgensen et al., 1995). Instead, leg weakness has been promoted as a visually detectable syndrome, representing various conformational deficiencies arising from problems in the musculoskeletal system (Van Grevenhof et al., 2012; Van Steenbergen, 1989). Osteochondrosis is considered to be the major underlying cause of leg weakness symptoms (de Koning et al., 2013; Jørgensen and Sørensen, 1998).

Subjective assessment protocols have been developed and are currently the only on-farm tool available for the quantification of leg weakness, including osteochondrosis (de Koning et al., 2012). Likewise, subjective gait/locomotion scoring systems have been developed for pigs (Main et al., 2000). However, there appears to be great

variability in the outcomes of studies investigating the causal factors of lameness in pigs, with several contradictory results over the last decades (Ytrehus, 2007; Stern et al., 1995, Simonsen, 1993; Grondalen, 1974). Several studies have found poor (Jørgensen, 2003 and 1995; Jørgensen and Andersen, 2000a; Goedegebuure et al., 1988) or very complicated (de Koning et al., 2012) associations between leg weakness symptoms and osteochondrosis lesions detected post mortem. One of the reasons for this variability could be the exclusive use of subjective methods to assess any clinical symptoms, since there may be a lack of repeatability in the classification of animals based on visual observation alone (Dyson, 2011; Waxman et al., 2008; Petersen et al., 2004).

Therefore identification of sensitive, accurate and objective clinical biomechanical parameters of locomotion could provide a superior means of detecting animals with osteochondrosis or other musculoskeletal weaknesses. In addition, such parameters may improve our understanding of the pathomechanics of the musculoskeletal system and may facilitate identification of those animals with abnormalities which were previously invisible to the observer. Evidence-based medical practice and large-scale modern livestock farms could benefit from automated biomechanical techniques as a means of herd health and welfare monitoring and early detection of imminent problems. Although subjective methods of scoring lameness have advantages, it is important to develop quantitative methods and identify gait parameters more sensitive to, or even able to distinguish between, the problems of interest (Gregoire et al., 2013). Such quantitative methods could complement or even replace existing subjective selection of future breeding animals (Serenius and Stalder, 2006).

The objective of this study was to determine whether there are detectable changes in joint and stride kinematics of clinically sound and unsound pigs, with or without post mortem joint lesions of osteochondrosis. Clinically unsound pigs had leg weakness and/or subtle lameness, as judged by visual observation.

The hypothesis was that pigs with conformational deficiencies, perceived walking abnormalities and/or joint disease would produce a different gait profile to clinically normal counterparts in one or more of the measured gait parameters (including left/right asymmetry). The gait parameters were chosen to represent a variety of geometric features of movement (angular, linear and spatiotemporal kinematics) and have been previously identified in various species as being relevant for differentiation between normal/abnormal gait and changes in gait over time (Bockstahler et al, 2007; Back et al., 1994).

5.3 Materials and Methods

5.3.1 Animals, management and experimental housing

All procedures on animals were in accordance with institution guidelines and UK animal welfare regulations. The experimental population at the Newcastle University pig unit consisted of 12 entire male and 12 female growing pigs of a commercial crossbred genotype (Large White x Landrace damline and Pietrain x Duroc sireline; Hermitage Genetics, Kilkenny, Ireland), clinically healthy and weighing 37 kg (SD 3 kg) at point of entry. The pigs were randomly selected from three double-litter groups previously housed on plastic slatted flooring. Only pigs with no signs of lameness were included, i.e. they showed no signs of limping when moving along a walkway and had no visible body surface abnormalities. The pigs were divided into groups of eight (four males and four females in each group) and randomly assigned to one of three pens within the same room in an experimental building with a controlled environment; pen area was 9.6 m² (Stavrakakis et al., 2014b). Pigs had free access to concentrate feed and water. During the following five weeks, pigs were habituated daily to separation from pen mates, close human contact, marker application and walking training. Pigs learned to follow a target led by a human operator to obtain a treat (a small piece of apple) when movement was considered acceptable (i.e. was regular, continuous and straight).

5.3.2 Data collection

A neighbouring building contained the motion capture area, along with a waiting pen and a preparation pen (each measuring 3.8 x 2.27 m). Each group of pigs was moved from their home pen to the waiting area before each capture session. Starting from a mean body weight of 63 kg (SD 7kg), every 7-10 days pigs were subjected to the bilateral application of 34 circular, reflective markers over anatomical landmarks by means of non-permanent adhesive tape. A uniplanar, linear kinematic model was used (Torres et al., 2010; Thorup et al., 2008) and the method had been previously validated for gait parameter repeatability. There were 1-2, 3-4, 3-4 and 4-5 degree mean differences between marker placements for carpal, tarsal, elbow and knee angle measures, respectively (Stavrakakis et al., 2014a). For step and stride lengths, mean differences were 6-13 mm. One pig at a time was moved from its pen group in the waiting area to the preparation pen, where it could move freely during marker

placement. Pigs became habituated to the procedure and allowed palpation of the landmarks without the need for crate confinement.

Once all markers were in position, the pig was moved into the motion capture area, a solid concrete walkway measuring 3.5 m long and 2.0 m wide. The motion capture system comprised six infrared cameras (Vicon T20, Oxford, UK) set up in an array at a 3.0 m distance to the centre of the walkway and connected to a PC. Movements were captured with motion capture software (Nexus v.1.7.1, Vicon, Oxford, UK) at a sampling rate of 125 Hz. At least two acceptable sequences of three to four strides in each direction (i.e. 4 trials including both sides; multiple trials constitute a session) were captured by the cameras on each occasion (capture). An acceptable sequence of strides was defined by the pig walking along the walkway in a regular and continuous fashion without changes in speed, stopping, stumbling or any other obvious distraction from steady movement.

When pigs achieved market liveweight (approximately 90 kg), they were transported to a local abattoir for slaughter. The motion capture period lasted six weeks allowing five captures for most pigs. Pigs that achieved market weight earlier (N=8) were not included in the fifth capture. Occasionally some pigs could not be motivated to move along the walkway or their sessions had to be discarded subsequently due to poor marker visibility, and therefore not all captures include all 24 pigs.

Pigs were weighed weekly and assigned a subjective score for conformational deficiency, i.e. leg weakness traits, and lameness at the same time. Scoring was undertaken by the same observer, the first author who holds a qualification in Veterinary Medicine. During this procedure, pigs were individually led into the solid concrete floor passageway between their home pens and inspected from both sides, and from both front and rear whilst standing and walking (taking approximately 4 minutes per pig).

For the scoring of conformational deficiency, points of consideration were the alignment of front and hind legs, evenness of claws and strength of pasterns (see Table 5.1). Pigs received a qualitative description of the observed conformation of front and hind legs which was further graded into mild, moderate or severe.

For the scoring of lameness/gait, an adaptation of the gait scoring system of Main et al. (2000) was used. A gait score was assigned (from 0 to 5, where [0] describes normal pigs with even strides and fluent walking, [1] stiffness and an abnormal stride length, [2] shortened stride and a detected lameness, [3]) minimal weight bearing on affected leg(s), [4] no weight bearing on affected leg(s) and [5] designates a pig unable to move.

For the scoring of joint lesions caused by osteochondrosis, after slaughter the major articulations of all four legs of each pig were inspected. With the exception of the interphalangeal joints, all joints for each pig were scored macroscopically for signs of osteochondrosis using a system adapted from Scott et al. (2006). The joint-level scale from 0 to 4 was score [0] no lesions, regular, even and opaque articular cartilage; score [1] thinning of cartilage; score [2] irregularity in cartilage (fissures and/or craters); score [3] extensive or deep irregularities (fissures and/or craters) and score [4] extensive erosion, ulceration and/or flaps in articular cartilage. The claws were also inspected for any abnormalities, but there were no notable lesions found. Thus, each pig was given an initial subjective score for conformational deficiency, lameness/stiffness and osteochondrosis, scores which would subsequently be used to place the pigs into particular categories (see *Animal categorisation* below)

Table 5.1: Definition of leg weakness traits examined in finishing pigs (adapted from Jørgensen, 1995)

Characteristic	Deficiency	Description
	Buck-kneed	Front legs buckling forward
	Sickle-hocked	Excessive bending of hind legs at hock
Alignment	Post-legged	Excessive straightness
of front and	Splay-footed	Toes/legs* turned out
hind legs	Pigeon-toed	Toes/legs* turned in
	Varus	O-shaped frontal profile of front or hind pair
	Valgus	X-shaped lateral profile of front or hind pair
Spine	Kyphosis	Humped back
	Lordosis	Broken back
	Weak pastern	Pastern touching ground
Claws	Upright pastern	Pastern angle too steep
	Unevenness	Uneven claws

^{*}It is possible that the inward or the outward positioning of the toes is caused by the phalanges themselves or upper leg segments turned out/in. In order to be accurate, these descriptions will clarify that for example a splay-footed pig was also checked for whether the splay was from the toes or a leg segment more proximal in the limb.

5.3.3 Data processing

For stride event detection, parameter calculation and time normalisation of joint angles, data were imported into the software Matlab (R2010b, Mathworks©, Natick, USA) and processed by a custom-written programme. The timing of toe-on and toe-off stride events was automatically determined from the vertical velocity graph of each toe marker (toe vertical velocity close to 0 m/s) and controlled through visual examination of the vertical toe marker position graph (minimal vertical position within a gait cycle). The toe-on event was used to define the beginning of a gait cycle of an individual claw, i.e. a gait cycle lasts from one toe-on to the next toe-on of the same claw. Toe-off points were used to define swing and stance time durations for individual claws. If at least one of the four claw displacement graphs was incomplete (e.g. due to marker occlusion), the complete trial was rejected. Pigs had already undertaken several strides when they entered the field of view of the cameras and continued to walk after leaving it. Therefore it was assumed that the strides filmed by the cameras were equal and not influenced by initial propulsion or terminal braking events. Joint angles between leg segments were calculated in degrees of flexion. Angle curves were normalised for stride time. This procedure enabled the within-pig summary of joint angle curves from different strides and allowed for comparison of angle-over-time curves between different pigs. Maximum and minimum values and the range of motion during stance and swing phases were obtained only from well-defined angle curves. Angle curves of all pigs and both sides were inspected and any obvious outliers (more than 1.5 SDs away from mean curve and/or with obvious stride phase displacements) were removed before within-pig curve summary to obtain a representative sample from each pig.

5.3.4 Animal categorisation

Conformation score. When more than one deficiency was present in an animal, it was categorised according to its most pronounced leg deficiency. The criterion for inclusion into a deficiency category was to score at least twice for the five overall capture occasions a non-mild (moderate or severe) version of the deficiency in question. Once assigned into a deficiency category, pigs were considered to represent the deficiency throughout the entire capture period, i.e. although conformation scores were given every week, a pig received a final score which was then used for every capture.

Lameness/gait score. Only gait score (1), representing stiffness/mild lameness, was detected in the current pigs, with more severe lameness not observed. At capture point three, the largest number of pigs (N=7) with stiffness was observed and some of these pigs were repeatedly given a score (1). Consequently, these pigs were used to represent stiff pigs at all time points.

Osteochondrosis lesion (OC) score. Due to occurrence of mild lesions in many different joints and for the recruitment of sufficient subject numbers for analysis, pigs were categorised based on the predominant lesions/or absence of lesions, without excessive sub-categorising. Generally, joint-level scores were used to categorise pigs into front and/or hind leg affected animals and mildly or moderately-to-severely affected animals. None of the pigs had lesions in the hip, metacarpophalangeal or metatarsophalangeal joints, hence these joints were not considered in categorisation. Joint-level score [1] was regarded as mild, score [2] as moderate and [3] and above as describing severe lesions. For example, a pig with a joint-level score [1] lesion in the left elbow (thinning), but score [2] in the right elbow (deeper crater/irregularity) and with score [0] for the left knee and score [2] in the right knee and no other joint affected, was labelled as a front and hind moderately affected animal. Note that a pig with similar lesions in the shoulder and tarsal joints would be classified the same on the animalbased level. Secondly, five joint-level scores per pig (shoulder, elbow, carpal, knee and tarsal) reflected the most affected body side and were used to investigate potential jointspecific movement effects of the lesions on a case-by-case basis (flexion graphs of individuals are included in appendices).

Clinical status. Pigs were categorised as clinical when they had at least one joint lesion and at least one conformational deficiency and/or lameness/stiffness, as defined above. Subclinical pigs had joint lesion(s), but no conformational deficiency or any lamenss/gait deficiency, as defined above.

5.3.5 Data analysis

In this analysis a selection of angular (elbow joint angle in front leg and knee joint angle in hind leg), linear (step length of front leg, step length of hind leg, stride length front and hind), temporospatial (walking speed) and two ratios (step-to-stride length and swing-stance time ratio) are presented. For analysis of the effect of osteochondrosis lesions on gait parameters at joint level, carpal and tarsal angles were included to investigate specific joint effects. Within-subject means were created for every gait

parameter and capture (i.e. session means) and, when originating from more than one trial, were weighted for the number of strides. Except for the asymmetry assessment, all results are based on trials obtained from the right side of the animals.

Differences in the gait of pigs with conformational deficiencies, stiffness and OC lesions were investigated for every capture separately in a one-way analysis of variance (ANOVA) (SPSS Statistics v.17.0 and Minitab v.16, USA). Scores, i.e. conformation, lameness/gait or OC scores, were used as a fixed factor and gait parameter as a response. Repeated measures of the same pigs during the experiment was not considered to be an appropriate analysis, since it was not the relative changes but the actual absolute differences for each capture that were of interest to this study. Furthermore, there were a few missing values for some pigs at different time points, and using a repeated measures analysis would have meant that these animals were excluded from the analysis thereby reducing the sample size.

Differences between left and right sides of the pigs were assessed using paired t-tests comparing left and right gait parameter within capture. The difference between left and right parameters in every capture was used as a response in a one-way ANOVA (or Kruskal-Wallis) with conformation, lameness/gait or OC score as a fixed factor to test whether score categories showed increased asymmetry. In the asymmetry analysis, carpal and tarsal angles were also included. Intraclass correlation coefficients (ICC) were used to assess the agreement of total clinical category (good or deficient conformation; stiff or normal gait) with joint lesion status (with or without joint lesion(s)). Agreement of conformation and lameness/gait score assignment from capture to capture was also evaluated using the ICC.

Continuous data were checked for normality using Anderson-Darling tests. Departures from normality entailed use of non-parametric equivalents (Kruskal-Wallis and Wilcoxon related sample tests). Significance was accepted at P<0.05 and a Tukey's test employed for post-hoc analysis of significant effects. To exclude factors which could cause gait differences other than the factors under investigation, all compared category groups were checked for absence of size (liveweight) and walking speed differences.

5.4 Results

Table 5.2 describes the associations of quantitative gait parameters with leg weakness traits and visually detected stiffness. Overall, leg weakness and stiffness caused more swing-phase related gait changes which, in most instances, were associated with

changes in the range of motion at joints. Pigs with stiffness at the third capture showed changes prior to stiffness and at the time of stiffness, but not afterwards, possibly due to recovery.

Table 5.3 summarises the changes in gait measured at five occasions before slaughter for pigs with osteochondrosis lesions detected post-mortem. The five motion captures were at 63, 76, 81, 87 and 90 kg average pig liveweight. Only pigs with moderate to severe lesions in either front or both front and hind legs generated significant differences in joint flexion patterns. Differences in pigs with moderate to severe front leg lesions were exclusively seen in increased flexion asymmetry at front leg joints and were in all instances stance-phase associated. These differences occurred at a younger age, while for pigs with front and hind leg lesions the differences became evident toward later captures. In the latter pigs, the differences were reflected in joint flexion values, i.e. peak flexion or range of motion at a joint, and often stance-phase related. The table further shows the number of pigs in each joint lesion category which also had a clinical deficiency, analysed by capture.

Table 5.4 shows irregularities detected in the step-to-stride length ratio when compared between pigs in different clinical categories, i.e. healthy, clinical and sub-clinical. This ratio suggests a link between asymptomatic pigs with lesions and those pigs with lesions and symptoms of leg weakness and/or observable gait abnormality, and differentiates both groups from the healthy lesion-free pigs. Similarly, Figure 5.1 shows the step-to-stride length ratio irregularity by capture and reveals that a substantial difference (P=0.018 for clinical and P=0.219 for subclinical pigs) occurred at capture two for this parameter, which might be an indication of the onset or peak of mechanical disturbance from the disease.

The intra-class correlation coefficient showed a low to moderate agreement between the joint lesion status post slaughter and the clinical appearance of the pigs. The ICC for conformational deficiency and joint lesion status was 0.221 (P=0.144); the agreement of repeated conformation scores amounted to 0.565 (P<0.001). For subjective gait scores the equivalent ICC measures were 0.143 (P=0.248) and 0.472 (P<0.001), respectively. The supplementary material (Figures 8.3-8.5) shows continuous joint flexion curves over normalised stride time for deficiency categories with significant flexion changes and individual pigs with and without lesions. These graphs demonstrate the value of assessing asymmetry and flexion patterns of entire waveforms rather than point values, such as peaks and amplitudes, extracted from the curves.

Table 5.2: Association between subjective conformation and lameness/gait scores with quantitative gait parameters

N	Ieasure r	nents			Quantitati	ve detection (Motio	on capture occasio	ons)	
Parameter	Limb	Joint	Phase	63 kg	76 kg	81 kg	87 kg	90 kg	$Value^{\ddagger}$
		elbow	ST		♦ BK				-10
	F		SW		lack BK, $lack Stiff$ (RoM)	♦ Stiff (RoM)	↑ PL (RoM)		-12;-6;-4;+7
.		carpal	ST						
Joint Flexion			SW						
Fiexion		knee	ST						
	Н		SW	₩ BK (RoM)			↑ PL (RoM)		-4; +4
		tarsal	ST						
			SW						
		elbow	ST						
	F		SW		↑ BK, PL (RoM)				+4
Flexion		carpal	ST	↑ BK					+2
Asymmetry			SW	↑ BK, Stiff					+1; +2
		knee	ST	↑ Stiff					+3
	Н		SW	↑ Stiff				↑ PL (RoM)	+2; +5
		tarsal	ST	↑ PL				↑ BK (RoM)	+7;+5
			SW		↑ PL (RoM)				+11
Step/Stride	F								
length ratio	Н					↓ BK			-0.03

Codes for conformation and gait: BK=buck-kneed front leg deficiency (N=5); PL=post-legged hind leg deficiency (N=5); Stiff=Stiffness (N=7, representing gait score 1 according to Main et al., 2000) Black symbols for significant differences $P \le 0.05$, greyed for tendencies (P > 0.05 but < 0.07).

All significant comparisons were against pigs with good conformation (N=14).

RoM=Range of motion.

ST=stance; SW=swing.

For angles the unit is degrees, ratios are dimensionless; semicolons separate values for groups as appearing in rows from left to right.

Table 5.3: Association between post-slaughter osteochondrosis lesions with quantitative gait parameters

Me	easurem	ents	<u>8</u>		$\overline{\varrho}$	uantitative detection (Me	otion capture occasions)		
Parameter	Limb	Joint	Phase	63 kg	76 kg	81 kg	87 kg	90 kg	$Value^{\ddagger}$
		elbow	ST						
	F		SW		↓ HF				-8
		carpal	ST						
Joint			SW						
Flexion		knee	ST			↑ HF (RoM)	·		+6
	Н		SW				↓ HF	A 1177 (D 116)	-14
		tarsal	ST				↓ HF	↑ HF (RoM)	-15; +9
			SW						
		elbow	ST	↑ F (RoM)					+4
	F		SW	- ,					
Flexion		carpal	ST	∱ F		↑ F, HF, mH			+2; +1.5
Asymmetry			SW						
		knee	ST						
	Н		SW						
		tarsal	ST						
a • 1a	_		SW						
Swing/Stance	F								
time ratio	Н			V mF, mH		V mH			-0.2, -0.2
Step/Stride	F							↓ mF	-0.06
length ratio	H								
						Subjective detection	$on(^1)$		
	BK			1 F	1F, 2HF, 1mH	1F, 2HF,1mH	1F, 2HF, 1mF,1mH	1F, 2HF,1mF 1mH	
Conformation	PL			1 F,1HF	2F, 1HF	1F,1HF, 1NL	2F, 1mF, 1NL	1HF, 1mF, 1NL	_
Gait score	Stiffne	ess		1 F,1HF,1mH	1F,1HF,1NL	3F,1HF, 2mF,1NL	1F ,1HF, 1mF, 1NL	1F, 2HF	

Codes for joint lesions:

F=moderately/severely affected front leg pigs (N=6).

mF=mildly affected front leg pigs (N=6).

mH=mildly affected hind leg pigs (N=4).

HF=moderately affected front and hind leg pigs (N=4).

Codes for conformation: BK=buck-kneed front leg deficiency; PL=post-legged hind leg deficiency.

ST=stance; SW=swing; RoM=Range of motion.

Black symbols for significant differences $P \le 0.05$, greyed for tendencies (P > 0.05 but < 0.09).

All significant comparisons were against lesion-free pigs (NL=no lesions; N=4).

(¹)Section 'Subjective detection' shows when the pigs with clinical abnormalities emerged by capture.

For angles the unit is degrees, ratios are dimensionless; semicolons separate values for groups as appearing in rows from left to right.

Table 5.4: Changes in the step-to-stride length relationship associated with pigs categorised as being lesion-free or affected by osteochondrosis joint lesions. Data are medians (interquartile ranges)

The data encompass front and hind legs and all captures. Pigs were primarily classified as having joint lesions or not; for pigs with lesions, these animals were secondarily categorised into clinical and subclinical based on the presence or absence of at least one of the subjective conformation or lameness/gait deficiencies. Subclinical pigs had joint lesion(s), but none of the subjective conformation or lameness/ stiffness deficiencies. Pigs with no lesions were considered to be healthy pigs regarding joint health. Numbers of pigs in the subjective observation columns are out of a total of 24 subjects. Columns under "subjective observation" correspond to lesion groups, healthy and clinical pigs, but not the subclinical pigs. A single pig may have both deficiencies and/or stiffness.

	Sub	jective obse	ervation		Kinematics			
	Leg we	Leg weakness Gait			ength ratio deviation from $0.5 (x10^{-3})^{\ddagger}$			
Lesion groups	Buck- kneed	Post- legged	Stiffness	Healthy pigs (N=4)	"Subclinical" pigs (N=7)	"Clinical" pigs (N=13)		
No lesions (n=4)	0	1	1	11 (3.1)				
Mild front leg (n=6)	1	1	0		14 (8.4) (N=4)	15 () (N=2)		
Mild hind leg (n=4)	2	1	2		19 () (N=1)	19 (13.5) (N=3)		
Moderate-severe front-leg (n=6)	2	2	3		16 () (N=2)	15 (7.2) (N=4)		
Moderate front+hind leg (n=4)	4	2	1			19 (22.6) (N=4)		
Total	9	7	7	11 ^a	16^{b} P=0.047	16^{b} P=0.062		

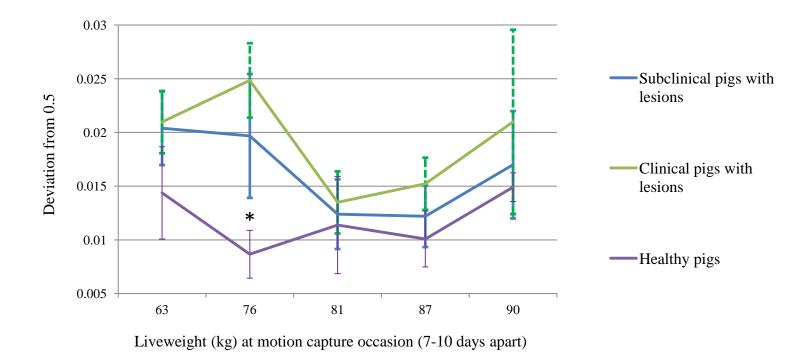
[‡]A ratio value of 0.5 would represent perfect symmetry between left and right step lengths. Deviations from this value denote increased stepping irregularity. Kruskal-Wallis and Mann-Whitney tests were used for statistical comparison.

Four subjects with mild (borderline) conformational deficiencies were included here (BK=2; PL=2) as clinical pigs to emphasise the detective power of the gait parameter.

Figure 5.1:

The step-to-stride length ratio deviation from 0.5 (maximal stepping symmetry) of pigs grouped according to their clinical status (presence of conformational deficiency and/or lameness/stiffness deficiency) and post-mortem presence of lesions of osteochondrosis after five motion captures from 63 kg to 90 kg liveweight. Error bars represent the standard error of the mean.

* denotes significant difference (P=0.018) between clinical and healthy pigs at the capture.



5.5 Discussion

The objective of this study was to determine whether there are detectable changes in joint and stride kinematics of clinically sound and unsound pigs, with or without post mortem joint lesions of osteochondrosis. Some gait variables could be the missing link between pigs with joint lesions, but without leg weakness/observable lameness and pigs without joint lesions, but with leg weakness/observable lameness. In the present study there was an insufficient number of lesion-free pigs with a clinical deficiency to investigate the latter scenario, yet there were differences in the gait of pigs from different clinical and subclinical categories. However, perhaps due to the dynamic nature of movement, the relatively low number of subjects and difficulties controlling experimental factors, some of these changes were intermittent in emergence across five different time points.

Ideally, the difference in a gait profile of a sub-clinical or clinical pig, both within and between measurement days, would be consistent over time (small within-pig variability), common among pigs with similar deficiencies (small within-group variability) and give a strong signal (magnitude of difference from normal pigs). In the present investigation, various sources of variability, such as walking speed, pig size differences (even though not significant) and methodological errors may have contributed to the variation in the results (Stavrakakis et al., 2014a; Chau et al., 2005). Nonetheless, despite all these sources of variability generally encountered in a clinical setting (Walker et al., 2010), the chosen gait parameters were successful in uncovering a pattern in a number of instances. For example, a functional deficiency, such as reduced stance flexion/extension at the elbow, could explain why buck-kneed pigs have an increased risk of developing joint disease or exacerbating lesions which are already present (Ratcliffe and Holt, 1997).

Overall, the level of clinical problems in this cohort of pigs was limited and, although many pigs showed some symptoms of being buck-kneed in the front legs as they grew heavier, most pigs had only mild versions of any deficiency. Nonetheless, at the second motion capture there was an abrupt rise in the presence of pigs with buck-kneed front legs. Some of the normal pigs also showed transient deficiencies (i.e. they returned to being scored as having good conformation) and some continued to have only mild versions after the second capture. Generally, some mild deviation from a normal,

slightly curved front and hind leg conformation (Jørgensen and Andersen, 2000a) was encountered in most pigs at some point.

Subjectively perceived abnormalities, such as conformational deficiency and subtle lameness, were mainly associated with differences in the ranges of motion during the swing phase, both at the elbow and the knee joints. It is perhaps not surprising that the difference in stiff pigs was discovered in the reduced elbow swing range of motion, as the latter would let a leg appear stiffer during locomotion (Bennet et al., 2012). It is interesting, however, that conformational deficiencies, thought to be the clinical expression of mainly joint disease, would affect swing rather than stance phases of the gait cycle. Nonetheless, Martens et al. (2008) also found altered swing phase motion in horses with conformational irregularities.

The swing range of motion at the knee joint tended to be smaller in pigs with buck-kneed front legs, but larger in pigs with hind leg deficiencies. Similarly, in some cases, pigs with front leg conformational deficiencies displayed greater asymmetry in the hind legs, and reciprocally for pigs with hind leg conformational deficiencies. These results suggest that compensation for a deficiency in one or more legs by the other legs could in some cases be more consistent and stronger in effect than the direct effect of the deficiency in the affected leg(s). This is in agreement with results from the gait analysis of pigs, rats and dogs with a locomotor impairment and substantial compensatory changes in unaffected legs (Pluym et al., 2013b; Bennet et al., 2012; Burton et al., 2008). Jørgensen (2000b) also found that osteochondrosis lesions in the front legs were associated with leg weakness and/or gait disorders in the hind legs and vice versa. This is an important point to consider, since consideration of only a single movement parameter could mask the true site of affliction. In addition, a suggested positive feature of joint movement, such as an increased range of motion at a particular joint (Brinkmann and Perry, 1985) could be misleading in the case of post-legged hind legs, as was found in the present study. This deficiency is characterised by an excessive straightness and, being a leg weakness trait, may be associated with underlying osteochondrosis (Nakano et al., 1987). Yet, Bockstahler et al. (2007) and Brady et al. (2013) also reported increased ranges of motion at the knee and hip joints for dogs at greater risk of orthopaedic disease and hence, the interpretation of a gait parameter value may depend on the specific condition in question.

Osteochondral joint lesions were more related to gait parameters during the stance phase of the gait cycle (strides) and most angular differences occurred in the pigs with moderate front and hind leg lesions. This indicates that more generalised conditions and/or the involvement of hind legs may cause more mechanical disturbance and gait alterations than even advanced lesions in one pair of legs and/or front legs only. This is in contrast to Jørgensen and Andersen (2000a) who found no clear correlations between the level of osteochondrosis in the tarsal joint, i.e. the hind leg, and clinical symptoms of leg weakness. Here, pigs with moderately affected front and hind legs had mainly tarsal lesions and produced detectable kinematic symptoms. Also, the significant stance phase variable involvement in pigs with osteochondrosis lesions is consistent with the expectation that a joint disease would affect the weight-bearing phase of the gait cycle (Khumsap et al., 2004). However, if the swing phase/stance phase differentiation between leg weakness/stiffness traits and osteochondrosis can be confirmed in future studies, then this could mean that conformational deficiencies arise from muscusloskeletal structures other than articular cartilage and are therefore not a good clinical trait upon which to make judgments about the level of joint pathology. Jørgensen and Andersen (2000a) found that while the level of osteochondrosis was unfavourably genetically correlated with daily weight gain in pigs, leg weakness traits were unfavourably correlated with lean meat percentage. This finding, in combination with the kinematic insight provided by this study, could point towards a muscular involvement in leg weakness and warrants further biomechanical research in this area.

Although the prevalence of osteochondrosis lesions was higher compared to the level of clinical deficiencies, i.e. 83% of the pigs had at least one minor lesion in one of 16 inspected joints, the severity of lesions was mostly mild. These findings are consistent with previous studies conducted by Jørgensen et al. (1995) and Jørgensen and Andersen (2000) who reported high prevalences of mild osteochondral lesions, which were poorly correlated to clinical leg weakness symptoms. In the current study, seven pigs (29%) without any conformational deficiency or stiffness had OC lesions in one or more joints. It is worthwhile mentioning that two of the most affected pigs were amongst those classed as being normal and thus clinically inconspicuous. This could further explain the transient nature of some kinematic differences among the pigs with subjectively perceived abnormalities, since these pigs were compared against pigs without clinical abnormalities, but with joint lesions potentially causing increased gait variability at different time points. It is challenging to obtain a consistent gait analysis

for pigs with osteochondrosis and other clinical abnormalities, when there are overlaps between them. For instance, pigs with conformational deficiencies can have other musculoskeletal issues such as osteochondrosis, and some pigs with osteochondrosis have a conformational deficiency, whilst others with osteochondrosis have no conformational deficiencies. These musculoskeletal conditions may all affect gait differently and while doing so, they may interfere with each other when evaluated at a category group level comprising pigs with not only a single condition. Assessing gait data of pigs individually and comparing these only to themselves over time, individual comparison against a 'normal' benchmark, or strictly matching the groups according to singular pathology/deficiency would facilitate detecting specific changes (Walker et al., 2010; Schoellhorn et al., 2002). Nonetheless, data from this current study and future studies can help to develop normative value ranges against which potentially abnormal gait data may be compared (Beynon et al., 2010). This will promote the ability to differentiate between expected values for key gait parameters and potential outliers. Due to a limited number of subjects and lesion categories, a threshold for subject classification could not be determined in the present study. However, future studies can select one of the identified sensitive gait parameters and investigate this parameter across a greater range of animals with varying levels of the same locomotor problem (Van Nuffel et al., 2009).

The step-to-stride ratio is proposed as one of the most sensitive pooled parameters, when many observations can be collected for an individual pig or a group of pigs and specific leg or joint involvement is not of interest. The step-to-stride length ratio (spatial gait measure) is the only parameter, among those considered in this study, which can be used as a collective outcome measure for different deficiencies and joint lesions. Angular measures should be considered by joint level for subjects grouped according to a specific lesion/deficiency related to a certain joint level, if possible. The only other parameter, the swing/stance time ratio (temporal gait measure) which could have also been used as a collective non-leg specific measure, varies more with walking speed compared to the step-to-stride length ratio, as was found in Stavrakakis et al. (2014b). The step-to-stride length ratio, in addition to subjective observation could increase the chance to detect pigs with osteochondrosis. Further, angular patterns might be specifically helpful in identifying the affected site in a pig, especially when complete joint flexion curves can be plotted and evaluated. The consideration of stance phase joint flexion variables could help detect pigs with osteochondrosis without scoring for

leg weakness, and/or help distinguish pigs with leg weakness and osteochondrosis lesions from those pigs with symptoms but without any lesions.

In conclusion, this study showed that monitoring of angular patterns, left and right leg movement symmetry and the step-to-stride length ratio may detect pigs with abnormalities, which previously were not apparent by visual observation. Whereas the presence of osteochondrosis lesions affected the stance phase of the gait cycle, conformational deficiencies mainly affected the swing phase and therefore conformational deficiencies may not be a good clinical trait to judge levels of joint pathology. For the purposes of selecting replacement breeding animals, gait measurements may be more successful than subjective classification in targeting longevity characteristics of pigs and preventing lameness. Yet, the importance of subclinical lesions of osteochondrosis and conformational deficiencies with respect to longevity has to be further elucidated. Biomechanical evaluation of pigs is still a very complex process that requires some simplification before it could be used at farm level. If they are included in studies evaluating factors associated with lameness in pigs, quantitative gait variables may help eliminate some of the variation seen in results based solely on subjective assessment of locomotion.

Chapter 6. Preclinical and Clinical Walking Kinematics in Female Breeding Pigs with Lameness – a Nested Case-Control Cohort Study

6.1 Abstract

Lameness is a major threat to welfare and longevity in sow herds. The objective of this study was to retrospectively investigate the gait profiles of a cohort of female pigs, in which N=24 developed lameness over a 17 month time in period. Furthermore, quantitative gait alterations during lameness were evaluated to identify the best quantitative lameness indicators. Pre-breeding gilts (N=84) were recruited over a period of six months and were subject to gait analysis. While younger gilts, starting from mean body weight 39 kg, could have a number of motion captures before entering the breeding herd, the oldest animals underwent just one motion capture. Gilts and sows were motion-captured again at eight weeks into pregnancy and on the day of weaning. During kinematic capture the pigs walked on the same concrete walkway and 3D coordinate data of reflective skin markers attached to leg anatomical landmarks were collected. Some 19 pigs which developed lameness were identified, and gait data from their earliest 1 or 2 motion captures 1-11 months prior to lameness occurring, and the day of lameness, were analysed. These pigs were compared to groups of sound control pigs of a similar age, body size and production stage for every point of comparison. Lameness detection and evaluation of the causal site were possible using spatial and temporal gait parameters, especially vertical head displacement (elevated in front and hind leg lameness; +20-38 mm, P≤0.050) and lateral pelvic displacement (elevated in multi-leg lameness; +10 mm, P≤0.050). Asymmetry in stride timing and the ipsilateral swing-to-stance time ratio were elevated in hind leg lameness ($P \le 0.050$). Step width, claw lift during swing (step height), stride length and walking speed were not different between lame and sound pigs, although, these have previously been suggested as reliable lameness indicators. The step-to-stride length ratio deviation from 0.5 (perfect symmetry) was elevated ($P \le 0.050$) in pigs with front and hind leg lameness and in young pigs who presented lameness later in their life (deviation ≥ 0.030). The sensitivity of this measure ranged from 50-75% and the specificity from 58-90%, depending on whether front and hind leg measures were pooled or considered separately and whether observations were separated according to pig age and capture experience. It was concluded that head and trunk movement and leg stance and swing asymmetry are

reliable lameness indicators. Lameness risk prediction in pigs appearing visually normal was possible using the simple and dimensionless step-to-stride length ratio.

6.2 Introduction

Lameness can pose serious problems impacting the welfare, health and production economics of sow herds (Willgert et al., 2014; Heinonen et al., 2013, Kroneman et al., 1993). The reported worldwide prevalence of lameness among gilts and sows generally varies from 5-20%, depending on the assessment method, production system and pig breed (Nalon et al., 2013a; Pluym et al., 2011, KilBride et al., 2009).

Lameness is a clinical symptom common to various structural and functional conditions and is defined by observable changes in gait (Weishaupt, 2008). Causes may be of a genetic, infectious or physical nature (Jensen et al., 2009). Whilst in young growing pigs degenerative joint disease and associated leg weakness is the predominant cause of lameness, in sows secondary degenerative changes and infectious arthritis are the most common problems (Kirk et al., 2005; Dewey et al., 1993). Group housing systems for female breeding pigs, which became mandatory in Europe in 2013, are likely to increase the prevalence of lameness due to the high demands on the locomotory system (Spoolder et al., 2009; Kroneman et al., 1993). However, while the prevalence of claw lesions has been reported to be 50-100% in group-housed gilts/sows (van Riet et al., 2013; Nalon et al., 2013a; Pluym et al., 2011), their presence does not sufficiently explain the observed lameness (Grégoire et al., 2013). Therefore injury or latent musculoskeletal weakness (such as degenerative joint disease) may also play a role in the acute emergence of lameness during group housing.

Subjective gait/locomotion scoring protocols have been developed and are currently the only on-farm tool available for the quantification of lameness (De Koning et al., 2012; Main et al., 2000). However, subjective lameness scoring is prone to bias and shows low to moderate repeatability across many animal species including pigs (Dalmau et al., 2010; Waxman et al., 2008; Keegan, 2007; Petersen et al., 2004). Subtle lameness is very difficult to detect and evaluate (D'Eath et al., 2012) and it is costly and time-consuming to observe individual pigs on farms (Nalon et al., 2013a). In fact, recent research has suggested that in order to obtain accurate lameness prevalence estimates for a farm, the entire population of animals would have to be examined (Mullan et al., 2009).

Identification of sensitive, accurate and objective clinical biomechanical parameters of locomotion could provide a superior means of identifying animals with lameness (Karriker et al., 2013; Sun et al., 2011). In addition, such parameters may improve our understanding of the pathomechanics of the musculoskeletal system and may facilitate identification of those animals with abnormalities which were previously invisible to the observer. Although the human perception is capable of integrating complex and multidimensional information simultaneously (Holcome, 2009), this skill comes at the expense of temporal and spatial resolution. Therefore it is important to develop accurate, quantitative methods and identify individual gait parameters more sensitive to subtle lameness problems (Grégoire et al., 2013). Evidence-based veterinary practice and large-scale modern livestock farms could benefit from the development and implementation of automated and continuous on-farm lameness monitoring systems (Cornou et al., 2008; Keegan 2007). Further benefits could arise from the development of additional tools for the selection of superior breeding animals.

There were two objectives in this study: (1) to determine current movement changes in gilts/sows with clinical lameness, based on an analytical biomechanical method, compared to those animals with clinically sound locomotion; (2) To investigate historical movement differences between juvenile pigs which subsequently developed advanced lameness and those that showed no lameness throughout the entire study period. There were three hypotheses: (1) there are similar changes in the kinematics of lame pigs, regardless of which leg(s) showed lameness; (2) simultaneous consideration of two or more quantitative gait variables can indicate the site of lameness; (3) early gait records of pigs developing lameness at a later time point can be differentiated from consistently sound control pigs. It was assumed that the majority of the diagnosed lameness was due to chronic and latent abnormalities and not due to acute injuries or infections in an otherwise healthy musculoskeletal system. The repeated occurrence of lameness in some of the animals and the high prevalence among the maturing gilts further supported this hypothesis.

6.3 Materials and Methods

6.3.1 Animals, management and experimental housing

All procedures on animals were in accordance with institution guidelines and UK animal welfare regulations. The experimental cohort consisted of 84 female replacement

gilts produced from the existing sow herd at the Newcastle University pig unit. A Large White x Landrace criss-cross breeding programme involved insemination with either Large White grandparent semen for Landrace-sows, or Landrace grandparent semen for Large White-sows. Recruitment of gilts to the study was gradual over a period lasting from January 2012 to July 2012 and all gilts from the grower stage to the first mating stage which were in the system during this period were included. The experimental population increased typically by five animals every three weeks, reflecting the threeweek batch rotation system used for sow management on the unit. The youngest gilts were 39kg (Standard Deviation (SD) 3.8) and the oldest 146kg (SD 13) at point of entry to the study. The oldest animals underwent just one data collection before they entered the commercial breeding herd. The pre-breeding gilts were housed on a solid concrete floor, typically in groups of five, in 9.6 m² pens in a separate building located 200m away from the main pig unit. The gilts had free access to concentrate feed and water. When considered ready for first service, at typically 220 \pm 10 days, they entered the breeding herd on the main unit. Gilts and sows in the breeding herd were managed in a straw-based, kenneled group-housing system with individual feeding stalls and groups consisting of the same five animals housed together pre-breeding. Upon entrance to the breeding herd, feeding was adapted to suit the stage of production according to standard farm protocols.

The motion capture facilities were located beside the gilt housing facilities and pigs could be moved along a walkway between the two buildings. Once in the breeding herd, transport of the pigs to the motion capture facility was achieved with a tractor-drawn trailer. Motion captures were applied to the same pigs at regular strategic intervals, i.e. initially every five weeks to build a database on gait development during gilt growth from grower stage (typically 40 kg or 4 months of age) to reproductive maturity (typically 140 kg or 8 months of age). Subsequently, each pig underwent one motion capture during mid-gestation (typically 8 weeks after insemination) and one on the day of weaning after 28 days of lactation. The study was terminated in July 2013 at which point the 15 oldest sows had given birth to and weaned their third litter.

6.3.2 Data collection

Pigs were initially habituated to close human contact/handling and learned to follow a human operator to obtain a treat (a small piece of apple) when movement was

acceptable (i.e. regular, continuous and straight). Then kinematic motion capture was applied in a group rotation fashion, always working from the oldest to the youngest group of gilts. Motion capture cycles were repeated such that the same pigs were typically captured again every five weeks. After every cycle of motion capture, typically 5-10 of the oldest gilts were transferred to the breeding herd on the main unit. A kinematic reflective marker model (described and validated in Stavrakakis et al., 2014a) was applied to the pigs over key anatomical landmarks and captured with a Vicon T20 (Oxford, UK) six-camera stereo-photogrammetric (3D) system providing full body kinematics of one body side at a time. The motion capture procedure was identical to that described in Stavrakakis et al. (2014b), with the exception that in the current experiment the pigs were not trained to follow a target. Instead, they followed a pan containing small apple pieces, and the pan was attached to a long handle convenient to be held by a human guide without the necessity to stoop.

In total, 29% (24/84) of the females developed spontaneous (i.e. not experimentally induced) clinical lameness, as described below. The condition occurred either at some point during the maturation period before they entered the breeding herd (17%) or in the breeding herd, typically during gestation housing (11%). One animal developed lameness during its time in the farrowing crate and was motion-captured on the day of weaning. Lameness was clinically diagnosed on the day of capture using a subjective scale from [0] to [5] (where [0]=normal, [1]=stiffness, [2]= reduced weight-bearing, [3]=minimal weight bearing on affected limb(s); adapted from Main et al., 2000). Animals showing grade [4]=pig may not place affected limb on the floor while moving and [5] =animal does not move, were not subjected to motion capture. A tentative diagnosis of the cause of lameness was made, by carefully examining the lame leg(s) and claws. The walls and whenever possible lower surface of the claws were inspected for lesions, such as cracks, haemorrhages, erosions and abscesses. Pressure tests including palpation on all joints in the affected leg(s) were made. Additionally, after each motion capture anatomical conformation of the musculoskeletal system and leg weakness symptoms were assessed with a qualitative description (adapted from Jørgensen and Andersen, 2000) and assigned a severity attribute (mild, moderate, severe). The considered traits are summarised in Table 3.1 in this thesis. A general impression of claw health, shape and size was recorded. Following motion capture and diagnosis of lameness, animals were treated with anti-inflammatory medication according to a standard farm protocol.

6.3.3 Data processing

Of the 24 pigs with clinical lameness, 19 had previous records of normal gait (as defined by absence of visual detection of gait abnormality) and 18 had a successful motion capture on the day of lameness. N=3 pigs were already lame at the first capture and N=2 had no usable early motion capture data. These 19 animals were included in this study to investigate whether there were predictive features present in their gait prior to lameness, when compared to sound matching control pigs of a similar age/size. Early gait records of the pigs developing lameness were separated into two size/weight groups, namely 63 kg median body weight (BW) gilts (range 45-77 kg; N=13) and 97 kg median BW gilts (range 84-123; N=11). This approach enabled the recruitment of at least ten subjects per size group, with the same individuals counted only once within each size group. Accordingly, gait data from matching normal control pigs from a similar body size and age/weight category with no perceived gait abnormality were used for comparison. Pigs developing lameness appeared only once in a particular body size category but, due to a second record prior to lameness, five pigs appeared in both body size groups. The gait data of the 18 gilts/sows with lameness were grouped according to site of lameness and compared to groups of normal pigs at an equivalent production stage, body weight and leg size. The 18 pigs with motion data during clinical lameness provided a dataset which was used to identify the most characteristic movement changes in pigs with acute lameness. Coordinate data was exported from motion capture software (Vicon Nexus v1.7.1, Oxford, UK) and imported into Matlab (R2010b, Mathworks©, Natick, USA) where data was processed by a custom-written programme. Stride event detection, gait parameter calculation and time normalisation of joint flexion curves was performed as described in Stavrakakis et al. (2014b).

6.3.4 Data analysis

Angular walking kinematics considered here were peak knee, elbow, carpal and tarsal flexion angles during stance and swing phases and the asymmetry of left and right body sides with regards to a particular equivalent joint flexion value. The four individual flexion values per joint were summarised to yield an overall stride flexion and asymmetry value. Temporal walking kinematics considered were stance times, swing times and duty factors (percentage stance time within stride) of individual legs and the swing/stance ratio of ipsilateral legs of affected and unaffected sides. Calculated spatial

walking kinematics included the step- and stride lengths and ratios of pairs of legs, the vertical displacement of the head and spine, the lateral displacement of the tuber coxae, the step height, step width and walking speed. Within-subject means were created for every gait parameter and capture and included left and right body sides (i.e. total session means). Front and hind leg gait parameters were analysed separately, except the stride lengths. For gait parameters for which asymmetry may increase in the presence of lameness, either the difference between left and right body side measures or the SD within session, were considered in addition to, or instead of, the mean. Using a threshold value for the step-to-stride length ratio (of 0.030 deviation from the perfect symmetry of 0.5, as derived from two significant findings and empirical observation of the data), observations of individuals were classified as abnormal/normal and with/without future lameness. Odds ratios, sensitivity and specificity of future lameness detection were then determined using this approach. To exclude factors which could cause gait differences other than the studied factors, all compared groups were checked for absence of differences in size (weight, leg length) and walking speed. Continuous data were checked for normality using Anderson-Darling tests. Departures from normality entailed use of equivalent non-parametric tests (Mann-Whitney tests). Significance was accepted at $P \le 0.050$.

6.4 Results

Body weight, leg length and walking speed were not significantly different between pigs with front and hind leg lameness and between pigs with multi-leg lameness/unclear leg involvement and their respective controls. Similarly, early gait records of pigs developing lameness were matching those of control pigs with respect to the above possible confounding factors. The following comparisons may therefore be based on the assumption that there is a unique effect of lameness/developing disease.

Lameness epidemiology. Lameness period prevalence over 17 months was 29%; however, lameness period incidence was 39%, since several animals were repeatedly diagnosed with lameness. Of the 24 clinically lame pigs, 14 were pre-breeding gilts, 9 were pregnant pigs of which 3 were pregnant with their second litter and one pig was weaned from its first litter on the day of its affected motion capture. Grade [2] lameness was observed in 13, grade [3] in 9 animals and grade [4] in 2 animals out of a total of 24 lame pigs. Proportionally, lameness severity was similar between pre-breeding and breeding animals. Clinical diagnosis of the lame leg(s) was possible in all, except five

cases, where either multiple leg involvement was observed or the site could not be clearly identified. Except in one pig, for which the tentative diagnosis of lameness was a lateral claw wall separation, in all other cases lameness appeared to originate more proximally. However, due to absence of any obvious swelling, redness and heat, identification of affected joints was not possible. Often, the pigs repeatedly withdrew the leg upon pressure on more than one joint. Some of the lame pigs showed signs of muscular weakness during motion captures, as indicated by regional muscle tremor and/or slight splaying (drifting apart) of the legs while standing. The latter would suggest a degree of muscular involvement in the observed lameness.

Angular kinematics. Table 6.1 shows joint angle differences detected in pigs with lameness compared to normal control pigs. Knee flexion was increased in pigs with hind leg lameness and lameness of uncertain origin (+3-4 degrees; $P \le 0.050$). The latter pigs also showed a tendency for increased knee joint angle asymmetry between left and right body sides (3 degrees).

Temporal kinematics. Generally, the difference between left and right claw temporal kinematics, i.e. stance time, swing time and duty factor (percentage stance time within stride) within a pair of legs, was greater for lame pigs than for controls (Table 6.2). Pigs lame in a front leg had accordingly increased front leg pair asymmetry, but no hind leg asymmetry, while pigs lame in a hind leg displayed asymmetry in both leg pairs. Pigs with unclear/multi-leg lameness had greater hind leg asymmetry and a tendency for front leg asymmetry, suggesting that these pigs were either more affected in hind legs or that multi-leg lameness causes greater mechanical disturbance in the hind legs than in the front legs. Overall, absolute stance time was not increased in the lame pigs (affected and unaffected limbs or both), except for the pigs with hind leg lameness, where this parameter was increased in the hind legs only (Table 6.3).

Spatial kinematics. The median vertical head displacement within stride was increased (+20-38 mm) in all lame pigs, but the most substantial increase was observed in pigs with front leg lameness (Figure 6.1). While all pigs with front leg lameness had a displacement greater than 54 mm, 67% of all lame pigs passed this threshold and therefore it is not only indicative of front leg lameness. Spine vertical displacement was increased in both front and hind leg lameness, however the increase was very small (+6-7 mm). Pigs with unidentified/multi-leg lameness presented greater lateral pelvic displacement (+10 mm). While 80% of all pigs with unidentified/unclear lameness showed a lateral pelvic displacement greater than or equal to 41 mm, 17% and 29% of the pigs lame in front and hind legs, respectively, and 18-29% of the normal control

pigs also crossed this threshold. Step width variability was increased in some cases, but the actual step width magnitude was not consistently affected (Table 6.4). Stride length was not different in lame pigs, but the step-to-stride length ratio was affected in all pigs with single-leg lameness.

Future lameness detection in early early gait records. The step-to-stride length ratio deviation from 0.5 (perfect symmetry) was ≥ 0.030 in both early data sets of the pigs which developed lameness 1-11 months later (Table 6.5). This deviation was greater by 0.007 (early group comparison) to 0.010 (later group comparison) compared to the control pigs. Odds ratios, sensitivity and specificity of the measure are displayed in Table 6.6.

Table 6.7 and 6.8 show differences in angular and trunk movement parameters in the early data sets of preclinical pigs.

Table 6.1: Joint flexion in lame pigs: Descriptive statistics of the walking gait of pigs with clinical lameness (reduced or minimal weight bearing) categorised as being either in front leg, hind leg or multi-leg/unclear compared to normal control pigs. Medians are displayed.

			Lameness		Control pigs			
		Front	Hind	Multi-leg				
		leg	leg	or unclear	Gilts	Gilts	Pregnant gilts	
	Gait parameter:	Gilts 140 kg	Gilts 137 kg	Pregnant gilts	94 kg	138 kg	206 kg	
Joint	(degrees flexion)	(N=6)	(N=7)	210 kg (N=5)	(N=11)	(N=17)	(N=16)	
Knee joint	Asymmetry [‡] Flexion [†]	6 ^{ab} 66 ^{abc}	5 ^{ab} 71 ^a	6 ^a 69 ^a	4 ^{(a)b} 65 ^{bc}	$4^{(a)b}$ 68^{b}	3 ^{(a)b} 65 ^{(b)c}	
Elbow	Asymmetry	4^{a}	4^{a}	3^{a}	5 ^a	5 ^a	5 ^a	
joint	Flexion	71 ^a	71 ^a	68 ^{ab}	71 ^a	68 ^{a(b)}	66 ^b	
Carpal	Asymmetry	1^a	1 ^a	1 ^a	1^a	1^a	1^a	
joint	Flexion	8^{a}	9^{ab}	9 ^{ab}	10^{b}	$10^{(a)b}$	10^{ab}	
Tarsal	Asymmetry	7 ^{a(c)}	8^{a}	2^{b}	3^{bc}	5 ^{ac}	3^{bc}	
joint	Flexion	33 ^a	36 ^a	35 ^a	34^a	34 ^a	33 ^a	

^{*}Mean asymmetry between left and right body sides for flexion extremes (minimum and maximum joint flexion) during stance and swing phases of the gait cycle.

[†]Mean of flexion extremes during stance and swing phases of the gait cycle including left and right body side.

abc Medians within a row not sharing superscripts differ significantly at $P \le 0.05$. Parentheses around superscripts denote tendencies when $P \ge 0.050$, but ≤ 0.090 . Mann Whitney tests were used.

Table 6.2: Temporal gait parameters in lame pigs:

Descriptive statistics of the walking gait of pigs with clinical lameness (reduced or minimal weight bearing) categorised as being either in front leg, hind leg or multi-leg/unclear compared to normal control pigs. Medians are displayed.

•	_		Lameness			Control pigs			
		Front leg	Hind leg	Multi-leg/unclear	Gilts	Gilts	Pregnant gilts		
	Gait parameter:	Gilts 140 kg	Gilts 137 kg	Pregnant gilts	94 kg	138 kg	206 kg		
Legs	Asymmetry‡	(N=6)	(N=7)	210 kg (N=5)	(N=11)	(N=17)	(N=16)		
Front	Stance time (s)	0.035^{a}	0.048^{a}	0.023^{ab}	0.025^{b}	0.014^{b}	0.017^{b}		
	Swing time (s)	0.044^{a}	$0.047^{a(b)}$	0.055^{a}	0.018^{bc}	0.010^{c}	0.016^{c}		
	Duty factor (%)	4.9^{a}	4.8^{a}	3.1 ^{ab}	2.3 ^{bc}	$1.3^{(b)c}$	1.4 ^{(b)c}		
Hind	Stance time (s)	0.016^{b}	0.059^{a}	0.071^{a}	0.013^{b}	0.017^{b}	0.021^{b}		
	Swing time (s)	0.029^{ac}	$0.071^{\rm b}$	0.044^{ab}	0.010^{c}	0.018^{c}	0.018^{c}		
	Duty factor (%)	2.1 ^{ac}	6.8 ^b	4.6^{ab}	1.5°	2.1°	1.8°		
Ipsilateral	Swing/stance time ratio	0.21 ^{ab}	0.30 ^(a)	0.09^{bc}	0.04 ^c	0.08 ^{(b)c}	0.03°		

[‡]Asymmetry as defined by the difference between the same parameter measured on left and right body sides.

**abc* Medians within a row not sharing superscripts differ significantly at $P \le 0.05$. Parentheses around superscripts denote tendencies when $P \ge 0.050$, but ≤0.090. Mann Whitney tests were used.

Table 6.3: Temporal parameters – absolute values:

Descriptive statistics of the walking gait of pigs with clinical lameness (reduced or minimal weight bearing) categorised as being either in front leg, hind leg or multi-leg/unclear compared to normal control pigs. Medians are displayed.

				La	meness					
		Front	Hind	Multi-leg or		None (control	pigs)			
Leg	Gait parameter	leg (N=6)	leg (N=7)	unclear (N=5)	94 kg gilts	138 kg gilts	206 kg gilts			
Front sound leg(s)	Stance time (s) Swing time (s) Duty factor (%)	0.56 0.35 60	0.53 0.40 56	0.59 0.33 ^a 62 ^a	0.57 0.40 58	0.52 ^a 0.37 56	0.59 ^b 0.40 ^b 59 ^b			
Front lame leg(s)	Stance time (s) Swing time (s) Duty factor (%)	0.51 0.40 55	- - -	0.58 0.40 59			- - -			
Hind sound leg(s)	Stance time (s) Swing time (s) Duty factor (%)	0.47 0.41 53	0.56 ^a 0.37 60 ^a	0.60 0.40 59 ^a	0.51 0.43 54	0.45 ^b 0.41 53 ^b	0.54 0.42 55 ^b			
Hind lame leg(s)	Stance time (s) Swing time (s) Duty factor (%)	- - -	0.50 0.43 52	0.50 0.44 56			- - -			

abc Medians of matching groups not sharing superscripts differ significantly at P≤0.05. Mann-Whitney tests were used.

Table 6.4: Spatial gait parameters in lame pigs:

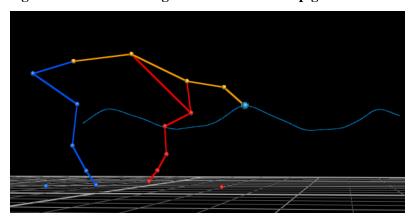
Descriptive statistics of the walking gait of pigs with clinical lameness (reduced or minimal weight bearing) categorised as being either in front leg, hind leg or multi-leg/unclear compared to normal control pigs. Medians are displayed.

	ier in nom reg, in	na reg or ma	<u> </u>	Lameness	ormar control pigs.		Control pig	gs
Body part	Gait parameter: displacement (mm)	Gait phase	Front leg Gilts 140 kg (N=6)	Hind leg Gilts 137 kg (N=7)	Multi-leg or unclear Pregnant gilts 210 kg (N=5)	Gilts 94 kg (N=11)	Gilts 138 kg (N=17)	Pregnant gilts 206 kg (N=16)
Head	Vertical	Stance Swing	$70^{a} \\ 70^{a}$	52 ^a 51 ^a	65 ^{a(c)} 49 ^{ab}	31 ^b 30 ^{bc}	32 ^b 30 ^{(b)c}	38 ^c 34 ^{bc}
Back	Vertical	Stance Swing	22 ^a 25 ^a	24 ^{ab} 24 ^{ac}	18^{ab} 19^{ab}	19 ^{(a)b} 18 ^b	21 ^{ab} 18 ^b	20 ^{ab} 19 ^{bc}
Pelvis	Lateral	Stance Swing	$31^{ad} \\ 36^{ab(e)}$	38 ^{a(bd)} 41 ^{a(b)e}	42 ^b 39 ^{abe}	30 ^{cd} 31 ^{bc}	29 ^{ac} 39 ^{acd}	32 ^{ac} 41 ^{de}
All legs	Stride length (Walking speed	(mm/s))	750 ^{abc} 890 ^{ab}	801 ^{abcd} 837 ^{ab}	776 ^{abcd} 836 ^{ab}	778 ^b 849 ^a	816 ^c 942 ^b	872 ^d 935 ^{ab}
Front legs	Step height Step width Step width SD		54 ^{abc} 147 ^{abd} 27 ^a	60^{ac} 162^{bd} 28^{a}	$78^{(a)b} 156^{bd} 30^{a}$	50° 117° 25°	58 ^{abc} 130 ^{a(b)c} 27 ^a	64^{ab} 163^{d} 25^{a}
Hind legs	Step height Step width Step width SD		61^{abd} 128^{ad} 41^{ab}	$62^{ad} \\ 123^{ac(d)} \\ 41^{a}$	70 ^{(a)be} 155 ^{bd} 30 ^{bc}	48 ^c 103 ^{(a)c} 22 ^{(c)d}	$54^{(c)d}$ $116^{ac(d)}$ 25^{cd}	65 ^e 144 ^d 26 ^{(b)d}
Front pair	Step/stride lendeviation from (0.067 ^a	0.028 ^(ab)	$0.021^{a(b)c}$	0.020^{b}	0.018^{b}	0.021 ^{bc}
Hind pair	Step/stride lendeviation from (0.034 ^{a(c)}	0.042 ^{ac}	0.032 ^{ab}	0.024 ^{bc}	0.019 ^b	0.25 ^b
Both pairs	Step/stride lendeviation from (0.049 ^a	0.041 ^a	0.026^{ab}	0.023 ^b	0.019 ^b	0.25 ^b

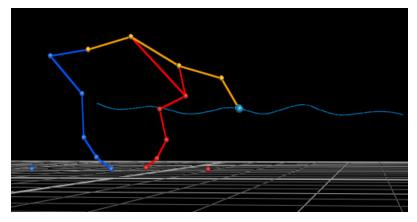
pairs deviation from 0.5

abc Medians within a row not sharing superscripts differ significantly at P \leq 0.05. Parentheses around superscripts denote tendencies when P \geq 0.050, but ≤0.090. Mann Whitney tests were used.

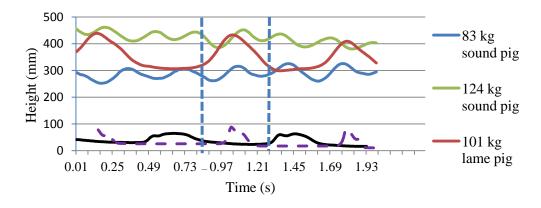
Figure 6.1: Head bobbing in lame and normal pigs



(a) Pig lame in the left front (LF) leg with a significant head bob. The head is lifted at the instant of impact of the lame limb.



(b) Normal pig with regular head movement.



(c) Vertical head movement in a lame and two normal pigs of different size. The lower solid and dashed lines are the vertical trajectories of lame and sound front claws of the lame pig, respectively. The vertical dashed lines enclose a plateau representing the stance phase of the affected limb and the corresponding head displacement phase above (solid red line). Also note how the swing phases (peaks of the lower trajectories) of the two legs are different, i.e. the sound dashed trajectory has a much sharper peak and mimics the trajectory of the head.

Table 6.5: Future lameness detection – the step-to-stride length ratio:

Irregularity in the step-to-stride length ratio, measured as deviation from 0.5 (perfect symmetry) in early gait records of pigs presenting lameness 1-11 months later. All pigs were clinically classed as showing no lameness/good locomotion on days of capture; control pigs additionally never developed lameness during the 17months follow-up period. Normal control pigs were matched to size. Medians are displayed. Older normal control pigs are added to demonstrate time effect on the ratio.

	Pigs develop	ing lameness		Normal control pigs				
	63 kg gilts	97 kg gilts	60 kg gilts	96 kg gilts	130 kg gilts	206 kg pregnant gilts		
Legs	(N=13)	(N=11)	(N=11)	(N=10)	(N=13)	(N=10)		
Front legs	0.028 ^{ab}	0.030 ^{a(b)}	0.026^{bc}	$0.020^{\rm c}$	0.018 ^c	0.021 ^c		
Hind legs	0.029^{a}	0.026^{a}	0.023^{a}	0.024^{ab}	0.019 ^b	0.025 ^{ab}		
Both legs	0.031 ^a	0.028^{ab}	$0.024^{bc(d)}$	0.023 ^{(b)cd}	0.019^{d}	0.025 ^{bd}		

^{abc}Medians within a row not sharing superscripts differ significantly at P≤0.05.

Parentheses around superscripts denote tendencies when $P \ge 0.050$, but ≤ 0.098 . Mann-Whitney tests were used.

Table 6.6: The step-to-stride length ratio as a diagnostic measure of future lameness:

Using a threshold value of 0.030 and considering lower values as normal, the step-to-stride length ratio deviation from 0.5 (perfect symmetry) provided the following diagnostic value:

	60-63 kg gilts Mean of front and hind leg deviation	96-97 kg gilts Front leg deviation only	60-97 kg gilts ^a Mean of front and hind leg deviation	60-97 kg gilts ^b Front & hind leg deviation
Odds Ratio [‡]	7.20	10.80	1.60	4.09
Sensitivity %	62	55	50	75
Specificity %	82	90	62	58

a, b This included all gilts at all time points available; the value of the measure in a pooled scenario is herewith demonstrated. Mean of front and hind leg deviation means that both together can be below 0.030, even if one was originally higher.

^b Both front and hind ratio deviations had to be below 0.030 for a pig to be considered normal.

[‡]The odd's ratio is a measure of strength of association and is a ratio of ratios, i.e. "condition positive & test positive divided by condition negative & test positive" divided by "condition positive & test negative divided by condition negative & test negative".

Table 6.7: Future lameness detection – angular gait parameters:

	Gait	Pigs develop	ing lameness	Normal co	ontrol pigs
	parameter	63 kg gilts	97 kg gilts	60 kg gilts	96 kg gilts
Joint	(deg)	(N=13)	(N=11)	(N=11)	(N=10)
	Flexion	66	62	68	65
Knee	Asymmetry	6	4	4	4
	RoM stance	25	26^{a}	26	$30^{\rm b}$
	RoM swing	46	47	47	46
	Flexion	72	75	71	72
Elbow	Asymmetry	4	5	8	5
	RoM stance	19	17	22	20
	RoM swing	39 ^a	35 ^(c)	43 ^b	39 ^(c)
	Flexion	9.1	9.4	9.3	10.5
Carpal	Asymmetry	1.4	1.3	1.4	1.3
-	RoM stance	1.5	1.6	1.9	1.6
	RoM swing	14.3 ^a	13.6	16.2 ^b	15.1
	Flexion	35	31	34	33
Tarsal	Asymmetry	5 ^a	4	3 ^b	3
	RoM stance	33	31 ^a	30	36 ^b
	RoM swing	38	40	41	43

^{abc}Medians of matching groups not sharing superscripts differ significantly at $P \le 0.05$. Parentheses around superscripts denote tendencies when $P \ge 0.050$, but ≤ 0.062 . Absence of superscripts indicates no significance. Mann-Whitney tests were used. RoM=Range of motion.

Table 6.8: Future lameness detection – head and trunk movement:

	_	Pigs developii	ng lameness	Normal co	ontrol pigs
Region	Gait parameter: displacement (mm)	63 kg gilts (N=13)	97 kg gilts (N=11)	60 kg gilts (N=11)	96 kg gilts (N=10)
Head	Vertical: Stance	28	37	29	34
	Swing	26	37	29	31
Spine	Vertical: Stance	16	22	18	19
	Swing	17	26 ^a	15	18 ^b
Pelvis	Lateral: Stance	27	34	26	31
	Swing	38	40 ^a	32	31 ^b

abc Medians of matching groups not sharing superscripts differ significantly at P \leq 0.05. Absence of superscripts indicates no significance.

Absence of superscripts indicates no significance. Mann-Whitney tests were used.

6.5 Discussion

The objectives of this study were (1) to determine current movement changes in gilts/sows with advanced lameness based on an analytical biomechanical method compared to those with clinically sound locomotion; (2) to investigate historical movement changes in the juvenile pigs with later clinical lameness in comparison with pigs that did not develop lameness at any point in the entire study period.

There are few studies with a detailed analysis of movement changes in pigs during lameness (Grégoire et al., 2013). Grégoire et al. (2013) averaged front and hind leg gait parameters which were not found to differ within-pigs to compare lame, mildly-lame and normal pigs. For pigs in the present study, front and hind leg gait parameters were kept separate to analyse potential site-specific changes in more detail. Temporal parameters differed not only between front and hind legs both in normal and in lame pigs, but especially between left and right body sides for the lame pigs. In order to increase resolution of an automated lameness tracking system, within-animal standard deviations or differences between left and right leg parameters should be used instead of whole-animal averages.

Joint flexion was tested for an indication of a joint origin of lameness, since it was not possible to clinically determine the origin of lameness beyond leg level. The increased knee flexion in pigs affected with lameness in hind legs may indicate that there was a predominant problem at this joint. Certainly, there is further research needed to determine whether flexion changes can be diagnostic of the origin of lameness. Particularly valuable would be further research to determine whether bacterial arthritis (treatable with antibiotics) and degenerative arthrosis (not treatable with antibiotics) can be discriminated with gait analysis. For example, Boettger et al. (2009) showed that gait abnormalities differentially indicated pain or structural joint damage in induced arthritis in rats. Taken together with the changes seen in the present study, these findings are encouraging of more targeted future research at the joint level.

Grégoire et al. (2013) established longer stance times for lame pigs (probably due to slow walking speeds); yet, in the present study lame legs often showed a shorter stance time than that of controls. Instead, stance times of contralateral and diagonally opposite legs were increased in the lame pigs, as was reflected in the difference in the ipsilateral swing/stance time ratio. This ratio on the non-affected side was frequently

observed to decrease below the median of the sound pigs, because both stance times on the non-affected side increased. Also notable were the substantial differences among the swing times of affected and non-affected legs, which were even greater than differences in stance times. This is in agreement with data from horses, for which reduced swing times are one of the most consistent findings of supporting limb lameness (Back and Clayton, 2001). Reduced impulse (time integrated force) during the stance phase can lead to a reduced propulsion of the affected leg. However, while lame horses have been found to maintain gait symmetry in leg pairs, asymmetry indices appear very important in lame cows and pigs (Duberstein et al., 2014; van Nuffel et al., 2009).

Absolute stance time, stride length and walking speed were generally not different between currently lame and sound pigs. The fact that pigs were awaiting treats and may have been more motivated to perform the walk, even when lameness was present, may explain why a lower walking speed was not observed. Willingness to follow for an apple treat did not differ between lame and non-lame pigs (Bos et al., 2013). Furthermore, lame pigs in the current study comprised both pigs with reduced and minimal weight-bearing lameness, which Grégoire et al. (2013) separated into mildly lame and lame pigs, respectively. This probably led to an increase in the variability of the walking speeds at which the present pigs were willing to follow. However, a decrease in stride length and an increase in stance time can be directly associated with a decrease in walking speed (Walker et al., 2010, Kirtley et al., 1985). Nervous pigs may hesitate and then quickly walk through an open gateway whilst a normal, confident pig may slowly stroll down a walkway. Consequentially, it is necessary to identify sensitive gait parameters in pigs which are size- and walking-speed independent. If such a precaution is not taken, specificity and sensitivity of diagnostic measures will be low.

Measures of asymmetry between the same measure of left and right legs, the overall within-leg-pair SD of a given gait parameter or the ratio of two variables with the same unit consistently differentiated lame from control pigs. The effects of lameness on these relative measures were often quite pronounced, i.e. 2-4 times higher than the control median. However, while the pattern of such differences varied between pigs with lameness in different legs and their respective control pigs, most of the parameters did not differentiate among the pigs with lameness in different sites. Only the lateral pelvic displacement and the hind leg temporal parameters distinguished some of the

affected groups. Yet, on an individual pig level within all three lameness groups, these were quite variable and therefore not suitable to reliably indicate the site of lameness. This suggests that there are considerable compensatory changes in non-affected legs and, also, that different pigs may employ different compensatory strategies to compensate for a lame leg. These results are consistent with Pluym et al. (2011), who also reported substantial stance compensatory changes in lame pigs.

In the current study, the vertical displacement of the head was clearly increased in 78% of all lame pigs and in 100% of the pigs with front leg lameness. This is consistent with Mustonen et al. (2011), who also referred to head bobbing as an advanced lameness indicator in pigs, but disagrees with Main et al. (2000) who argued that, with their short neck, pigs have a limited capability to compensate for lameness with changes in frontal body movement. It is possible that the relative change in pigs is smaller compared to other animal species, such as sheep and horses (Kaler et al., 2009; Weishaupt, 2008). Nonetheless, head bobbing was one of the most important clinical indicators of lameness in the present study, especially to determine front leg involvement.

One of the most important findings of this study was the prognostic value of the step-to-stride length ratio. A study by Hoffmann et al. (2010) in a rat model also showed that kinematic gait changes preceded overt arthritis, confirming that prediction of future lameness using kinematics is possible. In the longitudinal study of pigs by Stavrakakis et al. (2014b), the step-to-stride length ratio was shown to be independent of pig size, weight and speed variations within a walking speed range. This is again confirmed by the cross-sectional data from pigs of various sizes/ages included in the present study.

However, it has to be noted that irregularity in the step-to-stride length ratio can increase with instantaneous acceleration/deceleration during walking, when pigs don't walk in a straight line, but in a zigzag or curved fashion and possibly also with nervousness/insecurity. Although these effects could partly be quantified in this study, they were mainly noted during data processing. For example, one outlier among the control pigs was a particularly nervous animal and an outlier among the preclinical pigs with a low deviation from 0.5 was found to be the pig subsequently diagnosed with lameness due to toe wall separation. Unfortunately, since this study could not diagnose the exact cause of lameness, it was not possible to extract only those cases where a

preclinical biomechanical disturbance could be expected. Such conditions would include chronically developing degenerative joint lesions and weakness in the muscle-tendon structures.

In the first early dataset, the mean of front and hind leg deviation was significant, whereas in the later dataset, deviation in the front leg ratio only was significant. This leads to the conclusion that the step-to-stride length ratios should be calculated separately for front and hind legs and not be pooled for leg pairs. Attempts to predict the limb pair in which lameness would occur were successful in about half the cases. Consequently, it has yet to be clarified to what extent the irregularity in the step-to-stride length ratio is a primary or a secondary compensatory change in walking patterns. It is possible that the common expression of an irregular step-to-stride length ratio is the result of various causes in different pigs. Repeated measures may improve the specificity, since false positives may be ruled out when the repeated measures are inconsistent, due to nervousness for example.

In conclusion, this study showed that monitoring of relative gait measures can reliably detect lameness in pigs. The step-to-stride length ratio could detect up to 90% of the pigs which subsequently developed lameness. Gait measurements may be more successful than subjective classification in targeting longevity characteristics of pigs and assessing lameness risk. Equally, gait analysis may help eliminate variation seen in studies evaluating factors associated with lameness based solely on subjective assessment of locomotion.

Chapter 7. General Discussion and Conclusions

7.1 Discussion

7.1.1 Objectives of this PhD thesis

The objective of this PhD was to develop an objective motion capture method for growing pigs and assess (1) the repeatability and sensitivity of the method (2) the gait characteristics of pigs housed on different floor types and (3) gait differences in pigs with conformational deficiencies, joint disease and/or clinical lameness. Finally, the aim was to identify simple gait parameters for the development of a farm-friendly method of objective gait assessment (4). The goal of the experiments reported so far in this thesis was to parameterise normal and abnormal gait in pigs through the use of kinematic gait analysis.

7.1.2 Repeatability and sensitivity of the method

Repeatability and sensitivity are interrelated concepts in time-series gait analysis, since a lack of repeatability decreases sensitivity (Wilken et al., 2012), especially for absolute gait measures, hence measures which are not normalised to an equally biased measure (Torres et al., 2011; Noehren et al., 2010). Lack of repeatability, leading to inflated variability, remains a problem in gait research wanting to discriminate pathological from normal gait features (Steinwender et al., 2000). Particularly in kinematics, marker placement and skin movement remain compromises, and these could be responsible for any inconsistency in results concerning marker-derived, hence angular measures (Gorton et al., 2009; Benoit et al., 2006). While, in human kinematics, marker cluster techniques over centres of bone segments are applied to minimise skin movement error, these require a static trial before every data acquisition and the application of multiple markers per segment (Cappozzo et al., 2005). Simultaneously, it may be argued that tissue distribution between pigs is more similar than in humans due to genetic selection and uniform feeding, hence overall variability in tissue movement could be lower (Correa et al., 2006). Main plane motion is less affected by skin movement at a given joint, compared to secondary plane motion, thus subtle changes in a non-sagittal plane in farm animals are likely to be difficult to detect (Leardini et al., 2005). This was a reason why movement at hinge joints, rather than ball-and-socket joints, was of major

interest to the present studies. Instructing animals to perform calibration movements required for some of the more advanced gait analysis techniques is hardly possible in livestock animals.

Movement is dynamic by definition and therefore it is extremely challenging to obtain a series of samples under exactly the same circumstances both within subject and between subjects. Walking speed is almost never exactly the same and all related gait variables, such as stride length and the time components of stance and swing time, are accordingly variable (Walker et al., 2010). Controlling walking speed with a treadmill is not an option in a practical on-farm setting, since animals need to be thoroughly trained and, additionally, treadmills can induce unnatural walking patterns (Torres et al., 2013; Stolze et al., 1997). Applying correction algorithms to gait variables in an attempt to remove known effects of walking speed, leg length, conformation, weight, neuromotor control and muscle activation is not straightforward (Pierrynowski et al., 2001). First, exact relationships must be known for a given parameter in a species, both within- and between-animals, over a wide range of possible values in normal, healthy subjects and then in abnormal subjects (Kirtley et al., 1985). In the present series of experiments, comparisons across distinctly different subjects were not made, unless the distinction in a characteristic was of interest, for example the leg weakness traits. Focus in the current experiments was on maximising the number of animals from which repeated samples were taken and to investigate a wide range of gait parameters. However, some betweenanimal comparisons for confounding factors, such as walking speed and weight could be made. Some gait parameters in pigs were correlated to walking speed and some were independent of it. In an attempt to avoid effects of irrelevant factors within a group of pigs with a common characteristic of interest, relative within-animal gait parameters were calculated. Two measures taken from the same animal under the same circumstances remove some irrelevant variability. However, this may not be the case if one of the two variables changes in a different way with the confounding factor compared to the other variable. For example the swing-to-stance time ratio is not as stable as the step-to-stride length ratio, because the stance time may be expected to reduce with increasing walking speed.

The stronger the effect to be measured, the less of a problem is the "contamination" with confounding factors, such as walking speed, weight, size differences and misplaced markers (Chau et al., 2005). For example, lameness could be measured quite

easily, since it produced clear and strong differences in gait parameters. For the detection of subtle problems and changes, the repetition of within-animal sampling and averaging is therefore highly important. In the present studies, 2-3 repeated walks per sampling per animal were taken (as recommended by Ferrari et al., 2008), but repetitions of marker placement per sampling were not possible due to time constraints. On the other hand, the number of animals sampled and the repetitions over time per animal were relatively high compared to other biomechanical studies, typically sampling 1-40 subjects (McGinley et al., 2009). Overall, during this PhD project, 650 sessions were conducted with a session comprising marker application on both body sides and filming of one subject. Samples from 111 subjects overall were obtained.

The gain of information by gait analysis over visual gait assessment was clearly demonstrated by the identification of sensitive gait parameters, such as the step-to-stride length ratio. Joint flexion curves are also extremely useful in assessing the soundness of the musculoskeletal system (Boettger et al., 2011; Whittle, 1996). In the pigs of the floor treatment study, joint flexion had the best within-pig/between-pig variability relationship, thus joint flexion was consistent for repeated walks within a session and varied less than non-angular parameters. Future work should recruit subjects with well defined musculoskeletal conditions and compare specific joint flexion patterns using more powerful analytical techniques. For example, functional principal component analysis (fPCA) of continuous gait data, such as joint flexion curves is a promising method to identify a set of gait parameters which best explains variation in outcome measures (Richter et al., 2013). This approach can analyse gait more holistically without dissociation into isolated individual gait parameters (Lenhoff et al., 1999). However, the present work has shown that some isolated variables, such as the step-to-stride length ratio, may have sufficiently strong explanatory value for outcome measures of interest and are simpler measurements for use in a more practical context.

Consequently, gait analysis and the summary and comparison of gait data remains a challenging, yet necessary task, as visual assessment is limited to advanced conditions and has no reliable predictive ability (Whay et al., 2005; Jørgensen, 2000; Lopez-Serrano et al., 2000).

7.1.3 The gait characteristics of pigs housed on different floor types

No substantial longitudinal effect of pen floor surface on the musculoskeletal system, with a reflection in gait, was established in the current finishing pig. It is possible that there is no direct long-term biomechanical effect of hard pen floor surface or surface with reduced support area (slatted) when compared to a soft surface control, such as a straw-bedded floor (Scott et al., 2006). Consequently, there may be additional variables explaining the variability in the effect of floor surface on gait observed in different studies (Neuberg, 2003). Apart from the subjective methodology used in studies which have previously evaluated pen floor associations with leg problems, there may also be adaptation effects in pigs on less comfortable floors (Van Grevenhof et al., 2011; KilBride et al., 2010 and 2009; Jørgensen, 2003). Large epidemiological studies, which used subjective scoring of locomotion (KilBride et al., 2009), are likely to contain bias, since the scoring of pigs on site does often not allow for blind scoring. Similarly, in the present study, more pigs from un-bedded floors, particularly the fully-slatted, were observed as being deficient, though this was typically not significant. Reduced activity, and hence biomechanical exposure to the floor surface, in pigs on less comfortable or less motivating surfaces could be an explanation as to why there was no general floor effect measured in the present pigs (Scott et al., 2006). Decreased activity may primarily affect muscles rather than joints (Marchant and Broom, 1994). Therefore it is plausible that some of the current pigs in the floor treatment study developed leg weakness/stiffness, while there was no significant effect of floor on the presence of joint lesions. Varying degrees of slipperiness between the floors in different experiments are also likely to contribute to variability in results of lameness outcome measures (Thorup et al., 2008). Pigs are more likely to show injuries and abnormal gait when housed on more slippery floors, while straw-bedded surfaces provide more resistance and therefore a firmer footing. The currently used slatted and solid concrete floors were not slippery, as was judged visually and by personal exposure. However, finisher pigs housed on partly-slatted flooring in the present experiment (Exp 1, Chapter 4) generally had the severest joint lesions and gait differences, and hence, it is likely that floor type may aggravate pre-existing lesions. It was also observed that the present finishing pigs on the fully-slatted floor were the least active and such a factor may interact with floor type and joint lesions, although this was not quantified.

The absolute flexion change over time observed in the finishing pigs was not confirmed by studies of the pre-breeding females at equivalent time points. However, a tendency for reduction in the swing range of motion at the elbow joint over time was also seen in the replacement gilts. A possible explanation for elbow flexion differences between the two experimental cohorts is their different genetic background, i.e. the absence of Pietrain and Duroc genes and therefore perhaps reduced front leg weakness in the breeding females (Draper et al., 1992; Van der Wal et al., 1987). In the 24 pigs from the floor treatment study, there was a significant increase in overall elbow flexion from the second motion capture (76 kg) onward. Decreased flexion at the second motion capture was associated with buck-kneedness in the front legs and also with the partly-slatted floor, on which most of the buck-kneed pigs were housed. Gender differences in gait among the finishing pigs were not established. However, the pre-breeding females and the finishing pigs were fed different rearing rations, with reduced protein content in the diet of growing replacement stock. Mineral content was also higher in the diets of the gilts and therefore bones were likely to be stronger and possibly heavier in these animals (Varley et al., 2011). Consequently, clarification is needed on whether increasing front leg flexion is a characteristic during the grower stage of finisher pigs and not present in pre-breeding replacement gilts at the same growth stage. If this is the case, then further biomechanical investigation at this particular joint is needed to assess the effect or the origin of the flexion change in the musculoskeletal system. A reduced range of motion can be indicative of arthritis and generally, reduced flexion may imply reduced function of a joint (Boettger et al., 2011; Goldberg et al., 2006). It is important to confirm that a flexion difference generally exists between breeding and finishing pigs, because this may have a direct relationship with mobility and hence, welfare of the pigs. Future research should determine whether the mineral content of finishing pig diets needs to be reformulated to the requirements of uncompromised mobility at all growth stages until slaughter.

7.1.4 Gait differences in pigs with conformational deficiencies, joint disease and/or clinical lameness

One of the present studies (Chapter 5) could quantify whether there were common kinematic differences in pigs with two particular leg weakness traits, namely buck-kneed forelegs and post-legged hind legs. Potential kinetic differences and differences in tissue characteristics through the use of repeated biopsies and

microscopic/biochemical analysis were not determined. Therefore, judgement on whether misalignment of leg segments, as observed in leg weakness, was harmful to weight-bearing structures from a biomechanical perspective was possible with regard to kinematic aspects only. Further biomechanical investigation of leg weakness should use a more powerful and complex methodology, initially on a few subjects with pronounced leg weakness traits. Inverse dynamics is a method of Classical Mechanics which uses the Equations of Motion to calculate joint mechanics (Lanovaz and Clayton, 2001). Input parameters for inverse dynamics models are stance-phase ground reaction forces, typically measured by force plates, joint angle measures (angular kinematics) and body segment measures of mass, length and inertia. It should be noted that the currently measured angular kinematics would not be suitable without transformation as an input to inverse dynamics models, because the lines of force are not accurately represented. Hence, inverse dynamics are quite complex and can only be applied to a few subjects and a few strides at a time (Thorup et al., 2008). Heller et al. (2001) found good agreement between in vivo measures of joint loading in humans and predicted values using an inverse dynamics solution. However, human inverse dynamics models are readily available and can rely on patient cooperation during data acquisition. Perhaps instrumentation of joints with force sensors and/or muscles with EMG-sensors (electromyography) and hence, surgical preparation of pig test subjects for direct internal mechanical measurements would be required instead (Heller et al., 2001). There are at least three scientific sources now pointing toward a muscular, rather than skeletal pathogenesis of leg weakness (Stavrakakis et al., 2014; Jørgensen and Andersen, 2000; Draper et al., 1992). A more radical approach to the study of leg weakness is perhaps now justified, since better methods are available and a better understanding has been achieved. Based on present results, leg weakness symptoms should not be used as a clinical tool to judge levels of joint disease.

Leg weakness and osteochondrosis are widely seen as a result of selective breeding (Paxton et al., 2013; Rauw et al., 1998). Indeed, genes of the wild boar were found to reduce the prevalence of OC lesions and to lengthen rather than widen the bones of offspring (Andersson-Eklund et al., 2000). However, there are still substantial knowledge gaps in understanding the biomechanical effects of these presumed abnormalities, which may or may not lead to clinical lameness. Without a better understanding, potentially sound animals may be wasted, while other seemingly sound animals stay in the herd and may later develop lameness (Friendship et al., 1986). It is

possible that during natural selection, leg weakness traits disappear because they may lead to disadvantages in the locomotion/stamina required in natural environments due to increased locomotor inefficiency (Wilson et al., 2003). However, in indoor farming systems, new adaptations may be required by the animal and therefore the selection focus needs to strive towards the precisely required characteristics. Judging by the deviation from the appearance of a wild progenitor may not be an appropriate criterion.

It is generally supported that leg weakness and osteochondrosis are negatively associated with longevity due to causing irreversible locomotor problems (Anil et al., 2009; Yazdi et al., 2000; Jørgensen, 2000; Jørgensen and Sørensen, 1998; Dewey et al., 1992). Jørgensen (1995) reported a prevalence of 39% and 20% buck-kneed and postlegged slaughter pigs, respectively, which is similar to the findings among the finishing pigs in the current floor treatment study (29% and 21%, respectively). Subsequently, Jørgensen (2000) demonstrated that buck-kneed forelegs, swaying hindquarters and standing-under position in hind legs, observed at 6 months of age in gilts, had a negative impact on the longevity of the sows. However, the prevalence of leg weakness is generally higher than the prevalence of lameness (KilBride et al., 2009; Friendship et al., 1986). The period prevalence of lameness in the present breeding female cohort was 29% (Chapter 6). This is in agreement with the period prevalence found by Jørgensen (2000) which was nearly 30%. Yet, the breeding female cohort overall had negligible levels of clinical leg weakness compared to the finishing pig cohort (Chapter 5) in the current experiments, although this was not quantified in detail. Conversely, the finishing pigs showed more leg weakness, but no abnormal gait other than stiffness. Hence, there are animals which have osteochondrosis and/or lameness but no leg weakness (Goedegebuure et al., 1988), and pigs which have leg weakness but show no abnormal gait (de Koning et al., 2012). Serenius and Stalder (2006) concluded that sow longevity is genetically associated with prolificacy and leg conformation traits. Nonetheless, variable results from previous research have led to a lack of consensus among swine breeders concerning the valid methodology of estimating breeding values for longevity traits (Serenius and Stalder, 2006). It may therefore be anticipated that an improved understanding of the leg weakness syndrome and its biomechanical components will play a key role in the progress of selective breeding for longevity characteristics. Quantitative gait variables can increase confidence in classifying a pig as being in danger of compromised longevity through a more sensitive detection of those pigs having joint disease or developing lameness, ignoring their leg weakness status. Hence,

the currently identified gait measures may improve the availability of sensitive and objective methods to estimate breeding values for longevity.

It must be noted that, during the experimental period of the breeding female cohort study, 17 of the 84 gilts were culled (20%) and three animals had to be euthanized, two of them due to severe bilateral hind leg lameness. All culls were due to repeated failure of conception, and hence were attributed to reproductive problems (Dagorn and Aumaitre, 1979). As was mentioned in Chapter 6, period incidence of lameness was higher than the period prevalence and thus, several animals were repeatedly lame without a notable decrease in their reproductive ability. Consequently, most of the present 24 lame pigs experienced no impediment to longevity, but were compromised in terms of welfare during lameness and until rehabilitation (Whay et al., 2003). Regardless of the reason, the main interest of both swine breeders and farrow-to-finish farmers is the assurance of soundness of an animal. Hence, the goal of both interest groups is to exclude the incident of lameness and, therefore, the detection of truly negative pigs, with respect to musculoskeletal unsoundness, is crucial. False positive detection, i.e. low specificity, may likely lead to animal wastage, whereas false negative detection, i.e. low sensitivity, would lead to unexpected lameness cases. Consequently, both sensitivity and specificity of diagnostic measures of pathology are important in the case of lameness. Positive and negative predictive values of a test, however, will also depend on the prevalence of a condition in a population and these values should be subject of future investigations of entire populations.

Lameness, in the present experiments, was diagnosed based on clinical assessment and the exact underlying causes were not further identified by more specialised examinations. Based on the clinical appearance of the affected animals and their histories/age-group, non-infectious causes were likely to have produced the majority of the currently observed lameness. The fact that measurable gait alterations existed in the breeding females before lameness became clinical does not necessarily imply that the pathogenesis of lameness involved biomechanical factors (Heijink et al., 2012; Yusuf et al., 2011). To assess this further, more research is required to determine the internal consequences in joint loading caused by differences in kinematic characteristics and the adaptability of tissues to absorb higher forces if present (Forriol and Shapiro, 2005). The presently measured predictive parameters are likely to be an effect, and not the

cause, of a disturbance in the musculoskeletal system, which was nonetheless clinically unobservable.

Asymmetric stride timing during lameness in different legs showed that lameness involving hind legs caused a general disturbance in support timings, while lameness in the front legs did not. Similarly pigs with non-mild joint lesions in both front and hind legs produced significant gait differences, whereas pigs with mild lesions or advanced lesions only in front legs did not (except for joint flexion asymmetry). Thorup et al. (2008) and Carvalho et al. (2009) determined the weight distribution of pigs over front and hind legs and highlighted differences in mechanics between the two leg pairs. Differences, such as the front legs carrying more weight, may play a role in the compensation strategies employed during pathological changes in the locomotor system. Problems in the hind legs appear to redistribute demand for support more to the front legs, but not vice versa. Problems in the front legs are mechanically more redistributed to the frontal body, hence the head and neck. Shifting of the centre of mass of the body is important in the redistribution of mass over loaded supporting structures (Nauwelaerts and Clayton, 2010). Irregularity in the stride timing was generally elevated for the front legs, regardless whether front or hind legs were affected. Furthermore, front leg lameness caused substantial changes in the step-to-stride length relationship. This might be a reason why pre-clinical irregularity was particularly significant in the front leg step-to-stride length ratio at the second earliest motion capture, regardless of which limb developed lameness later on. Hence, gait compensation phenomena are extremely important and might be more easily detected than direct local changes.

Elbaz et al. (2014) demonstrated that spatiotemporal gait analysis objectively classified human patients with knee osteoarthritis according to disease severity. Their method correlated with radiographic evaluation, the level of pain, function and number of total knee replacements. Nalon et al. (2013b) assessed mechanical nociception thresholds in lame sows to quantify hyperalgesia (increased sensitivity to painful stimuli) as an accompanying symptom and potential proxy measure of lameness. Lame limbs were consistently found to exhibit lower tolerance to the mechanical stimulus (pressure) compared to non-lame limbs. Hence, for the evaluation of intervention/treatment success in response to lameness in pigs, nociceptive and gait analysis methods may provide useful tools to evaluate subtle changes during improvement. However,

compared to static force plate and nociceptive techniques, where animals may stand or even lie, respectively, gait analysis methods may have the advantage of using a biomechanically more sensitive sample, i.e. higher forces acting in the musculoskeletal system during the walk compared to standing (Tessutti et al., 2010; Bergmann et al., 2001). Using relative within-pig measures should compensate for an increase in variability due to the dynamics of gait.

7.1.5 Identification of simple gait parameters for the development of a farm-friendly method for objective gait assessment

Simple and sensitive gait measures of lameness in pigs were identified in the present work, and these could contribute to the development of a practical and farm-friendly lameness assessment tool. Single-device 3D motion sensors, such as the Microsoft Kinect (Zhengyou, 2012), may be used as an overhead device detecting irregularities in head and trunk movement of pigs in motion within a pen or along a walkway on farm. Claw stance and swing timing and the step-to-stride length ratio could be measured by alternative techniques, such as pressure mat systems (van Nuffel et al., 2013; Kim and Breur, 2007).

Yet, for an automated gait analysis approach, adequate software needs to be developed first. Before the automated use of any of the currently identified sensitive gait parameters, a customised software algorithm for pigs is required. Based on known normal ranges of stride timings and stride geometry, filters need to be created which would only sample useful stride sequences of pigs. In the present experimental series, all walks were performed under close human surveillance and films were processed with a high degree of visual control. Fully automated systems will require further development and may potentially have some accuracy trade-off (Maertens et al., 2011). Changes in walking speed while walking, i.e. propelling and braking attempts, walking in a curve trajectory, sidestepping, stopping and other irregularities need to be identified and if present, should not contribute to a sample.

7.1.6 Generalisation of the methodology to other species

The present method of data collection may be directly applicable to other vertebrate species, provided that skeletal landmarks project at equivalent sites in these animals

(Torres et al., 2010; Weller et al., 2006). Differences in gait parameters may exist and relate to scaling differences of the animals (Gasc, 2001; Biewener, 1983). McMahon (1975) proposed that body size is a determining factor of the structural design of species. Similarly, Biewener and Taylor (1986) suggested that bone strain could be a determinant of gait and speed in different species. Therefore collection of information on aspects of the structural design of different species along with measures of gait may enhance the present understanding of relationships between size, anatomical conformation and normal/abnormal gait. Since patients with joint disease tend to walk slowly and with a short stride, it is essential that normal ranges for gait parameters should be defined with reference to speed of walking (Kirtley et al., 1985). Lameness associated modifications of gait are likely to be similar in quadrupeds (van Nuffel et al., 2013; Flower et al., 2005; Back and Clayton, 2001), nonetheless, there are differences in gait compensation and pain alleviation strategies within and between species (Back and Clayton, 2001; Whittle, 1996). The choice of a method for gait data collection depends largely on the purpose, the movement of interest and the compatibility with the animal species. Dimensionless and relative measures of gait asymmetry are promising measures of abnormality in various species and can be measured by a variety of techniques (Pfau et al., 2007; Santos et al., 1995).

7.1.7 Future research

It should be mentioned that factors not studied in the present series of experiments, such as animal character, i.e. a dominant or submissive character, are likely to have an effect on lameness prevalence among a group. There may be complicated interactions between animal character, floor surface/activity level, feed intake, growth rate and lameness (Barnett et al., 1984). Hence, animal character and behaviour within a group may have biomechanical implications. These could be expressed as modifying the musculoskeletal system through activity and feed intake in such a way that it becomes predisposed to lameness during normal daily mobility requirements. However, character may be one factor causing lameness in otherwise biomechanically sound animals. Future research is required to determine the weighting of such animal-based factors, for example, whether the lowest pig in a pen hierarchy is at greater risk of developing lameness. This might be particularly relevant in pigs with a predisposition to lameness, such as genetic lines with increased leg weakness or osteochondrosis for example.

Pluym (2013c) suggested that aggression/fighting during initial group housing after lactation did not influence the development of lameness in sows, despite an increased incidence of lameness after lactation and in the insemination stalls or during early group housing. Hence, other risk factors in the environment or within-animal may be responsible for the occurrence of lameness at this particular production stage. During the present experimental work, kinematic gait data for consecutive gestation and lactation periods (parity 1-3) were collected from the breeding gilt/sow cohort described in Chapter 6, but this data could not be analysed due to time contstraints. However, the impact of intensive, productive cycling with a high demand on the musculoskeletal system due to mineral depletion and weight changes, which female breeding pigs undergo, should be addressed in future research.

In summary, based on the insights generated during the present work, future quantitative research into lameness in pigs should clarify: (1) associations of gait differences between finishing and pre-breeding pigs with mobility and welfare during growth, (2) the relationship between leg weakness and osteochondrosis and the impact of these two conditions on longevity and welfare, (3) associations between other animal-based factors, such as character/dominance with risk of lameness, (4) the implications of continuous cycling from lactation to gestation, with a short break of one week, on musculoskeletal integrity and mobility of female breeding pigs. Furthermore, (5) presently identified sensitive gait measures for pre-clinical and clinical lameness can be directly taken further to be measured by an alternative system for automated animal screening, following the development of appropriate software.

7.2 Conclusion

This doctoral thesis presents an approach to biomechanical gait assessment in pigs with a focus on longitudinal gait development/variability and detailed gait histories of lame and non-lame pigs

Using marker-based kinematic methodology, it was concluded that detecting a true gait deviation may require repeated marker placements for anatomical regions known to promote marker misplacement, such as the proximal hind limb. Gait measurements with low repeatability between marker placements, such as absolute joint flexion, should be interpreted with caution. However, joint flexion within marker placement shows a good within/between pig variability relationship and could be used in the research of long-term flexion effects on orthopaedic health.

The quantification of locomotion in a longitudinal observational study of pigs housed on floors differing in hardness and supporting surface could not confirm detrimental differences observed in previous epidemiological studies using subjective gait assessment. Measured changes in gait patterns over time were largely unaffected by the floor surface on which current growing and finishing pigs were reared. Nevertheless, elbow joint flexion during walking significantly increased in finishing pigs and this was not observed in pre-breeding gilts at equivalent growth stages. Hence, there are likely to be mobility differences in finishing and growing breeding pigs, which require further evaluation with respect to lameness, welfare, diets and genetics. Kinematic gait analysis could be used to re-evaluate mineral treatment effects *in vivo*.

The presence of osteochondrosis lesions affected the stance phase of the gait cycle, conformational deficiencies merely affected the swing phase and therefore conformational deficiencies may not be a good clinical trait to judge levels of joint pathology. For the purposes of selecting replacement breeding animals, gait measurements may be more successful than subjective classification in targeting longevity characteristics of pigs and preventing lameness. Yet, the importance of subclinical lesions of osteochondrosis and conformational deficiencies with respect to longevity has to be further elucidated.

Temporo-spatial gait parameters, especially asymmetry indices and head and trunk movement, could reliably detect lameness. More intuitive lameness indicators, such as short stride lengths and reduced stance times were not consistently measured in lame pigs. Comparison of the flexion at a joint against expected values may aid in the

identification of the affected site. The step-to-stride length ratio could detect up to 75% of the pigs which subsequently developed lameness.

Lastly, biomechanical evaluation of pigs is still a very complex process that requires some simplification before it could be used at farm level. However, if included in studies evaluating factors associated with lameness in pigs, quantitative gait variables may help eliminate some of the variation seen in results based solely on subjective assessment of locomotion.

It is concluded that kinematic gait analysis offers potential for automated prediction and early detection of lameness.

Appendices

Table 8.1 below shows some additional early data from replacement gilts of the cohort introduced in Chapter 6. Compared groups are the same as in the named Chapter. Stride lengths were not predictive of impending lameness. However, some measures relating to step widths were significant.

Table 8.1: Future lameness detection – step width and stride length:

Medians are displayed.

	Pigs develop	ping lameness	Normal control pigs		
Length measure (mm)	63 kg gilts (N=13)	97 kg gilts (N=11)	60 kg gilts (N=11)	96 kg gilts (N=10)	
Front leg step width	131 ^a	145 ^b	100 ^(c)	117 ^{ab(c)}	
SD	26	28	26	25	
Hind leg step width	108	108	97	103	
SD	30	35 ^a	28^{a}	22 ^b	
Walking speed	847 ^(a)	896	912 ^(a)	849	
Stride length	663°	802^{b}	681 ^a	792 ^b	

AbcMedians within a row not sharing superscripts differ significantly at P \leq 0.05. Parentheses around superscripts denote tendencies when P \geq 0.050, but \leq 0.098. Mann-Whitney test were used.

Table 8.2 shows differences in equivalent elbow flexion values between the finisher pigs described in Chapter 4 and replacement gilts, matched by weight, described in Chapter 6.

Table 8.2: Elbow flexion in finisher and pre-breeding gilts:

Right side flexion values are displayed. Finishers at 63 kg are 12 males and 12 females, while at 90 kg 5 females and 9 males. Differences between genders were not detected.

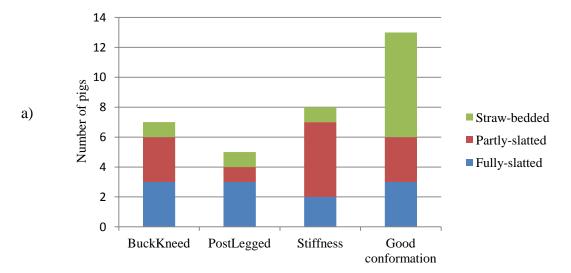
Gait parameter	Finisher pigs at 63 kg (N=24)	Gilts at 60 kg (N=11)	Finisher pigs at 90 kg (N=14)	Gilts at 94 kg (N=11)
Minimum stance	51 ^a	56 ^{a(b)c}	62 ^b	57°
elbow flexion (deg)	(1.4)	(2.8)	(1.7)	(2.0)
Elbow joint	40^{ab}	43 ^b	35°	39 ^{a(b)}
range of motion (deg)	(1.4)	(1.1)	(1.0)	(1.3)
Walking speed	0.86^{a}	0.88^{a}	$0.97^{\rm b}$	$0.88^{a(b)}$
(m/s)	(0.09)	(0.08)	(1.12)	(1.17)

Abc Means within a row not sharing superscripts differ significantly at P≤0.05. Two sample t-tests were used to compare means (SEM). Parentheses around superscripts denote tendencies when P = 0.076.

Table 8.3 and Figure 8.1 present the distribution of the finisher pigs, introduced in Chapter 4, with and without joint lesions and leg weakness by pen floor.

Table 8.3: Number of pigs with or without joint lesions housed on three different floors.

Osteochondrosis	Fully-slatted	Partly-slatted	Straw-bedded
No lesions	2	0	2
Mild lesions	4	2	4
Moderate-severe lesions	2	6	2



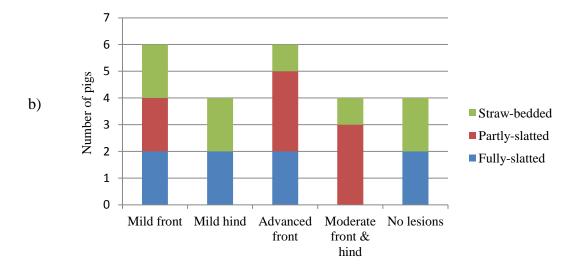


Figure 8.1 a-b:(a) Leg weakness and stiffness, subjectively observed in pigs housed on three different floor types (b) joint lesion characterisation in the same pigs by floor type (N=24)

Table 8.4 and Figure 8.2 show a preliminary analysis of data from the female pigs of the breeding cohort described in Chapter 6 of this thesis.

Leg stance double- and multiple-leg support phases were analysed by a custom-written programme in Matlab (Mathworks©, Natick, USA) and the results presented at the ISB Congress, 2013 (Reference 6 in List of Abstracts in Conference Proceedings).

Table 8.4: Leg support overlaps during walking in 5 lame and 5 non-lame control pigs

Support overlaps (% gait cycle) between (a pair of) feet (diagonal, ipsilateral and contralateral) in lame and non-lame pigs. L=left, R=right, F=front and H=hind. Score 3 lameness (N=5) was identified to arise from the left side (front or hind), except for one uncertain case among affected hind limbs. Increases in double support times of ipsilateral and contralateral legs in lame pigs may not necessarily entail increases in 3-leg and 4-leg support phases, but rather expand the duration of such multiple support phases, meaning that measuring simply the number of claws on the ground without considering the time may be misleading and result in underestimated lameness prevalence on farms.

	Diagonal Ipsilateral		Contraleral							
Lameness	RF LH	LF RH	LH LF	RH RF	RF LF	RH LH	LF RF	LH RH	3 leg support*	4 leg support*
Front	30 ^{ab}	34	27 ^a	26^{ab}	6 ^a	3 ^a	14 ^a	4 ^a	14%	55%
Hind	29^{a}	33	29^{a}	26^{a}	4 ^b	6^{b}	6^{b}	$8^{\rm b}$	43%	36%
None	32^{b}	35	22^{b}	22^{b}	4^{b}	2^{a}	7^{b}	4^{a}	34%	34%

^{*}Relative frequency of observations.

abc Within a column, means followed by a different superscript are significantly different at P<0.05

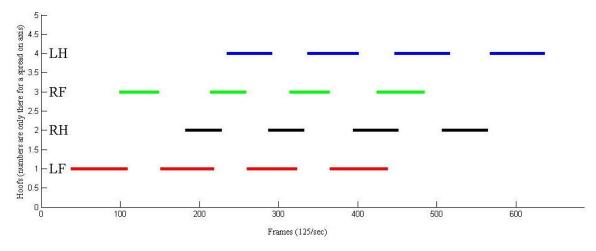


Figure 8.2: Stance times of all four legs in a lame pig over 3 strides.

L=left, R=right, F=front and H=hind. The pig was clinically diagnosed lame in RH and an uncertain involvement of LH.

1 able 8.			on behavioural indicators, d Behaviour of individual		n et al. (2000).
Score	Initial response to human presence	Response after opening gate	within group	Standing posture	Gait
0	Bright, alert and responsive (pigs rise immediately and approach inquisitively)	Inquisitive, will tentatively leave pen	Freely participates in groups activity	Pigs stands squarely on all four legs	Even strides. Caudal body sways slightly while walking. Pig is able to accelerate and change direction rapidly.
1	As for score 0	As for score 0	As for score 0	As for score 0	Abnormal stride length (not easily identified). Movements no longer fluent – pig appears stiff. Pig still able to accelerate and change direction.
2	As for score 0	As for score 0	May show mild apprehension to boisterous pigs	Uneven	Shortened stride. Lameness detected. Swagger of caudal body while walking. No hindrance in pig's agility.
3	Bright, but less responsive (may remain down or dog-sitting, before eventually rising).	Often last to leave pen	Apprehensive to boisterous pigs (usually remains separate from group activity)	Uneven posture. Will not bear weight on affected limb (appears standing on toes)	Shortened stride. Minimum weight-bearing on affected limb. Swagger of caudal body while walking. Will still trot and gallop.
4	May be dull (only rises when strongly motivated)	Unwilling to leave unfamiliar environment	Will try to remain separate from others within the group	Affected limb elevated off floor. Pig appears visibly distressed.	Pig may not place affected limb on the floor while moving.
5	Dull an unresponsive	No response	May appear distressed by other pigs but may be unable to respond	Will not stand unaided.	Does not move.

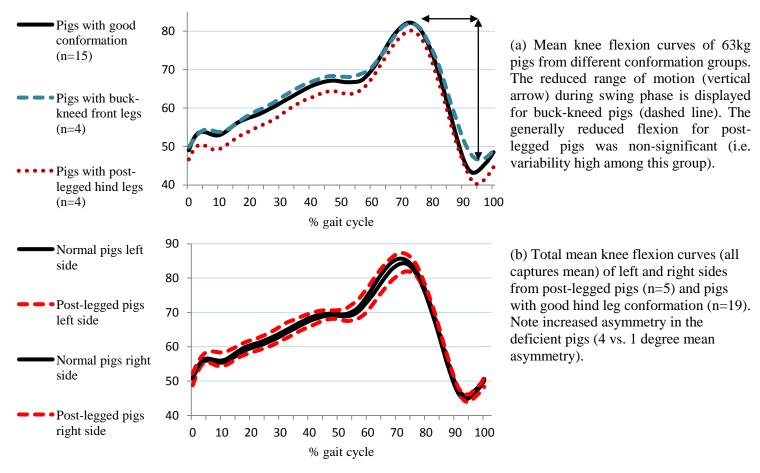
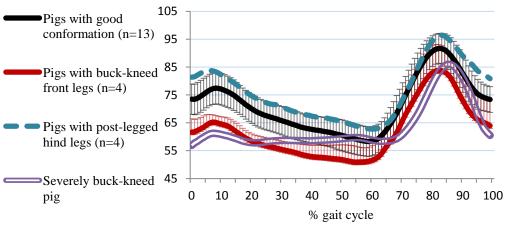
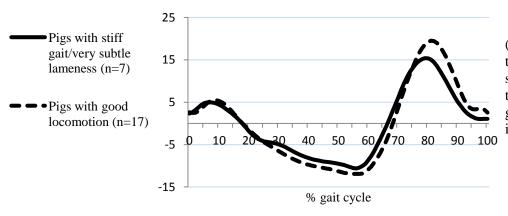


Figure 8.3 a-d: Angle-time graphs of pigs with musculoskeletal deficiency/subtle lameness vs. normal pigs. Total mean curves, group mean curves within capture, and an individual curve against group curves within capture are shown. Flexion angle degrees are displayed on the y-axis. Note that the stance phase is typically completed at 50-55% gait cycle.



(c) Mean elbow flexion curves from the second capture (76 kg) for three different conformation groups. Shaded areas outline the standard deviation. Note that in the single most affected pig (also included in the group mean) there is almost no extension at the elbow joint during the stance phase. This was a consistent feature of this pig over all captures.



(d) Mean elbow flexion curves from the third capture (81 kg) for two different subjective gait score groups. Note that the y-axis here was normalised to the group mean to emphasise the difference in the swing range of motion.

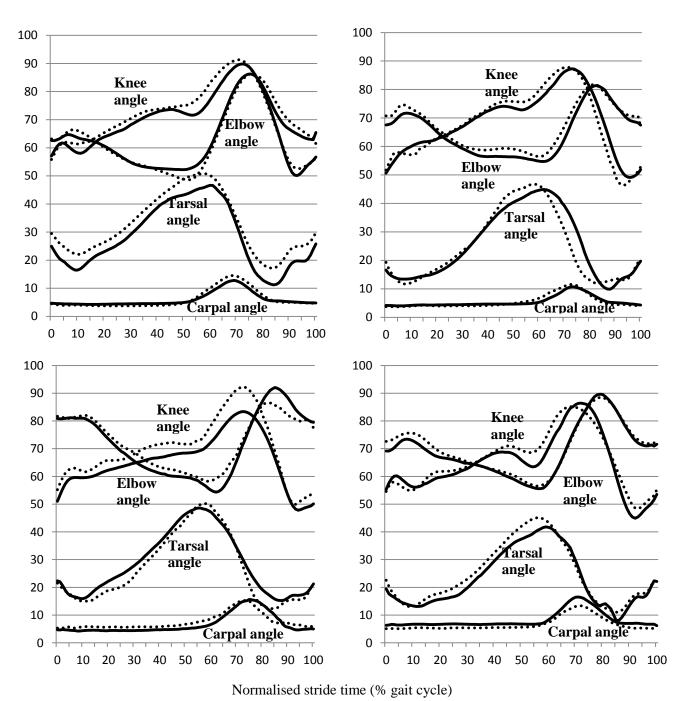
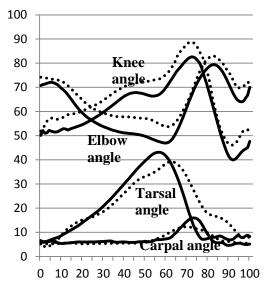
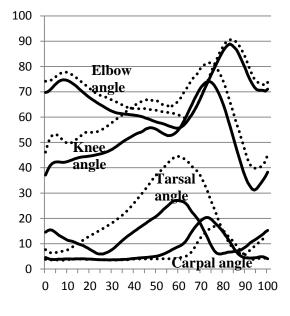


Figure 8.4 a-d: Total left (solid line) and right (dotted) side mean angle-time curves of pigs without lesions in knee, elbow, tarsal and carpal joints. Flexion angle degrees are displayed on the y-axis. Note that the stance phase is typically completed at 50-55% gait cycle.

The pig below had moderate shoulder and carpal lesions and arthritic changes in hips, right knee and right carpal joints.

The pig below had moderate lesions in the elbow and tarsal joints.

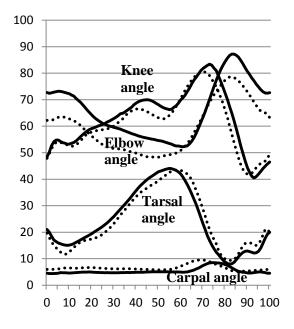


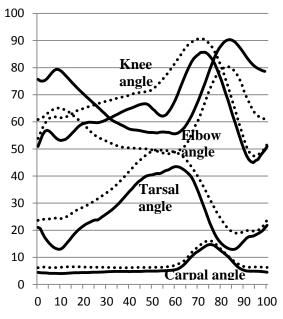


Pigs with moderate front and hind leg lesions

The pig below had a severe lesion in its right elbow joint (deep cartilage erosion).

The pig below had moderate lesions in shoulder and elbow joints and mild lesions in carpal, knee and tarsal joints.





Pigs with moderate to severe front leg lesions

Normalised stride time (% gait cycle)

Figure 8.5 a-d: Total left (solid line) and right (dotted) side mean angle-time curves of pigs with lesions in shoulder, elbow, carpal, knee and tarsal joints. Flexion angle degrees are displayed on the y-axis. Note that the stance phase is typically completed at 50-55% gait cycle.



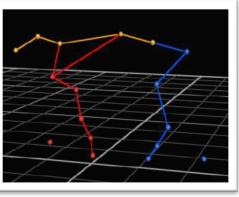


Figure 8.6 a-c: (a) Pig prepared with reflective markers (b-c) reflective markers reconstructed by motion capture software and kinematic model fitted.

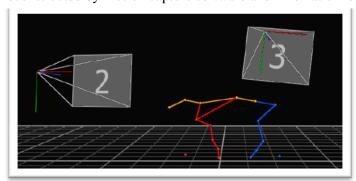




Figure 8.7: Vicon T20 camera set up for pig motion capture at the Newcastle University pig unit.

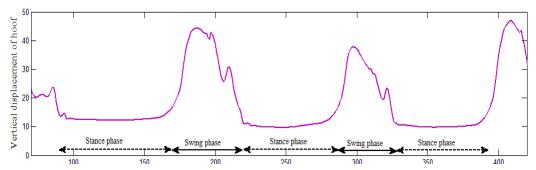


Figure 8.8: Hoof marker vertical trajectory with stance and swing phases identified. On the x-axis is time in frames (125/sec).

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