Rearing strategies to optimise gilt and sow welfare

UNIVERSITY of LIMERICK

OLLSCOIL LUIMNIGH

Rearing strategies to optimise gilt and sow welfare

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PhD thesis

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Declaration

This thesis is submitted to the University of Limerick in support of my application for the degree of Doctor of Philosophy. This thesis is composed by myself and has not been submitted previously towards any other degree. The work presented including animal trial work, laboratory analysis and data analysis was carried out by me the author.

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Publications, scientific awards and acknowledgments

Publications, scientific awards and acknowledgments

Scientific manuscripts

The following papers have been published:

- **P. Hartnett B. Younge, L. Boyle, and K. O'Driscoll.** The Effect of Group Composition and Mineral Supplementation during Rearing on Measures of Cartilage Condition and Bone Mineral Density in Replacement Gilts. Animals, 9(9), p.637.

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Abstracts

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Dedication

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List of Abbreviations

Abbreviation	Meaning
aBMD	Areal bone mineral density
ACTH	Adrenocorticotropic hormone
ADG	Average daily gain
ADFI	Average daily feed intake
BAP	Bone-specific alkaline phosphatase fragment of type II collagen
BMC	Bone mineral content
C2C	Collagen type II Cleavage
COMP	Cartilage oligomeric matrix protein
CON	Standard finisher diet – control
CPII	C-propeptide of type II Collagen
CS846	Chondroitin sulphate epitope 846
CTXII	Carboxy-terminal cross-linked telopeptide
CV	Coefficient of variation
Cu	Copper
DEXA	Dual energy x-ray absorptiometry
ELISA	Enzyme-linked immunosorbent assay
ESF	Electronic sow feeder
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
FEM	Female-only
GAMHRP	Goat anti-mouse horseradish peroxidase
GARHRP	Goat anti-rabbit horseradish peroxidase
HC	Humeral condyle
HRP	Horseradish peroxidase
HUM	Humerus
ICTP	Carboxy-terminal cross-linked telopeptide of type I collagen
IgG	Immunoglobulin G
IQR	Interquartile range
JLS	Joint lesion scoring
LW	Live-weight
MET	Metacarpal
MIX	Mixed-sex
Mn	Manganese
NRC	National Research Council
NTX	Amino-terminal telopeptide of type I Collagen
OC	Osteochondrosis
OCD	Osteochondrosis dissecans
OD	Optical density
PYD	Pyridinoline cross-links collagen
QC	Quality control
rpm	Revolutions per minute
RU	Radius/ulna
SUPP	Mineral supplemented diet
TMB	Tetramethylbenzidine substrate
TN	Trochlear notch
Zn	Zinc

Thesis abstract

Rearing strategies to optimise gilt and sow welfare

By Phoebe Hartnett

In Ireland, replacement gilts are often reared with finisher pigs, including entire males. This exposes them to sexual mounting and aggression, which is stressful and injurious. Moreover, finisher diets do not supply necessary minerals for optimal limb health which has a negative impact on welfare and performance. The objectives of this thesis were 1) to investigate two rearing strategies we hypothesised would improve limb health in developing gilts 2) to examine the condition of the gilts' bones and cartilage at breeding age 3) to assess carry-over effects of rearing treatments on sow welfare and performance and 4) to investigate levels of two potential biomarkers (C2C and CPII) of bone cartilage turnover, relative to bone cartilage condition and rearing treatment. Using a 2 × 2 factorial arrangement, we reared 288 gilts in either female-only (FEM) or mixed-sex (MIX) groups, and fed them either a standard finisher diet (CON), or the same diet supplemented with copper, zinc and manganese (SUPP). We expected improved limb health, welfare and performance both during rearing, and subsequently, in gilts in FEM and SUPP, and that the effects would be additive. During rearing, FEM gilts were less exposed to sexual mounting/aggression, and had improved hoof health (specifically less heel sole separation and white line lesions); the SUPP diet also reduced heel erosion. At breeding age gilts from SUPP had a greater aBMD than CON, and gilts reared in FEM had fewer cartilage lesions. The effect of rearing strategy on hoof health carried over into the early parities; FEM gilts had lower total hoof lesion and heel erosion scores, and SUPP gilts had lower horizontal crack scores. However, we found very few effects on levels of C2C or CPII. Thus, both rearing strategies had benefits for animal welfare, but validation of bone cartilage turnover biomarkers in pigs requires further research.

General introduction

General introduction

Replacement gilts are the most valuable animals in the herd, as they are an investment with the potential to generate a profit (Stalder *et al.*, 2003). Nevertheless, approximately 40 to 50% of sows are culled before their third or fourth parity (D'Allaire *et al.*, 1987; Boyle *et al.*, 1998; Schenck *et al.*, 2008); as sows only become profitable after their third parity (Stalder *et al.*, 2003), this means that potentially half of sows do not generate profit. Besides being an economic problem, high levels of involuntary culling could indicate welfare problems in the herd. Lameness in pigs is a significant welfare issue (Anil et al. (2007), and is a major reason for poor longevity in sows (Engblom *et al.*, 2007; Jensen *et al.*, 2010). Internationally, locomotory issues generally account for 13% of all sow cullings, and over half of these females have not attained their second parity (Lucia *et al.*, 2000). Indeed Dijkhuizen et al. (1989) suggested that the greatest economic losses related to culling were due to locomotory problems. However, in relation to Irish culling patterns the only available data showed that roughly 32% of animals culled for lameness had only produced a single litter (Boyle *et al.*, 1998). Although this paper is quite old, it is unlikely that this has changed greatly considering that the sow replacement rate in Ireland was 54.5% in 2017, the second highest of the InterPig countries (Interpig, 2018).

The aetiology of lameness in pigs is multifactorial, and risk factors include concrete flooring in general, and particularly slatted concrete; (KilBride *et al.*, 2009), stocking density and pen size (Willgert *et al.*, 2014b), housing type, growth rates, nutrition (Quinn, 2014) and group housing systems - especially those containing entire males due to added mounting and aggression (Boyle and Bjorklund, 2007; O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014). In recent years there has been scientific interest in the way replacement gilts destined to enter the breeding herd are reared, particularly with regard to investigating risk factors for lameness (Quinn *et al.*, 2015). This is because rearing conditions for replacement sows can affect their development and skeletal system which can affect their health in later life (Dewey, 2006). The majority of skeletal development occurs in young animals, which indicates that the most important time for preventing muscular-skeletal issues is during early development (Loitz and Zernicke, 1992; Umemura *et al.*, 1995; Iwamoto *et al.*, 2000). Nutrients and management can affect structural integrity (Nakano *et al.*, 1987; Crenshaw, 2006; Ytrehus *et al.*, 2007; Crenshaw *et al.*, 2013), and sow mortality can be associated with lameness due to structural complications (Kirk *et al.*, 2005; Anil *et al.*, 2009; Mote *et al.*, 2009).

In many countries replacement gilts are reared in a similar manner to finisher pigs and in mixed-sex groups up to breeding age (Boyle and Bjorklund, 2007; Quinn, 2014). This means that in countries that do not practice castration (e.g. Ireland) replacement gilts are kept with entire male pigs during their development, which exposes them to the high levels of aggressive and mounting behaviour that these animals perform (O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014). These behaviours increase the risk of injuries such as limb and cartilage damage (Andersson *et al.*, 2005) which are associated with lameness (Dewey *et al.*, 1993; Jensen *et al.*, 2007; Anil *et al.*, 2009; Quinn, 2014). Joint disorders such as osteochondrosis, and arthritis, and claw lesions, are considered to be the main disorders or diseases which cause lameness (Dewey *et al.*, 1993; Kirk *et al.*, 2005; Heinonen *et al.*, 2006; Anil *et al.*, 2008; Engblom *et al.*, 2008; Jensen and Toft, 2009). Osteochondrosis (OC) is an irregularity of endochondral ossification causing abnormal cartilage (Ytrehus *et al.*, 2007) and physical trauma to this cartilage results in separation of a segment of cartilage from the subchondral bone known as osteochondrosis dissecans (OCD) (Kinzel *et al.*, 1976; Kincaid and Van Sickle, 1981; Nakano and Aherne, 1988). Lameness is usually very difficult to treat, with the exception of infectious causes, where antibiotics can be used as treatment (Nielsen *et al.*, 2001; Heinonen *et al.*, 2013a; Willgert *et al.*, 2014a).

Pigs are intensively reared due to higher production levels as a result of genetic selection which can place a high demand on them physically, and consequently impact their health and welfare (Prunier *et al.*, 2010). Commercial pig breeds have been selectively bred for fast growth rates and lean meat deposition in finishers, and for larger litter sizes and increased weight gain in breeding sows (Arey and Brooke, 2006; Prunier *et al.*, 2010; Quinn, 2014). Finisher pig diets are formulated to maximise this genetic potential, and optimise growth-rate and high lean meat deposition (Harper *et al.*, 2002); they are thus not designed to meet the needs of growing replacement gilt. A gilt rearing diet should be formulated for fat deposition and fortified with specific minerals to establish strong bones and legs, and consequently increase longevity (Close and Cole, 2000; Quinn *et al.*, 2015). The addition of zinc (Zn), copper (Cu) and manganese (Mn) to the diet of breeding sows reduces the incidences of heel erosion, heel overgrowth, white line lesions (Nair and Anil, 2011), lameness, and leg abnormality prevalence (Ferket *et al.*, 2009). Thus these minerals could also prove to be beneficial to growing gilts; in improving structural integrity during a period of growth. Zn and Mn are particularly important for hoof horn production; Zn has a vital role in cell repair and replacement, and Mn in the formation and maintenance of cartilage and bone in cattle; deficiency is associated with short/weak bones (Mohamadnia, 2008; van Riet *et al.*, 2013). Minerals can play other important roles in promoting sow longevity; for instance Cu is essential for activation of enzymes (Tomlinson *et al.*, 2004). Copper is needed for activation of lysyl and thiol oxidases for structural integrity of cells (NRC, 2001). Of greatest importance thiol oxidase is necessary for the keratinizing of horn cells (O'Dell, 1990). Copper activates thiol oxidase enzyme, which is responsible for formation of the disulfide bonds between Cys residues of keratin filaments (O'Dell, 1990). This process is essential for structural strength on the cellular Although several studies have been published demonstrating the benefits of these minerals when supplemented during rearing, or during gestation, there is less work investigating how supplementation during rearing alone could have an impact on subsequent sow welfare and performance.

OC is a chronic degenerative joint disease (Dewey, 2006; Nalon *et al.*, 2013) which can result in leg weakness, and consequently difficulty in standing, an unsteady gait, and lameness (Ohata, 2001). Many studies suggest that OC is a cause of lameness, and that it is common in gilts and young sows (Arai *et al.*, 2009). It is a degenerative disorder, and thus if it is present during rearing, likely to persist into later life. However, OC lesions do not always produce clinical signs of pain and discomfort, as seen in horses even with severe pathological damage (Jeffcott, 1996). Thus biomarker identification may aid in the selection process of breeding animals against OC and help reduce the economic and animal welfare consequences of this disease (Frantz *et al.*, 2010). However, there are only a few biomarkers of bone or cartilage turnover that have been evaluated in pigs (Carter *et al.*, 1996; Weiler *et al.*, 2001; Shaw *et al.*, 2006). However, Frantz et al. (2010) found that CPII and C2C were significantly related to OC progression in pigs, when levels were evaluated relative to histological indicators of early OC development. Moreover, it is known OC is associated with pain (Frantz, 2006) and thus it can be assumed that progressive damage to the joints could ultimately cause chronic stress, even if animals are not demonstrably lame. Stress is caused by an inability to cope with the environment, and can cause subfertility and impair genetic potential (a delay in conceiving) (Dobson and Smith, 2000). Prolonged stress, can thus disrupt reproduction in female pigs (Turner *et al.*, 2005).

A stressful prenatal environment can lead to permanent postnatal changes in offspring metabolism (Armitage *et al.*, 2004; Wu *et al.*, 2006; Hill *et al.*, 2010). Both Haussmann et al. (2000) and (Otten *et al.*, 2000) indicated that prenatally stressed piglets (caused by Adrenocorticotropic hormone (ACTH) application to the mother) tended to be lighter than piglets from gilts not exposed to ACTH. Prenatal stress is also associated with low birth weights in rodents and humans (Welberg and Seckl, 2001). With regard to lameness specifically, Parada et al. (2017) found that this disorder in pregnant gilts had negative effects on their offspring's growth.

We hypothesised that rearing gilts in female-only groups and provided with a diet with supplementary levels of Cu, Zn and Mn would result in improved locomotory ability, higher bone mineral density and lower cartilage lesion scores, have a positive effect on bone cartilage turnover, reduce exposure to sexual and aggressive behaviours during rearing, and have carry-over benefits into breeding life, thus improving sow welfare, longevity and reproductive performance.

Ultimately we expected that application of both strategies would help to optimize the growth, physical development and welfare of young female pigs destined to enter the breeding herd. We focused on determining the condition of the gilts post rearing at breeding age to capture in detail the nature and amount of damage to the joint cartilage. We also aimed to develop an improved method of scoring joint surface lesions, and compare this to a previously published version. We then investigated the carry-over effects of these rearing strategies on aspects of sow welfare in their early parities, and on their lifetime performance. Additionally, we intended to validate the analytes C2C and CPII as biomarkers of OC to create a method of identifying animals for breeding selection by means of blood sampling live animals, and to determine whether rearing conditions affected the indicators of bone cartilage turnover.

Literature review

1 Literature review

1.1 Background

Intensification in commercial pig farming since the 1950s has altered farm productivity, pig physiology and animal welfare (Arey and Brooke, 2006; Mills and Marchant-Forde, 2010; Prunier *et al.*, 2010). Intensification associated changes to animal management, such as barren environments (Marchant-Forde, 2009), confinement (Fraser, 2005; Goldberg, 2016), indoor rearing and fewer production units but larger in size (Fraser, 2005). This intensification reduced production costs and labour requirements, and as a consequence there is an increased requirement for welfare monitoring associated with physiological challenges (Mills and Marchant-Forde, 2010; Prunier *et al.*, 2010). These challenges are linked to the fact pigs have been, and continue to be, selectively bred for fast growth and to produce larger litters (Arey and Brooke, 2006). Such increases to the pigs' physiological demands have led to health and welfare problems such as leg weaknesses and disease vulnerability (Arey and Brooke, 2006; Marchant-Forde, 2008; Prunier *et al.*, 2010), thus impacting sow health, reproductive performance and longevity. Therefore to optimise welfare and output, rearing strategies that promote long term good health and reproductive performance must be identified. For a pig farm to be efficient and profitable the lifetime performance of animals in the breeding herd is extremely important.

The most valuable animals in the herd are the reproducing females (Schenck *et al.*, 2008) as they are an investment with the potential to generate a profit (Stalder *et al.*, 2003). A pig producer will pay a gilts' direct costs (rearing, breeding, vaccinations, etc) which is estimated at €550 (Zinpro, 2018) with the expectation of prolificacy and longevity; however, approximately 40 to 50% of them are culled before their third/fourth parity (D'Allaire *et al.*, 1987; Boyle *et al.*, 1998). This invested cost is lost if the sow dies, although currently up to €150 can be recovered if sow was sold to the abattoir (Zinpro, 2018). Sows become profitable after their third parity so this is of significant economic concern for pig producers (Stalder *et al.*, 2000). Currently in Ireland the annual sow culling rate on a pig farm is 49.3% (PIGSYS, 2018).Voluntary culling is the removal of animals from the herd for reasons related to production e.g. age, milk conformation. An animal will be selected for removal to be replaced by an animal of a higher production potential. Involuntary culling is the removal of animals on the basis of health or fertility problems for example, lameness or not becoming pregnant after servicing. In Europe the sow replacement range is 35-55% (Zinpro, 2018) with the ideal replacement rate falling between 40-50% (Hartnett *et al.*, 2019b). A low replacement rate (below 40%) leads to too many old sows remaining on the farm beyond their peak productivity and a high replacement rate leads to too many gilts in the herd, as they are brought in to replace culled animals (O'Driscoll, 2015). Ireland has a very high replacement rate of 54% in comparison with other Interpig countries such as Spain, Netherlands and France ranging from 43-46% (InterPig, 2016).

Lameness is a common cause of poor animal welfare and an economic challenge for pig producers (Dewey *et al.*, 1993; Rowles, 2001; Dewey, 2006; Anil *et al.*, 2009). It is also the second most significant reason for the premature culling of sows, after reproductive problems (Hughes and Varley, 2003; Deen *et al.*, 2007). The most recent detailed survey of Irish culling patterns showed that roughly 32% of animals culled for lameness have only produced a single litter (Boyle *et al.*, 1998), indicating that it is a significant cause of involuntary culling. Thus reducing incidence and severity of lameness could yield significant economic and animal welfare improvements in Irish pig farming.

1.2 Replacement gilts

Gilts are female pigs that have not farrowed a litter and are intended for slaughter or breeding purposes, by replacing older/injured sows in the breeding herd. The lifetime potential for gilt productivity is dependent upon their growth and development, as this determines their ability to reach puberty, their lifetime reproductive performance and their structural soundness as they get older (Vallet, 2015).

In female pigs the age of puberty is usually between 190-230 days. Puberty can be defined as when the expression of behavioural oestrus coincides with ovulation (Knox, 2015). However, sexual maturity is considered different to puberty, as often gilts can express oestrus but do not ovulate or ovulate and not express oestrus (Knox, 2015). Therefore, sexual maturity happens after puberty. Since sexual maturity is difficult to measure, other factors are considered such as symptoms of oestrus and also the weight, age and body condition of the gilt (Knox, 2015). Ideal weight is approximately 136kg, around 200 days of age (Knox, 2015) with certain structural conditions also influencing a sow's lifetime performance in the breeding herd (Serenius and Stalder, 2007; Stalder *et al.*, 2008). Visual inspection can be used to identify structural problems such as "buck-kneed" front legs, straight rear pasterns and swaying hips - all of which have been shown to negatively affect sow longevity. Similarly, "soft" front pasterns, a condition shown to have a positive impact on sow lifetime performance in the breeding herd can also be seen by visual inspection (Serenius *et al.*, 2006; Stalder *et al.*, 2008). Training is required for gilt selection due to the task of selecting gilts who are structurally and reproductively sound (Stalder *et al.*, 2008).

Reproduction can begin once the conditions for maturity along with the external environmental conditions are suitable (Knox, 2015). Environmental conditions include air quality, ambient temperature, photoperiod, exposure to boar pheromones, floor type, floor space and stocking density (Levis, 2000; Knox, 2015). Air quality is affected by moisture levels, toxic gases, microorganisms and solid airborne particles (Hartung, 1993; Verstegen *et al.*, 1993; Levis, 2000), and it has been found that air quality has more of an effect on younger gilts with high ammonia levels delaying onset of puberty (Levis, 2000). Gases such as ammonia, and also dust can effect a gilts respiratory health and sensitivity to detect pheromones released by boars (Knox, 2015). Elevated ambient temperature can cause infertility in replacement gilts, due to heat stress and Flowers *et al.* (1989) found that chronic heat stress in replacement gilts from 150-230 days of age at 32 ◦C caused 80% of gilts not to cycle. The ideal photoperiod for developing gilts is suggested to be 10-12 hrs. a day of broad spectrum light (270 to 500 lux) by Levis (2000). Exposure to a boar is a well-known method of gilt puberty stimulation, which includes sight, sound, smell and physical contact between the replacement gilts and the mature boar (Levis, 2000). There has been an indication that rearing replacement gilts on partial slatted flooring is better than fully slatted flooring, due to a reduced risk of hoof and leg injuries (Levis, 2000) which can negatively affect longevity. It has been indicated that limited floor space is associated with stress and aggression, leading to delayed puberty (Knox, 2015). It has also been found that rearing gilts in groups of more than 50 pigs delays puberty (Levis, 2000).

When a gilt is ready for breeding at sexual maturity she will have an oestrus cycle (a recurring period of sexual receptivity and fertility, also known as 'heat'), and she should show normal oestrus expression; this involves a standing heat over 2 days, cycled in a regular 3 week interval. These maturation conditions must be accompanied by the physiological conditions of an ovulation and a uterus capable of holding piglets, accompanied by a first oestrus expression which is not serviced. This is to ensure the uterus and neuroendocrine tissues have already been exposed to progesterone before actual servicing at the second oestrus.

1.3 Animal welfare

Animal welfare is a multidimensional and compound concept (Mason and Mendl, 1993; Fraser, 1995). Any definition needs to state exactly the relevant dimensions under consideration, to allow for a supportive set of measures which can be used to assess the overall state of the animal with regard to its welfare (Tuyttens *et al.*, 2010). The Farm Animal Welfare Council, is an independent advisory body established in the UK in 1979, which has since been replaced by the Farm Animal Welfare Committee in 2011, and which safeguards and promotes welfare of farm animals. This committee set out the most well-known animal welfare indicators known as the 'Five Freedoms' (FAWC, 1993; Tuyttens *et al.*, 2010). These are 1) freedom from thirst, hunger and malnutrition, 2) freedom from discomfort, 3) freedom from pain, injury and disease 4) freedom to express normal behaviour and 5) freedom from fear and distress. These have been used extensively in research and have modelled a check list in terms of welfare standards (Tuyttens *et al.*, 2010). However, (Mellor, 2016) found that we need to consider more nuanced knowledge of biological processes that are fundamental to understanding animal welfare so therefore suggests the five freedoms is somewhat outdated with the aim to move away from "five freedoms" towards "A life worth living". The negative experiences such as thirst, hunger and discomfort are natural feelings which motivate animals to act in a particular way to obtain life sustaining resources and to avoid physical harm, in turn these mildly negative experiences can induce animals to engage in rewarding activities which generate experiences of comfort and pleasure (Mellor, 2016).

Welfare is an intrinsic characteristic of an animal, and it can vary from very poor to very good (Broom, 1991). Definitions of animal welfare vary, and factors such as cultural, scientific, religious and political background all contribute to these definitions (Swanson, 1995). Definitions based on politics are defined by legislations to create a minimum standard which is accepted by society that can be interpreted by authorities. Definitions of a public nature result from the knowledge of the society, their religious beliefs and their empathy for animals. Scientific definitions come from objective measures of welfare (Gonyou, 1993). The science of animal welfare is a relatively new scientific field, and although it is a complex subject however, the foundation of knowledge in this area is expanding thus there is increasing challenges and questions arising from research findings and also from societal needs

(Marchant-Forde, 2015). Welfare scientists continue to use both traditional and new welfare indicators (Marchant-Forde, 2015).

1.4 Lameness

Lameness is a term which covers a broad range of locomotory problems and is common in most animal production systems, including pig production. It is a significant health (Deen *et al.*, 2007) and welfare (Nalon *et al.*, 2013) concern on pig farms, affecting both breeding and fattening herds (Nalon *et al.*, 2013). Lameness isn't a specific disease; it is a clinical symptom of a variety of structural and functional conditions which result in observable changes to an animals' gait (Weishaupt, 2008; Nalon *et al.*, 2013) and posture, and which negatively affects locomotory ability due to pain and irritation (Nalon *et al.*, 2013).

Lameness thus reduces welfare not only because of the associated pain and discomfort, but because the pigs ability to interact with its environment and access resources is also hindered (Dewey *et al.*, 1993; Anil *et al.*, 2002; Jensen *et al.*, 2007). Economically, lameness results in an increased work load, increased veterinary expenses, and increased involuntary culling, thus leading to a reduction in farm productivity (Dewey *et al.*, 1993; Pluym *et al.*, 2011). It can also result in frequent use of antibiotics (Christensen *et al.*, 1994), and thus has effects on animal and human health and welfare which goes beyond individual animals and farms. The impact of high lameness levels can ultimately result in increased anti-microbial use, and thus contribute to the risk of anti-microbial resistance.

Lameness in sows is mainly caused by both claw lesion and joint pathologies, in contrast to other farmed species such as dairy cows and sheep, where hoof lesions are the primary cause (Mulling, 2019). Indeed joint disorders such as osteochondrosis and arthritis, and claw lesions are considered to be the main disorders or diseases which cause lameness (Dewey *et al.*, 1993; Kirk *et al.*, 2005; Heinonen *et al.*, 2006; Anil *et al.*, 2008; Engblom *et al.*, 2008; Jensen and Toft, 2009). Limb pathologies such as infectious arthritis and osteochondrosis can impact locomotory ability and result in lameness (Jensen *et al.*, 2007; Jensen *et al.*, 2009). Lameness can also arise from physical injuries such as claw lesions, joint lesions, muscle damage, tendon damage and bone fractures (Jensen *et al.*, 2009; Jensen and Toft, 2009). It is usually very difficult to treat, with the exception of infectious causes, where antibiotics can be used as treatment (Nielsen *et al.*, 2001; Heinonen *et al.*, 2013a; Willgert *et al.*, 2014a). Thus prevention and early identification are essential.

Lameness in gilts and young sows is highly prevalent. It has been recorded that between 15-20% are culled specifically culled for lameness before their third parity (D'Allaire *et al.*, 1987; Lucia *et al.*, 2000). The scientific literature suggests that in sows, lameness during pregnancy is common in Europe, with a prevalence range of 5-10% in countries such as England, Belgium and Finland (Heinonen *et al.*, 2006; Pluym *et al.*, 2011; Pluym *et al.*, 2013b; Willgert *et al.*, 2014a). As lameness can result in animals removed from the breeding herd prematurely, it can prevent young sows from reaching their full breeding potential, and ultimately increase the cost of production (Willgert *et al.*, 2014a).

1.5 Causes of Lameness

In the pig industry, lameness is seen as a condition which is associated with many inputs, which can be condensed into management factors and genetic factors. The primary causes can be broken down into three categories of leg disorders; Infectious (e.g. infectious arthritis), physical (e.g. fractures and claw lesions) and inherited disorders (e.g. osteochondrosis) (Jensen *et al.*, 2009). The most important management factors include overall herd health, nutrition, flooring type and group management. Sows are mainly kept on fully or partly slatted housing systems in Europe and many other countries (SVC, 1997). While such housing systems are desirable for the management of sows in terms of labour and hygiene, this system does not provide comfort for them (Tuyttens *et al.*, 2008). In group housing systems, especially those which contain entire males, physical trauma can be caused by sexual mounting and aggression (Boyle and Bjorklund, 2007; O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014) which is further complicated by housing on fully slatted floors, resulting in injuries as claws get caught or trapped in the slats (Gjein and Larssen, 1995a; Pluym *et al.*, 2011). Physical trauma due to management of pigs in groups with entire males poses a risk to pigs already suffering from osteochondrosis, as fractures or osteochondrosis dissecans (OCD) may occur. The prevalence of infectious arthritis or osteochondrosis is not thought to be affected by floor type (Jørgensen, 2003). Genetic factors such as structural soundness, leg conformation and claw conformation are important factors in terms of lameness. Claw size and hoof growth are genetic factors which influence lameness in sows, due to risk of claw lesions and injuries thus influencing sow culling (Pluym *et al.*, 2013a). Fan et al. (2009) identified that uneven claw sizes are linked to genetics with the suggestion that genetic selection of replacement gilts with even-sized toes may limit the occurrence of claw lesions. Hoof growth has been reported to be moderately heritable and is linked to an increased culling risk in sows (Quintanilla *et al.*, 2006; de Sevilla *et al.*, 2008; de Sevilla *et al.*, 2009) due to claw lesions (Pluym *et al.*, 2013a). Osteochondrosis is moderately heritable (Aasmundstad *et al.*, 2013) and can be exacerbated by physical trauma.

1.5.1 Lameness Identification

1.5.1.1 Locomotory ability

The most common signs of lameness in pigs include unwillingness to move, slowness, short/uneven strides, vocalisation, and increased swaying when walking (Okholm-Nielsen, 2011; Gregoire *et al.*, 2013). Most scoring systems involve scoring these aspects of locomotion, whether as part of a composite score, or individually. When a pig can put minimal/no weight on an affected limb or if they are completely unable to/refuse to walk, they are considered clinically lame (Main *et al.*, 2000). This is usually easy to identify, even by an untrained observer. However, sub-clinical lameness should also be monitored. This can be visually assessed by observing the locomotion of the pig, but it relies heavily on the ability of the trained observer (Nalon *et al.*, 2013).

Table 1-1: A typical locomotion scoring system, which has been used in experimental work (KilBride *et al.*, 2009)

Score	Description
0	Even strides, Caudal body sways slightly while walking, Pig is able to accelerate and change direction rapidly.
1	Abnormal stride length (not easily identified). Movements no longer fluent: pig appears stiff. Pig still able to accelerate and change direction.
2	Shortened stride. Lameness detected. Swagger of caudal while walking, No hindrance in pigs agility.
3	Shortened stride. Minimum weight bearing on affected limb. Swagger of caudal body while walking. Will still trot and gallop.
4	Pig does not place affected limb on the floor while moving.
5	Does not move.

1.5.2 Specific disorders

1.5.2.1 Osteochondrosis

Osteochondrosis is a non-infectious, degenerative condition which affects the articular-epiphyseal cartilage and growth plate, which causes secondary changes in the bone (Ytrehus *et al.*, 2004b). Osteochondrosis is common in domestic animals (Bohndorf, 1998) and has been investigated in pigs (Dewey, 2006), horses (McIlwraith, 1987) and cattle (Jensen *et al.*, 1981). Indeed it is well known as a common cause of lameness in young athletic horses (McIlwraith, 1987). It is also one of the most important underlying causes of lameness in growing pigs (Nakano *et al.*, 1987; Jørgensen and Andersen, 2016) and lesions can be observed in the joints by visual inspection post mortem. Symptoms include local thickening of articular cartilage, irregular cartilage surface, fissures between cartilage and subchondral bone, osteochondrosis dissecans and necrosis of subchondral bone (Reiland, 1978). Osteochondrosis dissecans (OCD) is described as a separation of a segment of cartilage from the subchondral bone (Nakano and Aherne, 1988).

The development of osteochondrosis (OC) is believed to be associated with genetic factors, growth rates, lean meat percentage and mechanical stress (Nakano and Aherne, 1988; Stern *et al.*, 1995; Kadarmideen *et al.*, 2004). If genetic selection focused on OC, together with an improved ability to detect pigs with this condition, there could be significantly reduced lameness prevalence in pigs on farms.

It is known that pain is associated with OC (Frantz, 2006) however, OCD lesions do not always produce clinical signs of pain and discomfort, as seen in horses even with severe pathological damage (Jeffcott, 1996). In a recent study by Uilenreef et al. (2019) it was found that there was a low correlation between joint damage and the degree of lameness scored and pigs with induced-osteoarthritis. Indeed, some were even considered ‘sound’ during visual assessment by trained observers.

1.5.2.1.1 Blood biomarkers

Biomarkers (biological-markers) are molecules that show an alteration in physiological state compared to the norm (Fung *et al.*, 2000) as a result of synthesis or degradation in certain tissues, in relation to a particular disorder. They are delivered into the blood stream, maintained in the tissue of interest, or excreted in bio-fluids such as urine (Garnero *et al.*, 2000). Biomarker levels are altered when there is an imbalance between the synthesis and degradation processes in a tissue (Garnero *et al.*, 2000; Garnero *et al.*, 2001). Therefore, it is possible that there are biomarkers which potentially correlate with the metabolic activities in the joint and underlying bone (Frantz, 2006). As OC is a degenerative joint disease, these biomarkers could be used for diagnostic purposes with the intention to reflect a disease state (Fung *et al.*, 2000). Indeed biomarker identification may aid in the selection process of breeding animals against OC and help reduce its economic and animal welfare consequences (Frantz *et al.* (2010). Biomarkers of joint metabolism are predictors of joint deterioration, and their identification could allow treatment intervention aimed at preventing disease progression. Moreover, biomarkers could then also be used to measure the effectiveness of treatment (Ofluoglu and Ofluoglu, 2005).

However, there are only a few biomarkers of bone and cartilage turnover that have been evaluated in pigs (Carter *et al.*, 1996; Weiler *et al.*, 2001; Shaw *et al.*, 2006).

In animal models it is possible to correlate biomarkers with physical changes in the joint (Lozada and Altman, 1999; Frantz, 2006). Frantz et al. (2010) evaluated a variety of metabolic biomarkers in the categories of synthesis, degradation, and bone turnover and bone formation. Levels of various analytes investigated have been measured in serum taken from pigs between 5 and 6 months old (Frantz *et al.*, 2010). In this study, the pigs were then slaughtered, their left hind limb extracted, and the stifle joint region (knee) was examined visually focusing on the femoral condyles. After visual inspection the distal end of each femur was sliced using a band saw for histological examination. Levels of the analytes were then compared in animals with and without osteochondrosis dissecans (OCD). The severity of the disorder was also estimated in the cohort of animals, and compared with analyte levels.

Table 1-2: List of analytes studied by Frantz et al. (2010) for biomarker analysis.

Category	Analyte
Synthesis	C-propeptide of type II (CPII)
	Chondroitin sulphate epitope 846 (CS846)
Degradation	Carboxy-terminal telopeptide of type II collagen 3/4-length fragment (C2C)
	Carboxy-terminal cross-linked telopeptide (CTXII)
	Cartilage oligomeric matrix protein (COMP)
Bone turnover	Carboxy-terminal cross-linked telopeptide of type I collagen (ICTP)
	Amino-terminal telopeptide of type I Collagen (NTX)
	Pyridinoline cross-links collagen (PYD)
Bone formation	Bone-specific alkaline phosphatase fragment of type II collagen (BAP)
	Osteocalcin

Frantz et al. (2010) found that CPII and C2C were significantly related to OC progression, when levels were evaluated relative to histological indicators of early OC development.

CPII has also been demonstrated to increase in horses with OC lesions compared to those without (Black *et al.*, 1989; Kollerup *et al.*, 1994; Lindqvist *et al.*, 2005), which suggests that CPII has the potential to indicate OC in swine as the disease process is similar to OC in other species. In an experimental model of arthritis, it was found that elevated levels of C2C in both serum and urine were related to progressive arthritis (Song *et al.*, 1999). Thus these metabolites have potential to aid in osteochondrosis detection in replacement gilts.

1.5.2.1.2 Markers of cartilage synthesis

High levels of cartilage synthesis biomarkers indicate that the body is producing high levels of analytes involved in the production of collagen components.

C-propeptide of type II Collagen (CPII)

CPII is a marker of collagen type II synthesis. Collagen type II is a fibrillar collagen, and the main component of cartilage. It represents around 90% of the collagen content of articular cartilage, which surrounds the end of long bones (Manicourt *et al.*, 2006). When cartilage breakdown is occurring, the articular cartilage attempts to repair the damaged tissue by increasing synthesis of collagen type II (Månsson *et al.*, 1995). There is a presence of a positive feedback loop, with degraded collagen type II stimulating collagen type II synthesis (Oesser and Seifert, 2003). Collagen type II is synthesised as procollagen and during type II procollagen synthesis, the C-propeptide of type II Collagen (CPII) and amino-terminal end of the propeptide are released into the circulatory system prior to mature collagen fibril formation (Nelson *et al.*, 1998).

CPII increases in horses with OC compared to those without (Frisbie *et al.*, 1999; Laverty *et al.*, 2000; Billinghurst *et al.*, 2004). Frantz (2006) suggests that high levels of CPII can be used as an OC indicator in pigs, due to other species presenting similar OC characteristics. It has been found that the progression of joint disease can be analysed through measuring synovial fluid CPII (Lohmander *et al.*, 1996).

1.5.2.1.3 Markers of cartilage degradation

Collagen type II Cleavage (C2C)

C2C is a marker of cartilage degradation. When cartilage is degrading, collagen is broken down into two cleavage products (Frantz, 2006). Type II collagen is the main component of articular cartilage and the rate at which articular cartilage is being degraded can be assessed by measuring the components released during the process (Billinghurst *et al.*, 1997). Increased levels of C2C indicate destruction of type II collagen (Frantz, 2006).

1.5.2.1.4 Blood sampling for biomarker analysis

Blood is considered a suitable bio fluid to use in disease detection due to its ease of collection, large volume, and the variety of markers which can be analysed, particularly compared to urine (Frantz, 2006) and synovial fluid (Manicourt *et al.*, 2006). The main disadvantage of using blood samples for analysing biomarkers is that some markers are present in tiny amounts and thus difficult to detect (Thonar *et al.*, 1993). This is as a result of processing before circulating in the blood stream, as a majority of biomarkers pass through the lymphatic system for degradation (Frantz, 2006). This is further complicated by differences in liver and kidney filtration rates affecting the maker concentrations present (Frantz, 2006). Nevertheless, blood samples can indicate physiological changes in an animal and can be analysed to detect a wide range of metabolites (Frantz, 2006; Manicourt *et al.*, 2006), so are widely used.

1.5.2.1.5 Joint surface examinations

The Elbow joint (figure 1-1) comprises of the humeral condyle (HC), which slots into the trochlear notch (TN) surface (also known as the trochlear groove). The HC is made up of the capitulum and the trochlea. In the elbow joint, OCD frequently occurs in the HC capitulum (Kaji *et al.*, 2015). The humeral condyle (HC) is the most common joint surface affected by OCD in pigs (Jørgensen, 1995).

In previous research abnormalities of the HC were examined for abnormal cartilage surfaces (e.g. invaginations, grooves, defects, and OCD) (Grondalen, 1974; Jørgensen, 1995; Ytrehus *et al.*, 2007; Kirk *et al.*, 2008; Jensen and Toft, 2009). Joint lesions have generally been scored on the HC from 1 (normal) to 4 (severe abnormality), with a score of 5 reserved for cases of osteochondrosis dissecans (OCD; (Jørgensen *et al.*, 1995; Christensen *et al.*, 2010; Busch and Wachmann, 2011; Quinn *et al.*, 2015).

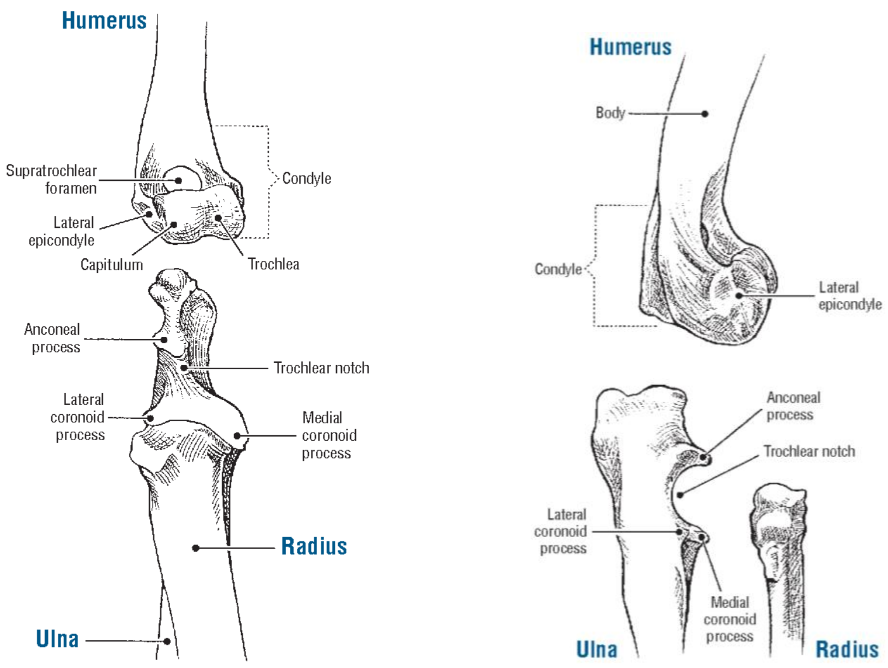

Figure 1-1: Diagram of elbow joint (Orchard Road Veterinary Surgery, 2008)

1.5.2.2 Claw lesions

Prevalence of claw lesions amongst gilts and sows can be as high as 99% (Gjein and Larssen, 1995b; Kirk *et al.*, 2005; Anil *et al.*, 2007; Knauer *et al.*, 2007; Pluym *et al.*, 2011; Pluym *et al.*, 2013b). Claw lesions have been reported to be accountable for 5 to 20% of sow cullings for lameness in a study carried out by Dewey et al. (1993). They are most likely linked to intensive farming strategies; rearing pigs on concrete slats, selection for highly productive sows and fast growth rates are all risk factors (Anil *et al.*, 2007). KilBride *et al.* (2009) stated that the key risk for abnormal gait in finishing pigs and pregnant sows is slatted floors without bedding, compared to soil or solid concrete floors with deep bedding. However, even with such a high prevalence, not all sows express signs of lameness (Pluym *et al.*, 2013a). Expression of lameness (Anil *et al.*, 2007), and reduced performance (Pluym *et al.*, 2012), may be dependent on the location and the severity of the lesion, as some areas of the hoof are more sensitive than others (Deen *et al.*, 2007).

Superficial lesions which are shallow enough to only cause slight damage to skin or horn may not cause the animal any pain and therefore would not be associated with lameness (Anil *et al.*, 2007; Deen *et al.*, 2007). However lesions which are present on the surface of the hoof and extend inwards to affect underlying sensitive tissues can result in irritation and pain. The corium is a modified vascular tissue of the hoof that is highly susceptible to sensitivity, as it is dense with nerves and blood vessels (Brennan and Aherne, 1986; Van Amstel, 2010). Lesions that penetrate this layer cause pain, pathogen entry and risk of a secondary infection (Penny and Hill, 1974; Gjein and Larssen, 1995a; Straw, 2013); (Zoric *et al.*, 2004). It is this type of painful lesion which can lead to overt signs of lameness (Pluym *et al.*, 2011). Moreover, hoof horn production and maintenance relies on the corium for oxygen and nutrients, and thus disruption caused by physical damage or inflammation can impair horn production and result in further lesions (Shearer *et al.*, 1996; van Amstel, 2011).

Various parts of the hoof can be affected by claw lesions depending on the cause of the injury (Quinn, 2014). Ossent (2010) describes the causal factors of hoof damage to be nutrition, aggression, laminitis and overgrown heels. Lesions occur mostly on the outer claws of hind feet; the hind hooves are most commonly affected by heel overgrowth and heel erosion (van Amstel and Doherty, 2010). The heel and the sole of the hoof are also susceptible to separation from one another, as this area is weak due to the meeting of the two types of tissue. Separation at the white line may also occur due to continuation of any separation at the heel sole junction (Ossent, 2010; Van Amstel, 2010). High levels of activity (mounting, aggression, and play) in pigs housed on concrete slatted flooring is known to be injurious to the hooves (Gjein and Larssen, 1995a; Pluym *et al.*, 2011; Quinn *et al.*, 2015); both horizontal and vertical cracks in the hoof horn may occur due to trauma. Indeed animals become more prone to cracks when their hoof or dew claws are overgrown (Ossent, 2010; Van Amstel, 2010), which can result from inappropriate nutrition or flooring. Overgrown hoofs and dew claws in turn are a risk factor for other injuries such as white line separation, dew claw tears and amputations (Ossent, 2010; Van Amstel, 2010; Pluym *et al.*, 2011).

It is possible to visually assess claw lesions in live animals (Nalon *et al.*, 2013). Many lesion scoring systems are adapted from (Gjein and Larssen, 1995b). This classification includes the examination and scoring of six aspects of hoof condition as follows; (i) side wall cracks (ii) white line (iii) heel–toe junction and heel–sole interface, (iv) toe overgrowth and (v) heel lesions and (vi) toe cracks. For each area, the severity of any disorder was scored on a scale from 1 (perfect) to 3 (severe). A more up-to-date version is the Feet First® Lesion Scoring System which is focused on the ability to visually rank the seven most common claw disorders (toe length, heel sole separation, heel overgrowth, white line separation, dew claw length/injury, hoof cracks both vertical and horizontal), based on the individual type of lesion/disorder and its level of severity (1 = mild, 2 = moderate, 3 = severe).

Table 1-3: Claw lesion scoring system (Zinpro Corporation, USA).

Scores	Toe length	Dew claw length	Dew claw injuries	Heel overgrowth and erosion	Heel sole crack	White line damage	Horizontal wall crack	Vertical wall crack
0	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1	≥1 toes slightly longer than normal: Difference detected only when sole is pressed flat	Slightly longer than normal	Short crack or cracks	Flaking of the skin of the heel	Separation at the junction has just begun; length of separation < 0.5 cm	Shallow and/or short separation along white line	Haemorrhage evident, short/ shallow horizontal crack in toe wall	Short/shallow vertical crack in wall
2	≥1 toes moderately longer than normal: difference obvious without flattening sole	Significantly longer than normal	Long but shallow crack or cracks in dew claw wall	Slight overgrowth and/or erosion in soft heel tissue	Separation slightly longer than score 1, but still shallow	Long separation along white line	Long but shallow horizontal crack in toe wall	Long but shallow vertical crack in wall
3	≥1 claws much longer than normal and/or the toes are torn and/ or partially or completely missing	≥1 claws much longer than normal and/or the claws are torn	Multiple or deep crack or cracks in dew claw and or/partially or completely missing	Numerous cracks with obvious overgrowth and erosion	Long separation at the juncture, which is deeper than score 2	Long and deep separation along white line	Multiple or deep horizontal crack or cracks in toe wall	Multiple or deep vertical crack or cracks in the wall
4	≥1 toes significantly longer than normal			Large amount of erosion and overgrowth with cracks	Long and deep separation at the juncture			

1.5.2.3 Bursitis

Adventitious bursitis is a common disorder of the hock in both finishing pigs and in the breeding herd (Penny and Hill, 1974). Bursas are fluid filled sacs which become enclosed by connective tissue cells known as fibroblasts (Mouttotou *et al.*, 1998). The fluid is made up of serum and blood, which is known as seroanguineous fluid – consisting of red corpuscles and lymphocytes. Bursas usually develop on the hind legs below the point of the hock or on the lateral sides of the elbow (Calderon Diaz *et al.*, 2013). Infection is not thought to be involved in the pathogenesis of adventitious bursitis (Smith, 1993); it is believed that the bursas result from pressure on the lymphatic vessels and capillaries, which causes damage to them. Slatted floors and a lack of bedding, which are predominantly used in Irish commercial farms (Quinn, 2014), are associated risk factors for bursitis (Smith, 1993; Mouttotou *et al.*, 1998; Mouttotou *et al.*, 1999). Gillman et al. (2008) and Mouttotou et al. (1999) found that bursa prevalence increases with age. This could be due to an increased amount of time lying on hard surfaces (Ekkel *et al.*, 2003), as well as increasing pressure as pigs grow heavier. Indeed pigs can spend up to 80% of their time lying down (Marx and Mertz, 1989; Ekkel *et al.*, 2003; Tuyttens, 2005).

Bursitis is also associated with other disorders; KilBride et al. (2009) found that there was an increased risk of abnormal gait associated with increasing callus, bursitis, and capped hock score on the limbs of finishing pigs. Thus the presence of bursitis on developing gilts should be monitored.

Bursa are scored by visual assessment and palpation of limb, and usually according to size as follows; 0 indicates no lesion present, 1 indicates a small bursa around the size of a hazelnut, 2 indicates a medium sized bursa around the size of a walnut, and 3 indicates a large bursa around the size of a hens egg (Mouttotou *et al.*, 1998). Other studies record bursa as either present or absent regardless of the size (Bottacini *et al.*, 2018).

1.5.3 Bone Mineral Density

Nutrition, age and mechanical stress are factors which influence bone strength (Hall *et al.*, 1991; Mills and Marchant-Forde, 2010). Bone strength is majorly influenced by bone mineral density, which determines an animal's susceptibility to bone fractures (Ammann and Rizzoli, 2003). Bone fractures in limbs can occur as a result of severe trauma (Engblom *et al.*, 2008), and are known to be highly associated with pain (Jensen *et al.*, 2012). Kirk et al. (2005) suggests that 16% of sow mortality by involuntary culling is as a result of fractures. Thus factors which increase bone mineral density, and consequently strength, should be considered when rearing gilts. Bone mineral density is measured most commonly by dual x-ray absorptiometry. This method is a two dimensional measurement of bone minerals within a certain area (Mitchell *et al.*, 1996; Mitchell *et al.*, 1998; Nielsen *et al.*, 2004; Nielsen *et al.*, 2007; Ryan *et al.*, 2011).

1.5.4 Risk factors for lameness

Management factors which reduce the risk of injuries, and a diet that optimises skeletal and muscular development, are extremely important in reducing the risk of lameness. Thus, as much as possible, strategies for prevention through the control and management of on-farm risk factors should be applied (Fan *et al.*, 2009; Pluym *et al.*, 2013a). Housing systems are a substantial risk factor for lameness. Concrete flooring in general, and particularly slatted concrete, represents a high risk and this is hypothesised to be mainly due to its role in the development of foot lesions (KilBride *et al.*, 2009). However, as the majority of Irish pig farms house their animals on slatted floors, this means that other risk factors besides flooring which could influence the occurrence or severity of lameness need be taken into account. The use of bedding material improves animal welfare (Andersen and Bøe, 1999) which reduces the risk of injury and leg problems (Spoolder *et al.*, 2009); however, due to the predominance of fully slatted floors in Irish pig farms, provision of bedding is not always possible, due to the potential complications regarding the under floor slurry collection system. Alternatively, rubber mats have been demonstrated to improve welfare by reducing limb lesions and lameness in sows (Calderon Diaz *et al.*, 2013). Historically, group housed sows were considered to be more at risk of lameness than stalled sows, and moreover in 2013 group-housing of sows during gestation became mandatory in the EU (Calderon Diaz *et al.*, 2014; Quinn, 2014; Maes *et al.*, 2016). It is known that lameness prevalence is higher in group housed sows compared with stalled sows (Maes *et al.*, 2016). Factors which affect lameness development in group housing are floor space per sow, group size, pen design and flooring. Another reason that group housing is a major lameness risk factor is due to mixing of unfamiliar sows leading to high levels of aggression (Gjein and Larssen, 1995a). However the aggression subsides once the hierarchy has been established in the group (Meese and Ewbank, 1973), and thus development of systems where sows can be maintained in the same static group could reduce this risk. Another risk factor linked to locomotory problems is lack of exercise

(Svendsen *et al.*, 1992; Stavrakakis, 2014), as exercise is linked to increased bone (Stavrakakis, 2014) and muscle strength (Marchant and Broom, 1996). There is more potential for sows to exercise in group housing than when confined in gestation and farrowing stalls, and thus the ability to move freely in group housing systems appears to have mitigated some of the potential negative effects of the system. Exercise is limited in intensive production systems due to confined space (Marchant and Broom, 1996), and thus large groups which can be fed by long trough or electronic sow feeders can provide more 'shared' space.

Several studies have reported that low parity sows are at a higher risk of lameness compared to more mature sows (Heinonen *et al.*, 2006; Pluym *et al.*, 2011). This is likely due to the fact that if a sow survives her early parities without an incident of lameness, she is likely to be somewhat resistant to developing the disorder. Thus employment of a strict culling strategy, whereby animals at risk of lameness are culled from the herd earlier, is somewhat the reason for this (D'Allaire *et al.*, 1987).

1.5.5 Lameness treatment and prevention

Treatment of lameness is dependent on the cause of the disorder. Infectious arthritis is caused by pathogens (Mycoplasma hyosynoviae, Erisipelothrix rhusiopathiae, Haemophilus parasuis and Streptococcus sp.) and can therefore be treated with antibiotics (Jensen *et al.*, 2009). Treatment of physical injuries may require an animal to be isolated from the herd to allow healing, and antibiotics may be required if infection is present. Osteochondrosis (OC) in swine is a highly problematic disease with no known treatment (Frantz, 2006). However, many studies suggest that OC can be treated and/or prevented by nutritional means (Nakano *et al.*, 1983; Frantz *et al.*, 2008; Johnson, 2010). Preventing physical injuries requires changes in housing and management systems e.g. flooring, group dynamics, stocking density and feeding space. To prevent inherited leg problems such as osteochondrosis the control of boar semen and managing growth rates could help alleviate the issue (Jensen *et al.*, 2009).

Ensuring proper prevention and management of lameness can improve sow welfare and production and consequently reduce involuntary culling rates and treatment costs of lameness within the breeding herd (Willgert *et al.*, 2014a).

1.6 Management strategies

1.6.1 Group Composition

In commercial Irish pig farms it is common practice that breeding gilts are reared from weaning onwards with pigs that are destined for slaughter, including entire males. Aggression and mounting behaviours are more frequent in male pigs than in female pigs (O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014), and thus gilts reared with males are exposed to higher levels of these behaviours than if they were in female only groups. This increases risk of injuries such as limb and joint damage, which are associated with lameness. Moreover, it is possible that in mixed sex groups, female animals experience higher levels of stress, which could result in increased cortisol secretion (Jong *et al.*, 2000; O'Driscoll *et al.*, 2013). In humans, increased blood cortisol caused by mental stress or increased physical effort can cause disturbances in bone mineralization and collagen synthesis (Stachowicz and Lebiedzińska, 2016). Thus this is a secondary way in which mixed sex groups could have a negative impact upon limb health.

1.6.2 Sexual behaviour

In Ireland, castration of entire males is not practiced. Although many countries do practice castration, due to welfare concerns (EFSA, 2004) there are significant attempts to phase out the practice entirely within the EU and across the EU (Kristina Andersson *et al.*, 2005). However, rearing entire males in groups does cause other welfare concerns (Kristina Andersson *et al.*, 2005). Compared to castrated males, entire male pigs are more sexually active and aggressive (Cronin *et al.*, 2003; O'Driscoll *et al.*, 2013) and this mounting and fighting can result in leg problems and skin damage (Kristina Andersson *et al.*, 2005)

As male pigs mature their levels of testosterone rise, and therefore it is not surprising that levels of sexual behaviours also increase as the animals age (Giersing *et al.*, 2000). Sexual behaviour in pigs is typically expressed as mounting behaviour. This behaviour poses a risk to animal welfare as it is associated with skin lesions and bruising (Faucitano, 2001; Harley *et al.*, 2014) as well as more serious injuries such as fractures of the legs and lameness (Rydhmer *et al.*, 2004). Sexual behaviour is much more common is mixed sex groups compared to female only groups (Rydhmer *et al.*, 2004; Björklund and Boyle, 2006). It is also likely that sexual behaviour disturbs all pigs in the pen, not only the one being mounted on (Rydhmer *et al.*, 2004).

1.6.3 Aggression

The predominant causes of aggression in pigs are attempts to gain access to resources, establishment of a relationship with a new animal, and maintenance of a hierarchy within a pen (Meese and Ewbank, 1973; Lynch *et al.*, 1984; Gonyou, 2005; Spoolder *et al.*, 2009). Due to increasing levels of testosterone, aggression increases with age in male pigs (Giersing *et al.*, 2000). Aggression can cause leg problems, especially when management systems do not allow animals to avoid interactions easily, and when floors do not provide sufficient claw support during aggressive interactions (Spoolder *et al.*, 2009). This is especially a risk on slatted floors, as claws can get caught in them, resulting in tearing, particularly to the dew claws. Dewclaws that have been completely torn off leave the corium exposed, which is believed to be extremely painful for the animal (Pluym *et al.*, 2011).

In pigs, aggression is greater in male only and mixed sex groups, than in female only groups (Boyle and Bjorklund, 2007; O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014). In other species such as rats, males also engage in more play-fighting (which is considered social play) than females (Pellis *et al.*, 1997). As male pigs mature their levels of testosterone rise, and therefore it is not surprising levels of mounting and aggression rose as these animals age (Giersing *et al.*, 2000). Some studies have suggested that aggression reduces as the animals get older due to a greater focus on mounting (Clark and D'Eath, 2013); however this may be due to space restrictions, limiting space for parallel pressing and therefore interfering with aggressive motor patterns (Straw, 2013).

1.6.4 Body lesions

The physical consequences of aggression can be seen in the form of cuts/scratches on the surface of the skin, and thus scoring of skin lesions is often used as a proxy for measuring levels of aggression. Lesions at the front of the body are associated with reciprocal fighting, whereas when a pig mainly tries to escape from fighting there are more lesions present towards the rear of the body (Turner *et al.*, 2006). Non-castrated males have more lesions than female pigs, believed to result from fighting and mounting (Rydhmer *et al.*, 2006).

Indeed previous studies found that pigs in mixed sex groups have higher body lesion scores than pigs reared in female only groups and this reflects higher levels of aggression, sexual behaviour and activity (Turner *et al.*, 2006; Teixeira and Boyle, 2014). Body lesions are physical injuries which are linked to limb health in both gilts and sows (D'Allaire *et al.*, 1987; Lucia *et al.*, 2000; Deen *et al.*, 2007; KilBride *et al.*, 2009; Pluym *et al.*, 2011; Calderon Diaz *et al.*, 2013). They are associated with a reduced sow performance and welfare and due to their obvious visibility they can help identify animal state (Deen *et al.*, 2007).

1.7 Nutrition

Specifically formulated diets are required to optimise the processes of bone modelling and remodelling (i.e. musculoskeletal growth and maintenance) in pigs. This is to ensure the processes are supplied with essential materials (van Riet *et al.*, 2013). There is evidence that osteochondrosis development can be influenced by the nutrition of the developing gilt and sow. For instance, Faba et al. (2018) found that the inclusion of micro minerals, such as Cu and Mn, can reduce joint lesion presence and severity, as did Quinn et al. (2015). Another significant risk factor for osteochondrosis development, which can be somewhat managed by the producer, is a high growth rate. As most Irish replacement gilts are managed with finisher animals, they consume a finisher pig diet which is designed to favour fast growth and lean meat deposition. Formulation of a specific gilt 'developer' diet has been shown to result in a more gradual weight gain, and reduce lameness incidence, joint lesions (i.e. osteochondrosis incidence), and claw lesions (Quinn, 2014). This was achieved by adjusting the energy: lysine ratio, as well as the inclusion of supplementary minerals.

1.7.1 Minerals

There are few established guidelines with regard to trace mineral requirement, and often gilt diets are developed using levels appropriate for finisher pigs, or gestating sows; in fact there are no trace mineral recommendations for developing gilts in the most recent edition of the Nutritional Requirements for Swine (NRC, 2012). Current published nutrient requirements by the NRC recommend levels for sows which were evaluated based on growth and reproductive performance. However, Van Barneveld and Vandepeer (2008) maintain that vitamin and mineral levels should be higher, to allow for the maintenance of optimum hoof health. Due to a lack of knowledge in this area it is difficult to state if excess mineral supplementation has a beneficial effect on hoof health (Pluym *et al.*, 2013a).

Nevertheless, it is known that nutrition plays a part in preventing claw lesions (Kroneman *et al.*, 1992; Kroneman *et al.*, 1993; Cameron, 2012) and recent studies suggest that supplementing copper (Cu), zinc (Zn) and manganese (Mn) may reduce claw lesion incidence in sows (Anil *et al.*, 2009). Nair and Anil (2011) found that the addition of supplementary trace minerals improved claw health in pregnant sows, and they also reduced the prevalence of lameness and leg abnormalities (Ferket *et al.*, 2009). Specifically, the addition of trace minerals Cu, Zn and Mn to the diet of the breeding sow herd resulted in the reduction of heel erosion, heel overgrowth, and white line lesions (Nair and Anil, 2011). Zn is particularly important for horn production and skin health due to its vital role in cell repair and replacement (Tomlinson *et al.*, 2004; Mohamadnia, 2008; van Riet *et al.*, 2013) . Van Riet et al., (2018) found that the addition of dietary Zn to a basal diet had minor albeit positive influences on claw health in sows. Mn plays a vital role in horn production and the formation and maintenance of cartilage and bone in cattle; deficiency is associated with short/weak bones (Mohamadnia, 2008; van Riet *et al.*, 2013). Finally, Cu is essential for activation of enzymes involved in keratinisation of horn walls (Tomlinson *et al.*, 2004).

There are negative impacts associated with excess trace minerals. Trace minerals are costly to the environment. Lopex-Alonso and Miranda (2012) stated that a significant proportion of minerals fed to livestock are excreted into urine and faeces, and this proportion increases as the margin of mineral supply over mineral requirement increases (Suttle, 2010). Intensive farming puts pressure on many ecosystems and contributes to global environmental problems coupled with excessive mineral supplies a ‘mineral footprint’ is created (Suttle, 2010). Therefore, it is important to understand the levels of mineral requirements for each animal when formulating diets.

1.7.2 Gilt nutrition

There is much understanding of gestating and lactation sow nutrition requirements; however there is a lack of knowledge with regards to optimum gilt rearing diets. Indeed Pluym *et al.* (2013b) stated that the dietary requirements for gilt development to promote hoof health require further understanding. It is widely known that replacement gilts are vulnerable to lameness due to inadequate nutrition during the developmental stage of rearing (Quinn, 2014). Nevertheless, Boyd et al. (2002) found that the majority of pig farmers feed finisher diets to breeding gilts up until service age, a diet which is formulated for fast growth, high lean meat deposition and cost efficiency (Harper *et al.*, 2002; Quinn, 2014). Gilts which are fed finisher diets during rearing may thus not be supplied with the correct balance of minerals to satisfy the nutritional requirements for long term reproductive performance, and cartilage and bone formation and integrity (Levis *et al.*, 1997; Gill and Taylor, 1999; Knauer *et al.*, 2012).

As an alternative to a finisher diet, and with good intentions with regard to optimising gilt nutrition, some producers feed gestating sow diets which have been formulated for fully developed sows, to developing gilts (Quinn, 2014; Quinn *et al.*, 2015). Gilts require a diet to meet their physical growth demands to support bone development and fat deposition (Quinn, 2014). Nutrients such as calcium (Ca) and phosphorus (P) are required for bone development and maintenance (van Riet *et al.*, 2013), so to establish strong legs and thus longevity (Close and Cole, 2000; Quinn *et al.*, 2015). Crenshaw et al. (2013) found that there is no requirement to increase dietary mineral supplementation with age as sows have the ability to retain trace minerals during gestation.

Thus a sow diet may not contain the required levels of Ca and P for a developing gilt (Quinn *et al.*, 2015). Nevertheless, although a gestating sow diet may not meet the requirements of developing gilt, it is closer to meeting these demands than a finisher diet. For example in the study carried out by Quinn *et al.* (2015) the ideal developer diet contained Ca 0.76; P 0.49 mg/kg while the nutrient levels were Ca 0.61; P 0.37 mg/kg in the standard finisher diet and Ca 0.69; P 0.46 mg/kg in the gestating sow diet, which were calculated from standard book values.

1.8 Reproductive performance

Producing gilts that portray sexual maturation, have the ability to retain a pregnancy at an early age and continue to conceive and produce an acceptable number of healthy, marketable offspring per service over multiple parities is a highly important objective of pig production systems.

Adequate nutrition during growth is required for development of reproductive females (Klindt *et al.*, 1999). Investigation into the potential for improvement in swine reproduction performance by provision of supplementary trace minerals in the diet has yielded limited findings. However, it has been reported that there are several beneficial effects when trace minerals are fed to dairy cows; improved fertility (Uchida *et al.*, 2001), reduction in mastitis and foot lesions (Nocek *et al.*, 2006; Siciliano-Jones *et al.*, 2008), and improved reproductive performance (Nocek *et al.*, 2006; Cope *et al.*, 2009) have all been observed. Additionally, it has been reported that Cu and cobalt (Co) deficiency can result in delayed puberty, decreased conception rate, and increased embryonic mortality in goats. Kreplin and Yaremcio (2009) also reported that cows grazing from a Cu deficient pasture had reduced conceiving ability.

Moreover, Mn is a co-factor in cholesterol synthesis, which is necessary for progesterone and oestrogen synthesis (Keen *et al.*, 2000; Bach *et al.*, 2015). There is affirmation that Zn plays important roles in reproductive function of dairy cows, such as reducing the incidence of abortion and abnormal oestrus (Dufty *et al.*, 1977) and also accelerating first oestrus (Campbell and Miller, 1998). Moreover, other authors found that Cu can also result in improved reproductive performances (Kappel *et al.*, 1984).

A sow needs to be managed well at all stages of her reproductive life. Previous research has highlighted the importance of management practices at different stages of reproduction (Koketsu and Dial, 1997), such as appropriately determining the optimal time for serving (Kaneko *et al.*, 2013), as well as importance of reducing environmental stresses (Iida *et al.*, 2015). Stress is caused by an inability to cope with the environment, and can cause subfertility and impair genetic potential (a delay in conceiving) (Dobson and Smith, 2000).

1.9 Sow performance

There is quite large variation between sows in terms of reproductive performance, even when reared under the same conditions (Iida and Koketsu, 2014). Evaluation, by a pig producer of sow performance in low parities is necessary to ensure appropriate retention and removal of sows (Iida *et al.*, 2015). Sow replacement rates in commercial pig herds are commonly between 40 and 55%, which relates to parity 3 and 4 at removal (Dagorn and Aumaitre, 1979; D'Allaire *et al.*, 1987; Lucia *et al.*, 2000) Consequently, breeding herds generally have high proportions of low-parity breeding females (Iida *et al.*, 2015). Thus, most involuntary cullings are from young breeding females (low parities), and this is generally due to reproductive failure (D'Allaire *et al.*, 1987; Dijkhuizen *et al.*, 1989; Patterson *et al.*, 1996).

Besides culling, poor limb health can have negative effects on other aspects of sow performance; for instance, osteochondrosis can ultimately progress to lameness in later life, and potentially chronic stress. Prolonged stress and associated elevated cortisol levels can disrupt reproduction in female pigs (Turner *et al.*, 2005). Good hoof structure and locomotion is associated with increased litter size, more piglets born alive, and fewer stillborn piglets (Pluym *et al.*, 2013b). Females with locomotary issues may produce less milk during lactation, leading to poor litter performance and increased piglet mortality (Lucia *et al.*, 2000).

1.10 Offspring performance

A stressful prenatal environment can lead to permanent postnatal changes in offspring metabolism (Armitage *et al.*, 2004; Wu *et al.*, 2006; Hill *et al.*, 2010). In pigs, several previous studies have found an effect of pre-natal stress on offspring performance, both when it is artificially applied (e.g. by application of ACTH (Otten *et al.*, 2007; Otten *et al.*, 2015), or when animals are submitted to social stress (Rutherford *et al.*, 2012), or affected by an illness (e.g. lameness (Parada *et al.*, 2017)). Both Haussmann et al. (2000) and Otten *et al.* (2000)indicated that prenatally stressed piglets (caused by ACTH application to the mother) tended to be lighter than piglets from gilts not exposed to ACTH. Prenatal stress is also associated with low birth weights in rodents and humans (Welberg and Seckl, 2001). With regard to lameness specifically, Parada et al. (2017) found that this disorder in pregnant gilts had negative effects on their offspring’s growth and behaviour. Rutherford *et al.*

(2012) found that socially stressed pregnant pigs can have offspring with negatively affected weight gain post-weaning.

Amdi *et al.* (2013) found that feed-restricted gilts had higher salivary cortisol levels during gestation, indicating greater levels of physiological stress, and subsequently these gilts gave birth to lighter piglets. Furthermore, the type and timing of a pre-natal stressor could affect the outcome for the piglets (Amdi *et al.*, 2013).

1.11 Conclusion

There is very little scientific knowledge available about how to balance appropriate nutrition for the promotion of good limb health, in developing sows. Currently, there are no longitudinal studies evaluating group composition and mineral supplementation in gilts which is required to understand the impact of these rearing strategies on gilt development, thus their lifetime performance.

The objectives of this thesis are to provide novel information on how to effectively manage developing gilts to reduce the incidence and severity of limb health problems, thus improving the welfare of the animals. It is important that we identify nutritional strategies that will help producers to reduce involuntary culling of gilts and sows. The aim of the study is to investigate if increased levels of trace levels (Cu, Zn and Mn) added to a standard finisher diet would improve limb health, relative to gilts without supplementation. In addition, we will investigate the effect of rearing replacement gilts separate to males during the growing period due to the well-established association between male pigs and increased sexual and aggressive behaviours. The application of both strategies during the gilt rearing period will be investigated and longitudinal data will be collected until the end of their lives.

Chapter 2 - The effect of group composition and mineral supplementation on gilt behaviour and welfare

2 The effect of group composition and mineral supplementation on gilt behaviour and welfare

2.1 Introduction

In Ireland replacement gilts are reared with pigs destined for slaughter, including entire males (Quinn, 2014). This means that they are exposed to high levels of aggressive and sexual (i.e. mounting) behaviour (Boyle and Bjorklund, 2007; O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014). These behaviours increase the risk of injuries such as limb and cartilage damage (Andersson *et al.*, 2005) which are associated with lameness, a major cause of culling in early parities (Dewey *et al.*, 1993; Jensen *et al.*, 2007; Anil *et al.*, 2009; Quinn, 2014). Old data on Irish culling patterns showed that roughly 32% of animals culled for lameness had only produced a single litter (Boyle *et al.*, 1998). It is unlikely that this has changed greatly considering that the sow replacement rate in Ireland was 54.5% in 2017, the second highest of the InterPig countries (Interpig, 2018).

Finisher pig diets are not designed to meet the needs of a growing replacement gilt, and may not supply appropriate trace minerals to promote longevity (Quinn *et al.*, 2015). The addition of copper (Cu), zinc (Zn) and manganese (Mn) to the diet of breeding sows reduces the incidences of heel erosion, heel overgrowth, white line lesions (Nair and Anil, 2011), lameness, and leg abnormality prevalence (Ferket *et al.*, 2009). Thus these minerals could also be beneficial to growing gilts. Zn and Mn are particularly important for hoof horn production. Zn has a vital role in cell repair and replacement, and Mn the formation and maintenance of cartilage and bone in cattle; deficiency is associated with short/weak bones (Mohamadnia, 2008; van Riet *et al.*, 2013). Cu is essential for antibody development and lymphocyte replication (Tomlinson *et al.*, 2004).

Previous work found improved locomotion scores, reduced weight gain, ADG and average daily feed intakes (ADFI) in gilts fed a restricted diet formulated for fat rather than lean deposition and supplemented with minerals (Quinn *et al.*, 2015). However, that study used terminal line gilts, and furthermore, restricted feeding is difficult to conduct in practice as most maternal line gilts are reared in finisher pig accommodation (Quinn *et al.*, 2015). We hypothesised that simple addition of trace levels of Cu, Zn and Mn to a standard finisher diet would improve limb health, relative to gilts without supplementation. In addition, we expected an added beneficial effect of rearing replacement gilts separate to males during the growing period because of the well-established associated reduction in exposure to sexual and aggressive behaviours.

Ultimately we expected that application of both strategies would help to optimize the growth, physical development and welfare of young female pigs destined to enter the breeding herd.

2.2 Materials and methods

2.2.1 Care and Use of Animals

This study was carried out in the 200 sow unit at the Pig Development Department in Moorepark, Fermoy, Co. Cork, Ireland between December 2016 and August 2017. The experimental work was authorized by the Teagasc animal ethics committee (Approval no.: TAEC136-2016) and licensed by the Health Products Regulatory Authority (License no.: AE19132-P057).

2.2.2 Experimental Design and Treatments

In August 2016, 52 sows in the Moorepark herd were served using maternal line semen from Landrace sires (0153H Longo and 0096H Grande from Hermitage Pedigree Pigs Ltd., The Hermitage, Sion Road, Kilkenny, Ireland). Four batches of sows were served to create four replicates, each three weeks apart (average 13 sows per replicate). A batch refers to animals going through the Moorepark pig farm system at the same time, the system runs in 3 week intervals as farrowing happens every 3 weeks. At farrowing, these sows provided a pool of 677 piglets for use in the experiment. The experimental period was from Feb 2017 (transfer to finisher house of the first replicate of offspring, at 81.3 ± 0.5 days of age; experimental day 0) to August 2017 (breeding age of the last replicate of offspring, 196.3 ± 0.5 days of age; experimental day 115). From this point forward all experimental time-points will be referred to as experimental days instead of days of age

All piglets were weighed and tagged at birth, then back-tested at 21.6 ± 2.38 days of age. For this, each piglet was lifted out the pen and placed on its back on a V board, then held in place by the experimenter. The right hand was placed on the piglet’s thorax with the forefinger between the front legs, and the hind legs were held using the other hand. The test started when the piglet remained still for 3 seconds, and lasted for exactly 60 seconds, determined using a stop watch. The number of resistance attempts (struggles), were counted. At weaning (27.4 ± 0.4 days of age), pigs were weighed, then blocked on the basis of sow, sire, sex, back-test score, wean weight and assigned to one of 32 (8 pens per treatment) separate groups of 12 pigs (96 ± 16.9 pigs per replicate). Each pen had an even distribution of sire (Longo or Grande) and back test score (av. 2, range 0 - 5), and a wean weight average of 7.8 kg ± 1.5. Half of the groups contained only females (FEM) and half of the groups were mixed (MIX, 6 males, 6 females). Four focal gilts were selected from each pen, according to their back test scores; one was a low responder (score 0 - 1), two were picked as medium responders (score 2 - 3) and one was a high responder (4 - 5). Any piglet with a score of 5+ was not included in the trial.

Pigs were assigned to their treatment groups in a 2 x 2 factorial design with the first factor being group composition (FEM or MIX as described above) and the second factor being mineral supplementation. The control diet (CON) represented a standard finisher diet used on commercial pig farms. This was fed to all pigs from entry to the finisher stage (day 0) until 5 weeks into the finisher stage (day 36) when pigs in half of the groups were switched to a similar diet, but supplemented (SUPP) with Availa®Sow minerals (Zinpro Corp, Eden Prairie, Minnesota USA; Table 2-1). This provided additional Cu, Zn and Mn (Table 2-2). Levels of Cu, Zn and Mn exceeded NRC (2012) recommendations for a finisher diet. The supplemented diet was formulated by taking the gestating and lactating sow NRC recommendations into account. All feed was in dry-pelleted form (3 mm).

Table 2-1 Details of the finisher (CON; fed to all pigs from day 0 to day 115) and supplemented (SUPP; for half of the pigs from day 36) diets which were used in the study

Ingredients	CON	SUPP*
Barley	50	50
Wheat	33.50	33.38
Soybean (47%CP)	12	12
Soya oil	1	1
Lysine HCl	0.4	0.4
dl-Methionine	0.1	0.1
l-Threonine	0.12	0.12
Premix[a]	0.1	0.1
Availa®Sow[b]	0	0.1
Phytase	0	0
Salt feed grade	0.5	0.5
Di-Calcium phosphate	1.3	1.3
Limestone flour	1	1
	100.02	100.00
Chemical composition		
Dry matter	89.8	89.8
Crude protein	15.56	15.56
Crude Fiber	3.74	3.77
Total oil	5.06	5.06
Ash	4.48	4.48
Lysine	0.969	0.969
Threonine	0.639	0.639
Methionine	0.337	0.337
Methionine and cysteine	0.639	0.639
Tryptophan	0.182	0.182
Calcium	0.779	0.779
Phosphorous	0.609	0.608
Digestible phosphorus	0.280	0.280
Digestible energy (MJ of DE/kg)d	13.50	13.49

* Supplemented with Availa®Sow (Zinpro Corp)

[a]Premix provided per kilogram of diet Cu, 15 mg; Zn, 30 mg; Mn, 0 mg; Fe, 24 mg; I, 0.15 mg; Se, 0.4 mg.

[b]Availa®Sow provided per kilogram of complete diet, Cu, 10 mg; Zn, 50 mg; Mn, 20 mg.

Table 2-2Mineral inclusion rates in the diets. Values are expressed as mg/Kg (i.e. parts per million, and as a % of NRC recommendations for gestating and lactating sows.

	NRC (mg/Kg)[1]	CON (mg/Kg)	SUPP (mg/Kg)	CON %[2]	SUPP %[2]
Mn	25	25.1	51.45	101%	206%
Zn	100	55.6	122.29	56%	122%
Cu	10	4.5	17.89	45%	179%

[1] NRC gestating and lactating sow requirements

[2] Values in the CON (control) diet and SUPP (mineral supplemented) diet as a percentage of the NRC recommendations.

The pigs were in the weaner stage for 6 weeks post weaning (i.e. until day 0), at which time they were moved to the finisher stage accommodation. They remained in the finisher accommodation in the same groups for 10 weeks (i.e. day 67); at this time half of their pen mates (all of the male pigs and 50% of the females) were sent for slaughter. The remaining 182 gilts stayed in the same pens (6 females per pen) until day 115 when 102 of these gilts were sent for slaughter (weighing 143.6 ± 12.8 kg). The remaining 80 gilts were kept for breeding as replacements.

In both the weaner and finisher accommodation pigs had access to fresh water ad libitum from a drinker, and to feed ad libitum from a single space trough feeder. Weaners were fed a starter feed for the first week, a link feed for the following two weeks, and thereafter a standard weaner diet. The weaner pens (2.4 × 2.6 m) had plastic slatted flooring, and the finisher pens (4 x 2.4 m) had fully slatted concrete floors. The rooms used for the trial contained either 10 or 30 pens. All rooms were mechanically ventilated. The temperature of the weaner rooms was kept at 27 – 28 ˚C for one week post weaning and reduced by 2 ˚C every 2 weeks. Heating was generated by hot water pipes (controlled via computer). Finisher rooms were maintained at 20 ˚C, by mechanical ventilation. Artificial lighting was on from 0700 – 1700 h. There was also natural lighting through the windows. Light to darkness cycle was approximately 12:12 (hours) to allow for normal circadian rhythm.

Weaner pigs were provided with an EASYFIX LUNA 117 (Perssepark, Ballinasloe, Co. Galway, Ireland) floor toy and finisher pigs were provided with two sources of enrichment – a wooden post attached to the side of the pen by a plastic holder and a rubber chew toy (EASYFIX ASTRO 200 Perssepark, Ballinasloe, Co. Galway, Ireland) hanging overhead on chains. Pigs were inspected twice daily and sick/injured animals were treated immediately; all antibiotic treatments were recorded. Gilts were continually monitored from weaning to the end of the experiment and all instances of disease, illness, and death were recorded.

2.2.3 Live-weight

All animals were weighed individually at entry to the finisher stage accommodation (day 0), when the experimental diet was applied (day 36), at slaughter (day 67), and for the remaining gilts, at breeding age (day 115).

2.2.4 Locomotory Ability

Gilts were locomotion scored every two weeks (14.1 ± 3.6 days) from day 0 until they left the experiment (i.e. either at slaughter day 67 or at breeding age day 115). Thus most gilts had at least 7 inspections, with those retained until breeding age having 2 additional inspections. Locomotory ability was scored while the gilts walked on solid concrete for a distance of approximately 30 m, from the front, rear and side of the animal. All observations were carried out by one trained observer who was blind to the treatments. Locomotion was assessed using an adapted version of Calderon Diaz *et al.* (2013) ranging from 0-5 (Table 2-3).

Table 2-3 Scoring system for locomotory ability (Hartnett *et al.*, 2019c) adapted from (Calderon Diaz *et al.*, 2013).

Score	Description
0	Even strides. Pig is able to accelerate and change direction rapidly
1	Pig appears stiff. Abnormal stride, which isn't easily identified. Movements no longer fluid but pig still able to accelerate and change direction rapidly. Caudal swagger evident.
2	Uneven stride. Sensitivity while walking detected on at least one limb. Pig able to accelerate and change direction. Caudal swagger evident
3	Uneven stride, with a stagger. Minimum-weight bearing on affected limb. Slow to move. Obviously lame even to the untrained observer
4	Pig may not place affected limb on floor
5	Does not move

2.2.5 Salivary Cortisol

One saliva sample was collected from each of the focal gilts once per month between 0930 h and 1000 h during the finisher stage (i.e. 4 samples/ gilt). Gilts remained in their home pens during saliva collection, and chewed on a large cotton bud (Salivette, Sarstedt, Wexford, Ireland) until it was thoroughly moistened for approximately 30 - 60 seconds. The samples were placed in tubes and centrifuged for 15 min at 2500 x g, then stored at -20 ◦C until analysis.

Saliva samples were analysed using a commercially available cortisol assay kit (Salivary Cortisol kit, Salimetrics Europe Ltd., Suffolk, UK). Cortisol was detected at a minimum concentration of < 0.003μg/dl. The inter-assay CV (n = 24) was 4.69%, and intra-assay CV (n = 794) was 7.68%.

2.2.6 Animal Behaviour

Animal behaviour was recorded by direct observation for one day every two weeks (14 ± 0.5 days) from day 10 of the experiment. All pens were observed for 5mins five times (i.e. 25 minutes in total/pen) between 9045 h and1708 h. All occurrences of aggressive, sexual and play behaviour were recorded (see Ethogram Table 2-4). Observations were equally distributed across pens for each recording period.

Table 2-4 Ethogram for the recording of behaviour in mixed-sex (MIX) and Female-only (FEM) groups of pigs on two different diets.

	Behavior	Description
Aggression	Inverse pressing	Inverse parallel pressing, the pigs' face front to front pushing their shoulders hard against one another throwing head against neck/flanks of other. Bites can be directed towards the head, neck, flanks of other.
	Parallel pressing	Parallel pressing, the pigs stand side by side and push hard with shoulders against each other throwing the head against the neck/ head of other pig. Bites can be directed towards the head, neck, flanks of other.
	Attack	One pig vigorously bites another and recipient attempts to get away without trying to defend themself/retaliate.
	Bite	Pig bites another pig with a vigorous movement of the head: mouth open, contact made with body.
	Head knock	As for threat but makes contact with head against recipient pigs body
	Threat	Pig swipes head vigorously in the direction of another pig
	Displaced	Pig with head in feeder displaced by another pig due to a threat, bite, climb etc.
Harmful	Tail bite	Pig forcefully bites down on another pigs tail – often reflected in reaction (vocalization, fast movement away) by the recipient
	Ear bite	Pig takes another pigs ear in its mouth and closes jaws around it – often associated with vocalization or swift head movement to extract its ear by recipient pig
	Flank/shoulder bite	Open mouthed bite directed to pigs flank or shoulder
	Bite other	Biting aimed at another part of the body e.g. leg. Not to be confused with aggression
	Belly nosing	Vigorous nosing of another pigs belly
	Climb	Pig places front legs on the back of another either to displace from feeder
	Vocalization	Loud, shrill 'scream' made by pig in response to aggression or being mounted
Sexual	Sexual mount	Pig mounts another, by placing both forelimbs on either side of the recipients flanks, sternum rests on

		mounted pigs loins, may be accompanied by thrusting movements and/or vocalizations by the mounted pig
	Sexual nudging	The boar noses the flanks and vulva of female prior to mounting.
Play	Head jostling	Two pigs engage/toss their heads together as they move around, mouths often open but not biting
	Individual play	Pig prances or jumps up in the air and often changes orientation of the body in mid air
	Locomotory play	Pig canters from one side of the pen to another often associated with head tossing and prancing

2.2.7 Body Lesion Scoring

Focal gilts were scored for body lesions caused by aggression every two weeks from experimental day 1 until day 99 (i.e. 7 recordings per gilt). Body lesions were scored on the back, neck, shoulder, flank and hind quarter on each side of the body, and then summed so that there was one score per gilt at each inspection. The trained observer was blind to the treatments of the gilts being scored. The scoring system is described in Table 2-5.

Table 2-5 Scoring system for body lesions (O'Driscoll *et al.*, 2013)

Score	Description
0	No lesion
1	1 small lesion
2	> 1 small lesion, or 1 red lesion
3	> 1 red lesion
4	1 deep red lesion. Skin has been broken and either fresh blood or scabbing present
5	> 1 deep red or 1 big lesion. Big lesions are as per deep red, but cover a greater area than a linear lesion
	> 1 big lesion

2.2.8 Hoof and bursa scoring

Hoof scoring was carried out by visual inspection on three occasions on day 36, day 70 and day 113. The trained observer was blind to the treatments of the gilts being scored. The gilts were raised 0.75 m above the ground using a hydraulic chute (FeetFirst Sow Chute; Zinpro Performance Minerals, Eden Prairie, Minnesota, USA). The medial and lateral toes, medial and lateral dew claws, sole and heels of both hind feet were inspected and severity of the following lesions was scored; heel erosion, heel-sole separation, white line separation, dew claw length, dew claw cracks, toe length and vertical and horizontal toe cracks. The scoring system was a modified version of the FeetFirst claw lesion scoring guide from Zinpro Corporation. Details of the scoring system are described in Table 2-6.

While the animals were raised in the crate, the hind legs were palpated and bursas were scored as follows: 0 = none, 1= hazelnut, 2 = walnut, 3 = hens egg (Mouttotou *et al.*, 1998). If more than one bursa were present each was scored separately and was summed together for analysis.

Table 2-6 Scoring system for hoof lesions (adapted from Zinpro Feet First® Lesion Scoring System)

Scores	Toe length	Dew claw length	Dew claw crack	Heel erosion	Heel sole separation	White line separation	Horizontal crack	Vertical crack
0	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
1	One or more toes slightly longer than normal: Difference detected only when sole is pressed flat	Slightly longer than normal	Short crack or cracks	Flaking of the skin of the heel	Separation at the junction has just begun; length of separation < 0.5 cm	Shallow and/or short separation along white line	Hemorrhage evident, short/ shallow horizontal crack in toe wall	Short/ shallow vertical crack in wall
2	One or more toes moderately longer than normal: difference obvious without flattening sole	Significantly longer than normal	Long but shallow crack or cracks in dew claw wall	Slight overgrowth and/or erosion in soft heel tissue	Separation slightly longer than score 1, but still shallow	Long separation along white line	Long but shallow horizontal crack in toe wall	Long but shallow vertical crack in wall
3	One or more claws much longer than normal and/or the toes are torn and/ or partially or completely missing	One or more claws much longer than normal and/or the claws are torn	Multiple or deep crack or cracks in dew claw and or/partially or completely missing	Numerous cracks with obvious overgrowth and erosion	Long separation at the juncture, which is deeper than score 2	Long and deep separation along white line	Multiple or deep horizontal crack or cracks in toe wall	Multiple or deep vertical crack or cracks in the wall
4	One or more toes significantly longer than normal			Large amount of erosion and overgrowth with cracks	Long and deep separation at the juncture			

2.2.9 Statistical Analysis

Data were analysed using SAS v 9.4. The treatment group was the experimental unit for analysis. Results were deemed statistically significant when α level was below 0.05, and a significant tendency was considered when α level was between 0.05 and 0.1. The Tukey-Kramer adjustment was used for multiple comparisons where least squares means (LSmeans) were determined and P-values were adjusted. Degrees of freedom were estimated using Kenwood-Rogers adjustment. Data are presented as LSmeans and standard errors. PROC UNIVARIATE was used initially for evaluating data distribution.

For all models, fixed effects included group composition (MIX v's FEM), dietary treatment (CON v's SUPP) – where diets were applied (day 36), and the interaction, as well as replicate. Inspection day was included as a fixed effect for LW, behaviour, cortisol and hoof score analysis. For all analysis only female pigs were included, and for cortisol and body lesions only focal gilts were scored. Pen was included as a random effect.

In all analysis where the mixed procedure was used, residuals were examined to verify normality and homogeneity of variances. General linear mixed models (PROC MIXED) were used to analyse LW, cortisol, behaviour, body lesions and total hoof scores. Generalised linear mixed model (PROC GLIMMIX) was used for locomotion, and bursitis. And (GENMOD) was used for individual hoof disorders.

Locomotion scores were analysed using a generalised linear mixed model (PROC GLIMMIX) as the data were ordinal in nature. As there were extremely low numbers of gilts with a score of greater than 2 (n=16), these were re-categorised as greater than or equal to 2. A multinomial distribution was specified. The model could not converge when data from all weeks were included as repeated measures, and as such each week was analysed separately, and p-values adjusted post-hoc using a bonferroni adjustment. Fixed effects on all inspection dates included group composition (MIX v's FEM) and replicate. Dietary treatment (CON v's SUPP), and the interaction were included for inspection dates after the minerals were added to the diet.

Salivary cortisol was analysed using a general linear model (PROC MIXED). For this analysis fixed effects were as above, with the addition of plate as random effects. We used a log transformation to normalise the data. Inspection day was included in the analysis as a repeated measure.

Behaviour data were also analysed using a general linear model (PROC MIXED). Behaviours were summed into 4 categories; 1) aggressive, 2) harmful, 3) sexual and 4) play (categorised in Ethogram; table 2-4) Statistical analysis for 'aggressive, harmful and play' behaviours were analysed individually. Behaviour data were summarised per 5/min recordings and divided by the number of pigs present in the pen. Fixed effects included group composition, diet and replicate. Pen was included as a random measure and week as a repeated measure. Due to its low occurrence in FEM groups, sexual behaviour could not be statistically analysed, so the average performance of behaviour was calculated instead.

Body lesion score analysis was carried out using a general linear model (PROC MIXED). Fixed effects included group composition diet, interactions and rep. Inspection day was included as a repeated measure.

A total hoof score for each female pig was generated by summing the score for all hoof disorders identified. These were analysed using linear mixed models (PROC MIXED). The individual hoof disorders were also analysed individually using generalised linear models (PROC GENMOD) as the data were ordinal, using a similar model to that used for locomotion scores. Gilts were categorised based on their maximum scores for each disorder. Fixed effects included group composition, diet, replicate and their interactions, with inspection day being a repeated measure.

Bursitis scores were analysed using a generalised mixed model (PROC GLIMMIX) as the data were ordinal. Fixed effects included group composition, diet, rep and interactions, with a random effect of inspection day.

2.3 Results

2.3.1 Live-weight

There was no effect of either diet or group composition on LW. However, there was a tendency for an interaction between group composition and experimental day; gilts in the FEM groups tended to gain more weight than gilts in the MIX groups by the end of the experiment ($P < 0.1$; Figure 2-1).

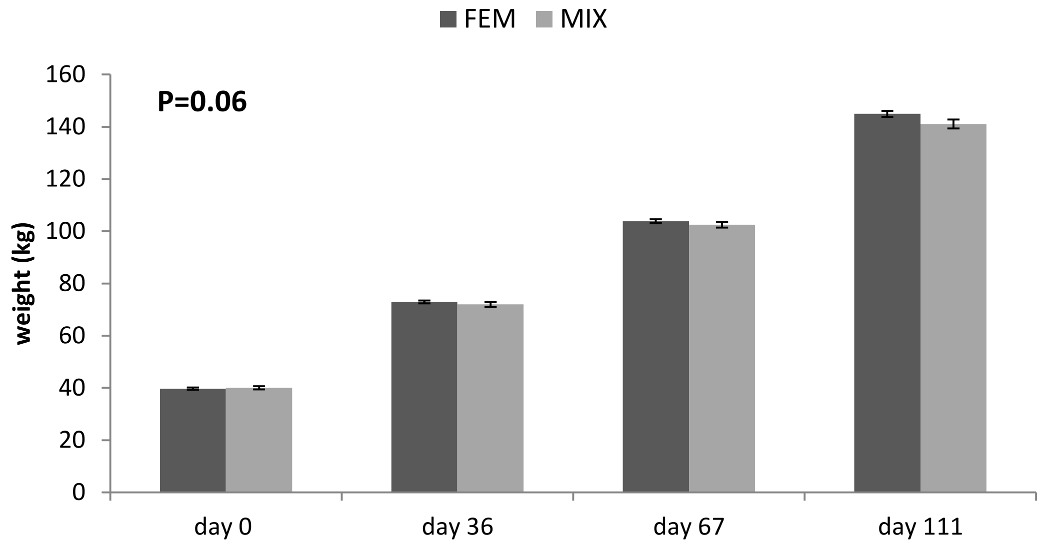

Figure 2-1 The live-weight (kg) of gilts on the FEM (female-only) or MIX (mixed-sex) treatments on each recording day. There tended to be an interaction between group composition and inspection day (P < 0.06).

*Experimental day 0 was at 81 days of age.

2.3.2 Locomotory Ability

There was no effect of either group composition or mineral supplementation on locomotion score on any observation day. The median and interquartile ranges during the experiment were all 1.

2.3.3 Salivary Cortisol

There was no effect of group composition or mineral supplementation on cortisol levels, although there tended to be an interactive effect between group composition and sample point ($P < 0.1$; Figure 2-2). There was an effect of inspection day on cortisol levels, but there was no discernible pattern over time ($P < 0.001$).

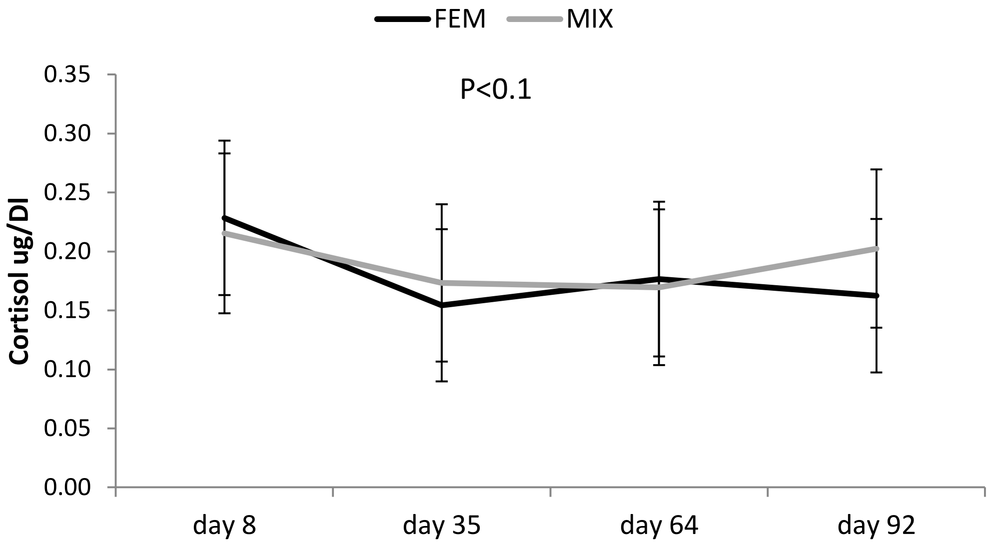

Figure 2-2 Salivary cortisol levels in gilts reared either in FEM (female-only) or MIX (mixed-sex) groups from weaning. There tended to be an interaction between diet and inspection day (P < 0.1).

*Experimental day 8 was at 89 days of age

2.3.4 Animal Behaviour

The amount of aggression observed was greater in the MIX than FEM groups (Table 2-7). There was an effect of week (P < 0.01), and in general aggressive behaviours increased over time (weeks 2 - 6), however they declined in weeks 8 to 10. There was no effect of group composition or diet on the performance of harmful behaviour, although there was an effect of week with week 4 being the highest. Play behaviour was observed more often in the MIX pens (P < 0.01; Table 2-7). There was an effect of week on play behaviour with play behaviour peaking in week 6 and declining in weeks 8 and 10. The mean number of sexual behaviours performed in FEM was 0.000 ± 0.005 /5min/pig compared to 0.036 ± 0.104 /5min/pig in MIX groups.

Table 2-7 Average number of occurrences of aggressive, harmful, play and sexual behaviours performed per 5 minute observation of pens of pigs in either mixed-sex (MIX) or female-only (FEM) groups and on a standard finisher diet (CON) or on a CON diet supplemented with minerals (SUPP).

	Diet			**Group composition**		
	CON	**SUPP**	**P-value**	**MIX[1]**	**FEM**	**P-value**
Aggression[2]	0.495±0.031	0.566±0.031	0.1191	0.589±0.031	0.472±0.031	0.0127
Harmful[2]	0.436±0.029	0.4819±0.029	0.2518	0.4851±0.029	0.4328±0.029	0.1934
Play[2]	0.03791±0.006	0.0299±0.006	0.1911	0.04414±0.006	0.02366±0.006	0.002
Sexual[3]	-	-	N/A	0.036±0.104	0.000±0.005	N/A

1 GC = Group composition

2Results are the least square means and standard errors of the number of occurrences over a 5min period divided by the number of pigs in the pen

3 The mean number of sexual behaviors per 5min/pig with standard deviation.

2.3.5 Body Lesion Scoring

Gilts in the MIX groups had higher body lesion scores than gilts in the FEM groups ($P < 0.001$). However there was no effect of diet on body lesion scores. There was an effect of inspection day on lesion scores ($P < 0.001$), although no clear pattern over time. There was an interaction between group composition and inspection day ($P = 0.05$; figure 2-3) and a tendency for an interaction between diet and inspection day ($P = 0.1$).

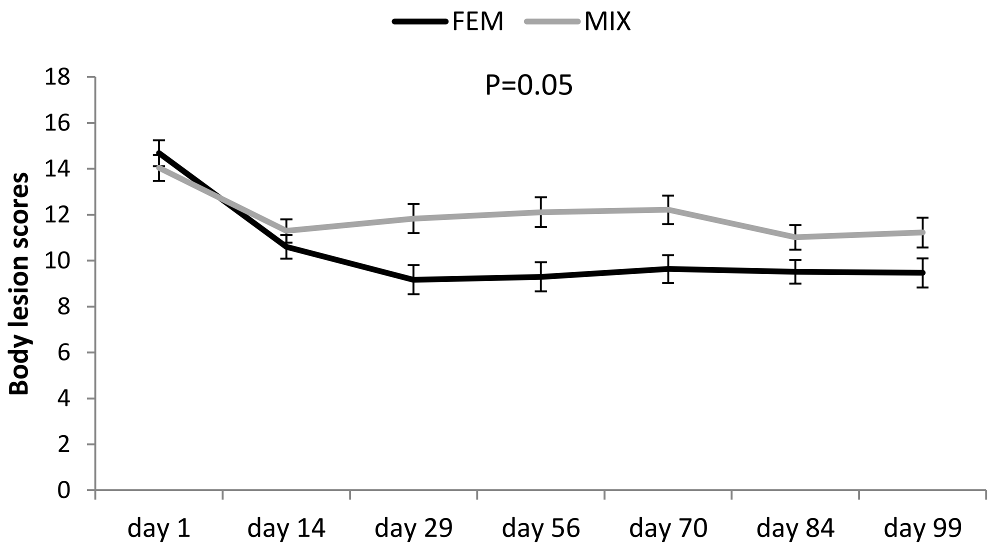

Figure 2-3 Total body lesion scores of gilts reared in female-only (FEM) or mixed (MIX) sex groups at seven inspection days during the finisher period (day 81- day 196 of age).

*Experimental day 1 was at 82 days of life.

2.3.6 Hoof scoring

Total hoof scores were higher (i.e. worse) in the MIX (8.01 ± 0.15) gilts than in the FEM (7.70 ± 0.12) gilts ($P < 0.02$). There was also an effect of inspection day, with hoof scores increasing over time (inspection day 1, 6.39 ± 0.1; day 2, 7.69 ± 0.13; day 3, 9.48 ± 0.20; $P < 0.0001$)

Gilts on the CON diet had higher heel erosion scores than SUPP gilts ($P < 0.05$; table 2-8). There was also an interaction ($P = 0.05$) between diet and inspection day with regard to heel erosion scores where scores increase over time with CON gilts scoring higher than SUPP gilts at each inspection day. There were no other effects of mineral supplementation.

Heel sole separation and white line separation scores were higher in gilts reared in MIX groups compared to FEM reared gilts ($P < 0.05$ for both; table 2-8). There was a tendency for an interaction between diet and inspection day on white line separation ($P = 0.1$) where the percentage of animals with a score of 1 and 2 are increasing over time. Heel erosion, white line separation, dew claw cracks, vertical toe cracks and toe length all increased with time ($P < 0.001$); however horizontal toe cracks fluctuated over time ($P < 0.0001$).

Table 2-8 Individual hoof disorders of gilts reared in either mixed-sex (MIX) or female-only (FEM) groups and on a standard finisher diet (CON) or on a CON diet supplemented with minerals (SUPP).

	Diet			Group composition		
	CON	**SUPP**	**P-value**	**MIX[1]**	**FEM**	**P-value**
Heel Erosion[2]	1 (1-2)	1 (1-1)	0.02	1 (1-1)	1 (1-1)	0.70
Heel sole separation[2]	0 (0-1)	0 (0-1)	0.94	0 (0-1)	0 (0-1)	0.04
White line separartion[2]	0 (0-1)	0 (0-1)	0.51	0 (0-1)	0 (0-1)	0.03
Vertical cracks[2]	0 (0-0)	0 (0-0)	0.16	0 (0-0)	0 (0-0)	0.76
Horizontal cracks[2]	0 (0-0)	0 (0-0)	0.68	0 (0-0)	0 (0-0)	0.95
Dew claw length[2]	0 (0-0)	0 (0-0)	0.48	0 (0-0)	0 (0-0)	0.72
Dew claw cracks[2]	0 (0-0)	0 (0-0)	0.67	0 (0-0)	0 (0-0)	0.70
Toe length[2]	1 (1-1)	1 (1-1)	0.38	1 (1-1)	1 (1-1)	0.82

16 males and 6 females per group

2 Data are provided as median and interquartile range

2.3.7 Bursa scores

There was no effect of group composition or mineral supplementation on limb lesion scores. However, there was an effect of inspection day on bursa scores ($P < 0.001$), with bursa size increasing over time.

2.4 Discussion

Improvements in the management of replacement gilts during rearing could help to improve longevity, primarily through reduced culling for lameness with associated benefits to animal health and welfare. This study investigated the effect of a supplementation with additional Cu, Zn and Mn, and the effect of rearing in female-only or mixed-sex groups on behaviour and welfare of replacement gilts. In general, few effects of mineral supplementation were found while rearing gilts in female-only groups reduced their exposure to aggression and sexual behaviour with associated benefits to their health (reduced injuries to the body and hooves).

As expected, the amount of aggression observed was greater in the mixed-sex groups than in the female-only groups, which is in line with previous studies on pigs (Boyle and Bjorklund, 2007; O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014). There is generally more activity in mixed-sex pens including play behaviour. In other species such as rats, males engage in more play-fighting (which is considered social play) than females (Pellis *et al.*, 1997). As male pigs mature their levels of testosterone rise, and therefore it is not surprising levels of mounting and aggression rose as these animals age (Giersing *et al.*, 2000). However, aggression decreased in week 10, which may correlate with studies which suggest that aggression reduces as the animals get older due to a greater focus on mounting (Clark and D'Eath, 2013). An alternative hypothesis is that this may be due to space restrictions, limiting space for parallel pressing and therefore interfering with aggressive motor patterns (Straw, 2013).

There was no effect of group composition or diet on the performance of harmful behaviour, although there tended to be an interaction between group composition and week. Sexual behaviour was not statistically analysed, but the averages were calculated and the performance of these behaviours was much lower in female-only groups compared to mixed-sex groups. This is in agreement with several previous studies (Rydhmer *et al.*, 2004; Björklund and Boyle, 2006).

We hypothesised that gilts reared with males would have higher levels of cortisol either due to; firstly the stress of being mounted and exposure to aggression, or secondly, due to underlying pain associated with hoof and leg injuries resulting from these behaviours. We also expected that these effects could be modulated by the dietary treatments, as any benefits to limb health through mineral supplementation could have reduced the impact of rearing with males. However, although the behaviour observations confirmed that gilts were exposed to higher levels of mounting and aggression when reared with male pigs than when in female-only groups, we did not observe any corresponding increase in cortisol, although there was a tendency for an interaction between group composition and inspection day. This was due to an increase in cortisol in gilts from the mixed-sex groups at breeding age, relative to gilts from the female-only groups. Even though this was after the males had left the groups and gone to slaughter, there may have yet been on-going physical effects on the gilts. In an associated published study (Chapter 3) based on the gilts from the same groups which were slaughtered at day 115 (196 days of age), we found more damage to the cartilage on the surface of the humeral condyle joint and trochlear notch, as well as a higher incidence of OCD in gilts reared in the mixed-sex groups (Hartnett *et al.*, 2019c). It is known that pain is associated with OCD (Frantz, 2006) and thus it can be assumed that with overlying biomechanical stress or trauma, this type of damage to the joints could progress to pain and lameness. OCD lesions do not always produce clinical signs of pain and discomfort, as seen in horses even with severe pathological damage (Jeffcott, 1996). This may be similar to our gilts as we saw no difference in locomotory ability, in gilts from which we identified OCD lesions and cartilage damage post-mortem. In line with this, in a recent study by Uilenreef *et al.* (2019) it was found that the there was a low correlation between joint damage and the degree of lameness scored and pigs with induced-osteoarthritis were even considered 'sound' during visual assessment by trained observers. Damage of this nature could have contributed to the slight rise in cortisol in gilts reared with males, as they aged.

Gilts in the mixed-sex groups have higher body lesion scores than gilts reared in female-only groups which is in line with previous studies and shows a reflection of higher levels of aggression, sexual behaviour and activity (Turner *et al.*, 2006; Teixeira and Boyle, 2014). There was an interaction between group composition and inspection day on body lesion scores. Both treatments had similar levels at the first inspection day, and then lesion scores in gilts reared in mixed-sex groups remaining higher than gilts reared in female-only groups for the rest of the experiment. This is likely due to on-going integument damage due not only to aggression, but mounting and play.

Hoof scores were higher in mixed-sex groups, which are likely due to the higher levels of activity (mounting, aggression, and play) in pigs in these groups on the concrete slatted flooring which is known to be injurious to the hooves (Gjein and Larssen, 1995a; Pluym *et al.*, 2011; Quinn *et al.*, 2015). Hoof scores also increased over time, which is likely due to general wear and tear with age, as the hoofs must support more weight as pigs increase in size. Hoof lesions are associated with pain and irritation as the lesions on the surface of the hoof can affect sensitive tissues, and secondary infections can occur as a result (Zoric *et al.*, 2004). Nevertheless, none of the hoof disorders we observed were severe, and this could be why we did not observe differences in locomotion score.

Nair and Anil (2011) found that the addition of trace minerals Cu, Zn and Mn to the diet of the breeding sow herd resulted in the reduction of heel erosion and white line lesions. Similar to this, we found that gilts reared without the addition of minerals had higher heel erosion scores than supplemented gilts.

In this study there tended to be an interactive effect of group composition and experimental day on LW, with gilts in the female-only groups tending to gain more weight than gilts reared in the mixed-sex groups by the end of the experiment. We previously showed that gilts coming from the same mixed-sex groups had more mechanical damage to cartilage on the elbow joint than gilts reared in the female-only groups (Hartnett *et al.*, 2019c) so it is possible that this type of injury could have contributed to a lower growth rate. However, Faba *et al.* (2018) found that incidence of lameness increased as body weight increased, and lame gilts had lower growth rates.

Surprisingly, there was no effect of any treatment on locomotion score. This may be due to hoof and bone disorders not having yet developed to a stage where impaired locomotion is evident, although several previous studies did find improvements in locomotory ability in gilts which were supplemented with similar levels of Cu, Zn and Mn as employed in this trial (Quinn, 2014; Quinn *et al.*, 2015; Faba *et al.*, 2018). Quinn *et al.* (2015) included the mineral supplement in a diet formulated for fat rather than lean meat deposition, (i.e. a developer diet) which had a higher energy to lysine ratio than a standard finisher diet. A restricted diet was fed, with the aim of slowing growth rates, which necessitated housing the gilts individually; individual housing means that gilts were likely less active than animals in our study. This could explain why the previous authors found an effect of diet.

In a similar study (Quinn, 2014) compared finisher and gestating sow diets to a 'gilt developer' diet. Faba *et al.* (2018) reared gilts on 4 diets; a standard finisher diet, and that same diet with the addition of minerals (Cu, Zn and Mn), methionine supplemented and a methionine plus mineral supplemented diet. That study found that there were a higher percentage of lame gilts when fed the control diet during the rearing period compared to all other treatments. A significant difference between both (Quinn, 2014; Faba *et al.*, 2018) and the current study is that half of all gilts in the current were reared in mixed-sex groups. Thus the effect of minerals could have been masked by the effect of management with male pigs.

Clearly, our findings indicate that supplementation of a finisher diet with minerals associated with promotion of hoof health is not enough to influence locomotory ability. This was the case even though aspects of hoof and limb health are improved by the treatments, as gilts had a higher areal bone mineral density and reduced number of OCD lesions when fed the supplemented diet in a companion study (Hartnett *et al.*, 2019c). In fact, locomotory ability was generally very good across all treatments and no lameness was detected in any of the trial animals.

2.5 Conclusion

Gilts reared with entire male pigs were exposed to higher levels of aggression and sexual mounting compared to gilts reared in female-only pens. As a consequence they had higher body lesion scores and more hoof damage. Supplementation with the minerals Cu, Zn and Mn had only a minor albeit positive effect on heel erosion. Rearing gilts in female-only pens reduces their exposure to behaviours which can injure the hooves and therefore increase the likelihood of culling for lameness in later life.

Chapter 3- The effect of group composition and mineral supplementation during rearing on measures of cartilage condition and bone mineral density in replacement gilts

3 The effect of group composition and mineral supplementation during rearing on measures of cartilage condition and bone mineral density in replacement gilts

3.1 Introduction

Lameness in pigs is a significant welfare issue Anil *et al.* (2007) and is a major reason for poor longevity in sows (Engblom *et al.*, 2007; Jensen *et al.*, 2010). In general, locomotory issues account for 13% of all sow cullings, and over half of these females have not attained their second parity (Lucia *et al.*, 2000). The aetiology of lameness in pigs is multifactorial and risk factors include flooring - concrete flooring in general and particularly slatted concrete (KilBride *et al.*, 2009), stocking density and pen size (Willgert *et al.*, 2014b), housing type, growth rates and also nutrition (Quinn, 2014). In recent years there is interest in the way replacement gilts destined to enter the breeding herd are reared as a risk factor for lameness (Quinn *et al.*, 2015).

In many countries replacement gilts are reared similar to finisher pigs and in mixed-sex groups up to breeding age (Boyle and Bjorklund, 2007; Quinn, 2014). This means that in countries that do not practice castration (e.g. Ireland) replacement gilts are kept with entire male pigs which perform high levels of aggressive and mounting behaviour (O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014). These behaviours increase the risk of injuries such as limb and cartilage damage (Andersson *et al.*, 2005) which are associated with lameness.

Additionally, diets designed for finisher pigs may not meet the physiological needs of the replacement gilts (Quinn *et al.*, 2015). In particular, such diets may not supply the correct balance of minerals to satisfy the nutritional requirements for reproductive performance, cartilage and bone formation and integrity (Levis *et al.*, 1997; Gill and Taylor, 1999; Knauer *et al.*, 2012). There are few established guidelines with regard to trace mineral requirement, and often gilt diets are developed using levels appropriate for finisher pigs, or gestating sows; in fact there are no trace mineral recommendations for developing gilts in the most recent edition of the Nutritional Requirements for Swine (NRC, 2012). Nevertheless, Ferket *et al.* (2009) reported that copper (Cu), zinc (Zn) and manganese (Mn) reduce lameness and leg problems in sows. Zn is particularly important for claw horn production due to its vital role in cell repair and replacement (Mohamadnia, 2008; Tomlinson *et al.*, 2008; van Riet *et al.*, 2013). Mn plays a vital role in horn production and the formation and maintenance of cartilage and bone; in cattle deficiency is associated with short/weak bones (Mohamadnia, 2008; van Riet *et al.*, 2013). Minerals can play other important roles in promoting sow longevity. For example, Cu is essential for antibody development and lymphocyte replication (Tomlinson *et al.*, 2004). Studies with pregnant sows found that the addition of these trace minerals to the diet improved claw health (Nair and Anil, 2011) and reduced the prevalence of lameness and leg abnormalities (Ferket *et al.*, 2009). A similar study reported improved locomotion scores, higher bone mineral density and lower cartilage lesion scores in gilts fed a restricted diet formulated for fat rather than lean deposition with increased levels of Cu, Zn and Mn (Quinn *et al.*, 2015). However, that study used terminal line gilts, and furthermore, restricted feeding is difficult to conduct in practice as most maternal line gilts are reared in finisher pig accommodation. Hence we hypothesised that supplementing a standard finisher *ad lib* fed diet with Cu, Zn and Mn and separating gilts from male pigs during the rearing period would result in improved locomotory ability, higher bone mineral density and lower cartilage lesion scores.

In addition, previously used joint scoring methods which we found in the literature are relatively subjective in nature (e.g. 1 = normal to 4 = severe abnormality (Quinn *et al.*, 2015)). Thus with the aim of capturing more detail on the nature and amount of damage to the joint cartilage, and improve the objectivity of the scoring system, we also aimed to develop an improved method of scoring joint surface lesions, and compare this to a previously published version.

3.2 Materials and methods

3.2.1 Care and use of animals

This study was carried out in the 200 sow unit at the Pig Development Department in Moorepark, Fermoy, Co. Cork, Ireland between March and August 2017. The experimental work was authorized by the Teagasc animal ethics committee (Approval no.: TAEC136-2016) and licensed by the Health Products Regulatory Authority (License no: AE19132-P057).

3.2.2 Experimental design

Details of the experimental design including the treatments that were applied to the gilts during the rearing stage are outlined in Chapter 2. In brief, a two by two factorial arrangement was applied to 288 gilts. The first factor consisted of group composition, whereby half of the gilts were reared in female-only groups of 12 animals (FEM) from weaning onwards, and the other half were reared in mixed-sex groups (MIX). The second factor consisted of mineral supplementation; half of each of the group composition treatments was fed a standard finisher diet until breeding age (CON) and the other half were fed the same diet, but with supplementary levels of Cu, Mn, and Zn applied (SUPP). Gilts were reared across three replicates, born three weeks apart.

At slaughter the right forelimb was removed at the shoulder from all 102 carcasses in the chill room and then frozen at -20°C until for further analysis.

3.2.3 Measurements

3.2.3.1 Locomotory ability

For the purposes of this study, only locomotion scores from the 102 gilts that were slaughtered at breeding age and had their right forelimb removed are considered. The gilts were locomotion scored every two weeks (14.1 ± 3.6 days) from day 81.3 (i.e. the day they entered the finisher accommodation) until day 165.8 of age (i.e. slaughter age). Locomotory ability was scored while the gilts walked on solid concrete for a distance of approximately 30 meters, from the front, rear and side of the animal. All observations were carried out by one trained observer who was blind to the animals treatment groups. Locomotion was assessed using an adapted version of Calderon Diaz *et al.* (2013) ranging from 0-5 (Table 3-3).

Table 3-1 Scoring system for locomotory ability (adapted from Calderon Diaz *et al.* (2013)).

Score	Description
0	Even strides. Pig is able to accelerate and change direction rapidly
1	Pig appears stiff. Abnormal stride, which isn't easily identified. Movements no longer fluid but pig still able to accelerate and change direction rapidly. Caudal swagger evident.
2	Uneven stride. Sensitivity while walking detected on at least one limb. Pig able to accelerate and change direction. Caudal swagger evident
3	Uneven stride, with a stagger. Minimum-weight bearing on affected limb. Slow to move. Obviously lame even to the untrained observer
4	Pig may not place affected limb on floor
5	Does not move

3.2.3.2 Areal Bone Mineral Density (aBMD)

To determine areal bone mineral density (aBMD; g/cm2) the right forelimb was scanned by dual energy x-ray absorptiometry (DEXA) using a Hologic QDR-4500 Elite bone densitometer and analysed using Apex software version 2.3.7. A total of 306 scans were performed on the 102 limbs collected from the factory. Three areas of each limb were scanned and analysed; the humerus (HUM), radius/ulna (RU) and metacarpal (MET). The scanning mode varied for the different parts of the limb; for the humerus and the metacarpal the ‘lumber spine’ analysis option was used and for the radius/ulna the ‘forearm’ option was used. These scanning modes provided the best fit after carrying out test scans on each option for each part of the limb. For analysis of the HUM and RU, the limbs were placed on their side and positioned according to the scanning mode such that the laser was in the correct place (approximately over the elbow joint at the top of the limb). For analysis of the MET, the limb was positioned on a Styrofoam™ plinth as the leg needed to be positioned with the hoof (sole side down) flat on the table to ensure that the bones of the hoof did not overlap. The scans were analysed for bone mineral content (BMC; g) and aBMD (g/cm^2) for the total area of the specific bone which was outlined using the software. Each leg was scanned from frozen. The scanner was calibrated every day using the software daily quality control (QC) exam; a weekly QC was also carried out which included a radiographic uniformity test.

3.2.3.3 Cartilage surface lesions

Dissection of the limb at the elbow joint was carried out to expose the humeral condyle (HC), which is made up of the capitulum and the trochlea and the trochlear notch (TN) surface. Two types of scoring methodology were used by one trained observer who was blind to the animals treatment groups. First, the HC and TN were examined and given one score (Joint lesion score (JLS)) for any abnormalities present (i.e., invagination, thinning, overgrowth (Figure 3-1.A) and fractures; cartilage score). The scoring system used was as per Quinn *et al.* (2015) and ranged from 1 (normal) to 4 (severe abnormality). The number of areas of osteochondrosis dissecans (OCD; detachment of the cartilage from the underlying bone (Figure 3-1.B)) that were present were also counted (OCD prevalence).

The second scoring method was developed to capture more detail on the nature and amount of damage to the cartilage present. The number of areas of thinned cartilage (thinnings), invaginations and overgrowths on both the HC and TN were counted, as was the number of areas of OCD. All limb dissections and analysis was carried out by one trained observer.

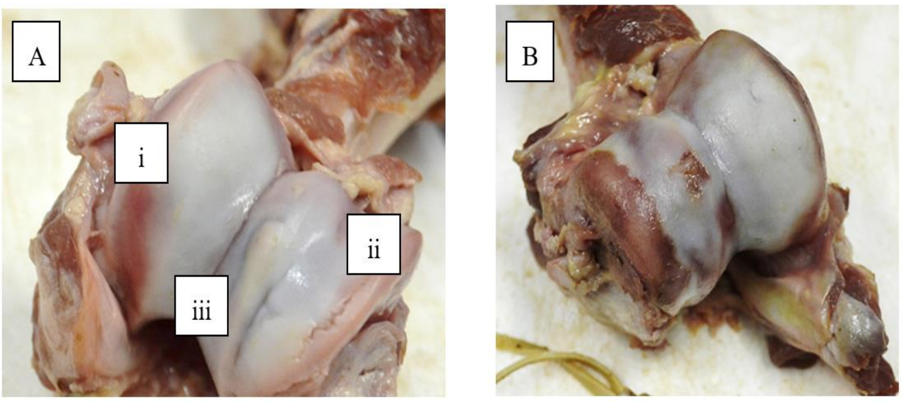

Figure 3-1 A) **(i) Thinning of cartilage, as evidenced by red bruise-like area (ii) Invagination of the cartilage and (iii) overgrowth, and** B) **OCD (separation of cartilage from the underlying bone).**

3.2.4 Statistical analysis

Data were analysed using SAS v 9.4. The treatment group was the experimental unit for analysis Locomotion scores were analysed using a generalised mixed model (PROC GLIMMIX) as the data were ordinal in nature. As there were extremely low numbers of pigs which had a score of greater than 2, these were re-categorised as greater or equal than 2. A multinomial distribution was specified. Fixed effects included group composition (MIX v's FEM), dietary treatment (CON v's SUPP), and the interaction, as well as replicate. Pen was included as a random effect. The model could not converge when data from all weeks were included, and as such each week was analysed separately, and p-values adjusted post-hoc using a bonferroni adjustment.

Areal bone mineral density (aBMD) was analysed using a general linear model (PROC MIXED). Within each leg, each bone type was analysed separately.

The two different systems of cartilage scoring were initially compared, to determine whether the newly developed system was reflective of increases in damage, similar to the previously published one. Pigs with cartilage which had at least one area of OCD were removed from the analysis. Analysis was carried out using a general linear model (PROC MIXED), with JLS as the fixed effect.

The JLS scores assigned to each limb using the method of (Quinn *et al.*, 2015) were analysed using generalised linear models (PROC GLIMMIX). Cartilage scores (1-4; multinomial distribution) were analysed separately to the number of areas of OCD (0, 1, 2 or 3; Poisson distribution).

For the second scoring methodology, the numbers of lesions were summed within lesion type and area of the cartilage (HC or TN). The overall sum for all cartilage areas, as well as the overall sum for the HC was analysed using linear mixed models. All other measures, other than overgrowths (incidence too low to be analysed statistically), were analysed using generalised linear mixed models. In instances when there were low numbers of individual scores, scores were combined with the next level on the scale.

For all models, fixed effects included group composition (MIX v's FEM), dietary treatment (CON v's SUPP), and the interaction, as well as replicate. Interactions were excluded from the model if found to be not significant. Cold weight was included in the models as a covariate. Pen was included as a random effect. In all analysis where the mixed procedure (PROC MIXED) was used, residuals were examined to verify normality and homogeneity of variances. Differences in least squares means were investigated using the t-test, following Tukeys adjustment for multiple comparisons. Degrees of freedom were estimated using Kenwood-Rogers adjustment. Statistical differences were considered significant at $P \leq 0.05$. Tendencies towards significance ($0.05 < P \leq 0.10$) are also presented. Data are presented as LSmeans and standard errors.

3.3 Results

3.3.1 Locomotory ability

There was no effect of either group composition or mineral supplementation on locomotion score at any observation. The median and interquartile ranges during the experiment were all 1.

3.3.2 aBMD

Gilts from the SUPP treatment had a greater aBMD ($P < 0.05$; Figure 3-2) in the HUM, and there was also an interaction between group composition and diet ($P < 0.001$; Figure 3-3). Gilts from the FEM treatment on the SUPP diet tended to have a higher aBMD than those from MIX groups on the SUPP diet ($P = 0.1$), and had a higher aBMD than FEM groups on the CON diet ($P < 0.001$). Within the CON treatment, there was a tendency for gilts from the MIX groups to have a greater aBMD than gilts from FEM groups ($P = 0.06$).

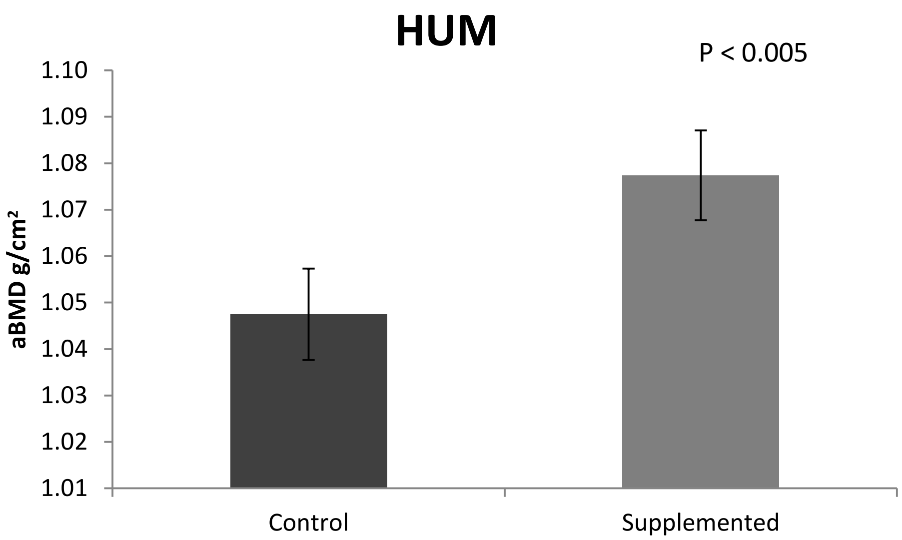

Figure 3-2 Humerus aBMD values in gilts fed a control finisher diet (Control) or the same diet supplemented with Cu, Mn and Zn (Supplemented).

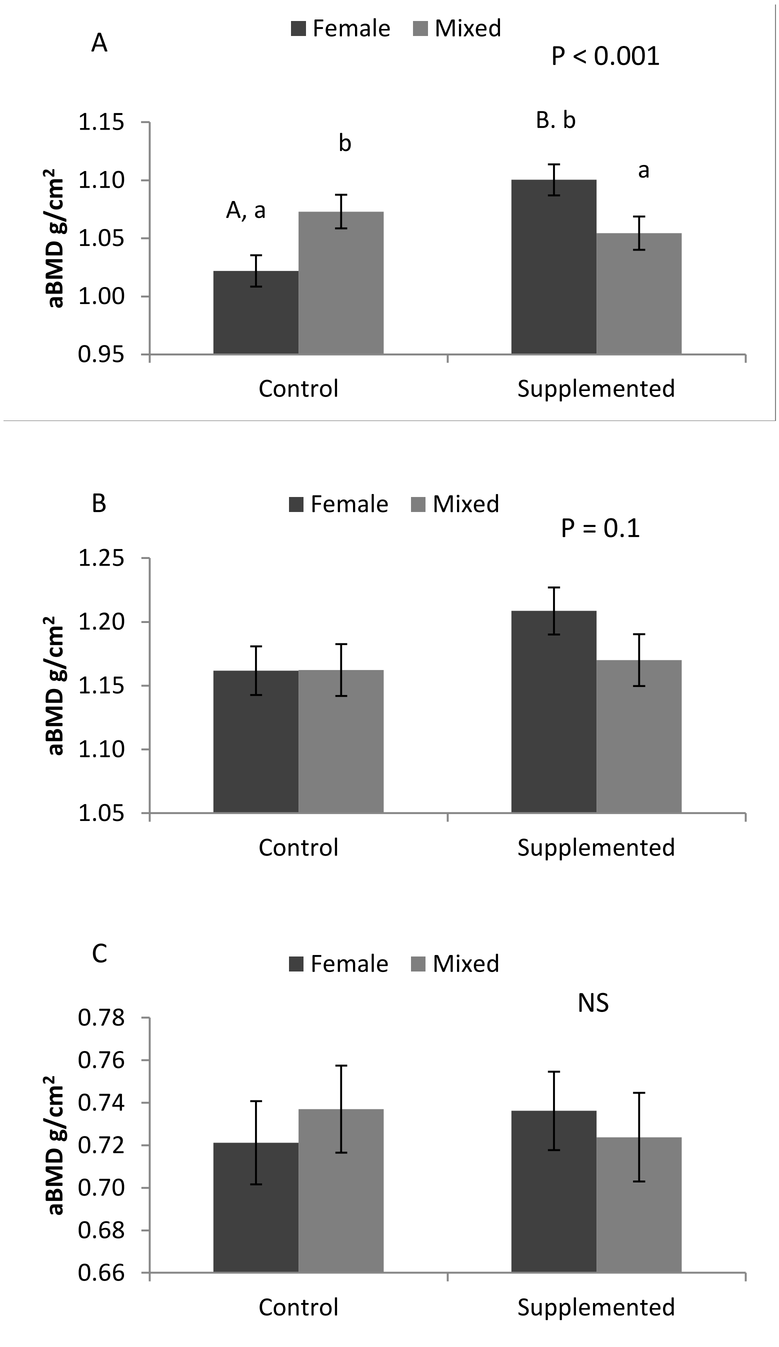

Figure 3-3 aBMD values for the A) Humerus, B) Radius/Ulna and C) Metacarpal. The P values represent the interactive effect between group composition and diet. The lower-case letters (a,b) indicate a tendency for a difference of *P* < 0.1 between group composition and within dietary treatment, whereas upper-case (A,B) superscripts indicate a difference of *P* < 0.001 between dietary treatment and within group composition.

There was also a tendency for an effect of mineral supplementation in the RU, with SUPP gilts having greater aBMD (P = 0.1). There was no effect of either diet or group composition on the aBMD of the META (P > 0.05).

Numerically we found that fractures were more common in mixed-sex groups. However, the average aBMD in pigs without fractures FEM (0.99 ± 0.21) and MIX (0.98 ± 0.21) was lower than the aBMD of pigs with fractures; MIX (1.01 ± 0.22) and FEM (1.15 ± 0.36). As only 5 pigs had fractures (4.8% of all trial pigs), this could not be statistically analysed.

3.3.3 Cartilage surface lesions

When comparing the two scoring systems (JLS *v's* counting the lesions), there was an overall effect of JLS category (P < 0.01; Figure 3-4). Although the only significant difference between JLS categories was between 1 and 3, and 1 and 4 (P < 0.01 for both), there tended to be a difference between scores 1 and 2 (P = 0.1), 2 and 3 (P = 1), and 2 and 4 (P = 1).

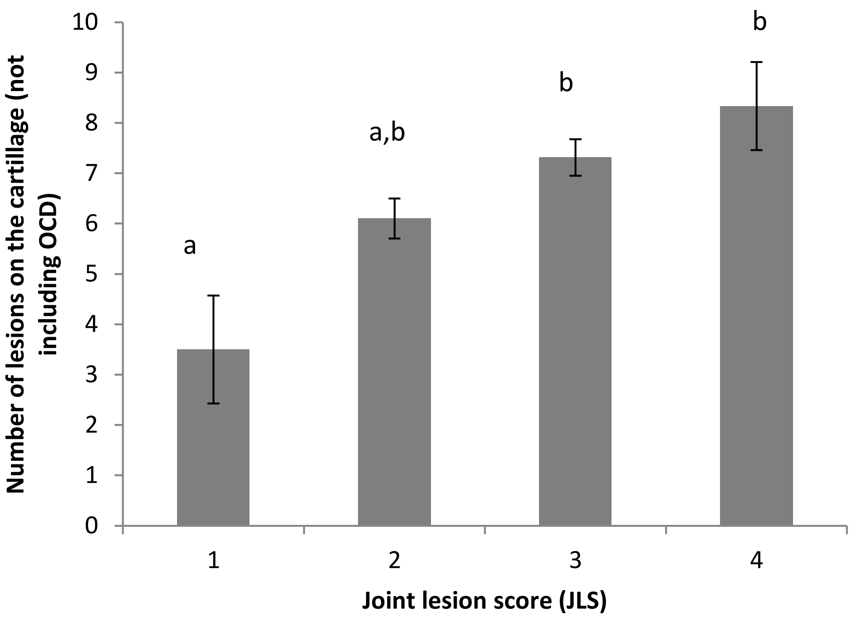

Figure 3-4 Comparison of the number of lesions on the joint cartilage relative to the overall joint lesion score (JLS) assigned. Levels of the JLS were 1 = normal, 2 = mild abnormality, 3 = moderate abnormality and 4 = severe abnormality a, b indicates a significant difference in the number of lesions present, between JLS categories.

There was no effect of either diet or group composition on JLS, or of group composition on the number of OCD lesions. However, gilts on the SUPP diet tended to have a lower number of OCD lesions than those on the CON diet ($P = 0.09$; Table 3-4). All gilts with OCD lesions in more than one location were on the control diet.

Table 3-2 Cartilage damage and OCD presence in the joints of gilts reared in either mixed-sex (MIX) or female-only (FEM) groups and on a standard finisher diet (CON) or on a CON diet supplemented with minerals (SUPP).

	Diet		Group Composition	
	CON	SUPP	MIX[1]	FEM
No. animals	51	50	45	56
Cartilage damage				
No. animals without OCD lesions	35	39	31	43
Sum of cartilage scores	88	103	82	109
OCD lesions				
No. gilts with 1 area[2]	10	11	10	11
No. gilts with 2 areas[2]	3	0	1	2
No. gilts with 3 areas[2]	3	0	3	0
Total no. gilts with OCD	16	11	14	13
No. gilts with fractures	2	3	4	1

[1]6 males and 6 females per group [2] Data are provided as the number of occurrences of OCD lesions. 1 = OCD lesion present on HC, 2 = Two OCD lesions present on the HC, 3 = Two OCD lesions present on the HC and one present on the TN.

With regard to the number of lesions on the cartilage, there was an effect of group composition; gilts reared in MIX groups had a higher (worse) total score when disorders (thinnings, invaginations and overgrowths) present on the cartilage, when summed together (Table 3-5).

Table 3-3 The number of instances of thinning, invagination, and overgrowth of the cartilage in the joints of gilts reared in either mixed-sex (MIX) or female-only (FEM) groups and on a standard finisher diet (CON) or on a CON diet supplemented with minerals (SUPP).

	Diet			Group Composition			Interactive Effect
	CON	SUPP	*P*-value	MIX[1]	FEM	*P*-value	*P*-value
No. animals	51	51	.	46	56	.	.
Total*[2]	7.21 ± 0.36	6.42 ± 0.36	0.12	7.39 ± 0.38	6.24 ± 0.34	0.03	NS
Thinnings[3]	4 (2–5)	4 (2–5)	0.37	4 (3–5)	3.5 (2–5)	0.45	0.13
Invagination[3]	2 (2–3)	3 (2–4)	0.60	3 (2–4)	2 (1–3)	0.27	NS
Overgrowth [4]	17	8	.	13	12	.	NS
HC total[2]	5.94 ± 0.39	5.48 ± 0.31	0.28	6.23 ± 0.33	5.19 ± 0.30	0.02	NS
HC thinnings[3]	3 (2–4)	3 (2–4)	0.72	4 (2–5)	3 (2–4)	0.20	0.14
HC invagination[3]	2 (1–3)	2 (1–3)	0.47	3 (2–3.5)	2 (1–3)	0.26	NS
HC overgrowth[4]	12	7	.	10	9	.	NS
TN total[2]	1.26 ± 0.16	0.94 ± 0.15	0.16	1.14 ± 0.16	1.06 ± 0.15	0.76	NS
TN thinnings[3]	1 (0–1)	1 (0–1)	0.44	1 (0–1)	1 (0–1)	0.80	NS
TN invagination[3]	0 (0–1)	0 (0–0)	0.52	0 (0–1)	0 (0–0.5)	0.79	NS
TN overgrowth[4]	5	1	.	3	3	.	NS

[1]6 males and 6 females per group [2]Analysis carried out using linear mixed models. Results are provided as least square means and standard errors.[3]Analysis carried out using generalised mixed models. Results are presented as median and interquartile ranges. [4]Due to the low incidence number, no analysis was carried out. Data are provided as the sum of the number of each disorder. *Sum of the number of all disorders (thinnings, invaginations and overgrowths).

When considering only the HC, gilts reared in MIX groups also had higher total scores (Table 3-5). There were no other effects of either diet or group composition on the number of disorders. Overgrowths were too few to statistically analyse, but numerically there were more instances in gilts on the CON diet than on the SUPP diet (Table 3-5). Fractures of the HC were found in 5 gilts (4.9% of all examined gilts). Four of these were gilts reared in MIX groups (Table 3-4).

3.4 Discussion

This study investigated the effect of a mineral supplemented diet and group composition on the limb health of replacement gilts with the ultimate aim of improving gilt longevity and welfare, and reducing culling for lameness. Supplementation of the gilts' diet during rearing with the trace minerals Cu, Zn and Mn led to increased bone mineral density, while rearing gilts in female-only pens reduced the number of joint lesions associated with cartilage degradation, and there were numerically fewer incidences of fractures in the humeral condyle (HC) joint. Female-only rearing and mineral supplementation combined resulted in an important beneficial effect on bone mineral density of the humerus.

Several authors found improvements in locomotory ability in gilts on mineral supplemented and/or specially formulated developer diets with similar levels of Cu, Zn and Mn as employed in this trial (Quinn, 2014; Quinn *et al.*, 2015; Faba *et al.*, 2018). In contrast, we observed no beneficial effect of mineral supplementation on locomotion scores. Indeed there was a tendency at day 148.3 ± 8.0 (the last locomotion scoring point before the gilts were slaughtered at day 196.3 ± 0.5) for gilts in the mineral supplemented groups to have higher locomotion scores. However, Quinn *et al.* (2015) included the mineral supplement in a diet formulated for fat rather than lean meat deposition (i.e. a developer diet). The diet was restricted fed, with the aim of slowing growth rates, which necessitated housing the gilts individually. This was compared to a standard finisher diet. In the other study by (Quinn, 2014) un-supplemented finisher and gestating sow diets were compared to a supplemented developer diet which also had a higher energy to lysine ratio compared to the other two diets, and terminal line gilts were used. Thus although similar to the current study, the mineral supplemented diets also had other critical differences to a finisher diet which could have promoted limb health. Faba *et al.* (2018) reared gilts on 4 diets; control, mineral supplemented (Cu, Zn and Mn), methionine supplemented and a methionine plus mineral supplemented diet. Additionally gilts were reared in groups of 10, which is a lower number than our study. In the current study all gilts were on a standard finisher diet, and in contrast to (Quinn, 2014) and Faba *et al.* (2018), half of them were kept in mixed-sex groups. Clearly, our findings indicate that supplementation of a finisher diet with minerals associated with bone/limb health alone is not enough to influence locomotory ability. On the other hand locomotory ability was generally very good across all treatments and no lameness was detected in any of the trial animals.

In line with Quinn (Quinn, 2014; Quinn *et al.*, 2015) we found a beneficial effect of mineral supplementation on bone mineral density. In the study by Quinn *et al.* (2015) the effect was only numerical which could be explained by the later stage at which the supplementation was applied (i.e. day 130 vs. day 117.5 ± 0.6), and the fact that the animals were individually housed, so restricted in their movements. In the other study by Quinn which applied the supplementation at a similar age to the current work (~112 days of age), and the animals were managed in groups, there was a significant difference in aBMD in developer diet fed gilts compared to finisher diet fed gilts (Quinn, 2014).

Mineral supplementation had the greatest impact on the bone mineral density of the humerus in the forelimb. Bio-mechanically, the forelimbs carry more load (Mitchell *et al.*, 2001), have higher peak forces when walking, and have a longer stance phase (Thorup *et al.*, 2007).The humerus is the biggest bone in the forelimb and it is possible that this bone bears a greater proportion of weight relative to the radius and ulna, where two bones are involved, and the metacarpals where there are multiple digits. The lack of an effect of mineral supplementation on the other bones examined could also partially be explained by the relative difficulty in scanning these bones relative to the humerus. The latter bone, because of its larger size, was very easily positioned for obtaining a clear scan by the DEXA scanner. In contrast, the radius and ulna may overlap, which can influence the aBMD reading (Schenck *et al.*, 2008). Schenck *et al.* (2008) separated the fibula and tibia for DEXA scans for better accuracy of aBMD readings. The metacarpal was the most difficult bone to scan not alone because of the risk of overlap between the metacarpals but also because of difficulty positioning it at a consistently stable angle on the table. However, most studies used the metacarpal for analysis of aBMD (Kornegay *et al.*, 1981; Hall *et al.*, 1991; Shaw *et al.*, 2006), as it believed to be a sensitive bone to mineral changes in diets (Eklou-Kalonji *et al.*, 1999; Brady *et al.*, 2003; Varley *et al.*, 2010; Varley *et al.*, 2011). Garg and Kharb (2013) stated that the femur is used to analyse aBMD in humans - which when compared with all three bones in our study is most similar to the humerus. In a sow study Crenshaw *et al.* (2013) used the humerus to determine BMC by bone ash mineral analysis.

Interestingly, and in contrast to our hypothesis, when fed the standard finisher diet, gilts from the mixed-sex groups tended to have a greater aBMD than the gilts from female-only groups. Weight bearing exercise is associated with improvements to aBMD in humans (Margulies *et al.*, 1986; Greene *et al.*, 2005) and in fact there is evidence that increased exercise in pigs also increases it (Burr *et al.*, 2002). In another study it was found that osteochondrosis (OC) risk is reduced in young foals by regular but limited exercise (Lepeule *et al.*, 2009). In fact, in Chapter 2, we observed increased levels of aggression and mounting in mixed-sex pens, as well as play behaviour (Hartnett et al., 2019 - under *review*) so increased activity in general. Stavrakakis (2014) stated that bone remodelling allows for the ability to adapt according to the environment and the loading the animal is exposed to. Therefore a pig experiencing more mechanical stress could increase bone mass accordingly. However, overloading and the stress of heavier body weight can cause a disturbance in metaphyseal blood flow which can cause joint lesions on immature cartilage (Grondalen and Vangen, 1974) additionally; this mechanical pressure can cause a disturbance in blood flow at bone-cartilage junctions causing OC lesion prevalence (Nakano *et al.*, 1987).

We compared both systems of scoring joint lesions so that we could confirm that the newer system which we developed (counting all lesions) produced similar data, with the same direction and magnitude of differences between scores, to the previously published version (JLS; Quinn *et al.* (2015)). The newer scoring system was developed in an attempt to improve the objectivity of the scoring system, and also so that we could determine whether any differences between treatments were due to particular pathologies.

Nevertheless, it was important that we compare both so that results from our study can be discussed in the context of with previously published work. Data from both systems corresponded with one another, in that as the JLS score increased, so did the number of lesions on the cartilage. Moreover, counting the lesions enabled us to discern differences between treatment groups in several measures, whereas the JLS system did not. As there were only 4 joints with a score of 1, and 6 with a score of 4, it is likely that with a larger sample size some of these differences may have been significant. Thus, for this type of study, counting the different types of lesions present may improve the ability of the researcher to detect treatment effects.

Female-only rearing also had a beneficial effect on the number of joint lesions observed. This is consistent with our hypothesis, as in mixed-sex groups females are subjected to potential trauma caused by sexual mounting and aggression performed by entire males (Boyle and Bjorklund, 2007; O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014). This is exacerbated by housing on fully slatted floors (Gjein and Larssen, 1995b; Pluym *et al.*, 2011). Indeed four of the five gilts with fractures in the humeral condyle at slaughter were reared with entire males in mixed-sex groups. This equates to 5% of all the gilts on trial and does not differ greatly from the 10% prevalence of fractures in cull sows (Kirk *et al.*, 2005; Engblom *et al.*, 2008). As fractures occur during falls, fights and attempts to free a leg (Muirhead and Alexander, 2002; Dewey, 2006; Nalon *et al.*, 2013) trauma associated with the behaviour of entire males cannot be discounted as a possible cause. While the injuries may have occurred during transportation or in the slaughterhouse, the finding still suggests an increased susceptibility for breakage associated with mixed-sex rearing, possibly because of pre-existing damage.

The presence of fractures in 5 of the gilts, as well as the high prevalence (100%) of joint lesions and the fact that over a quarter of the animals had OCD lesions irrespective of treatment raises concern for gilt welfare. This is complicated by the lack of an association between locomotion scores and joint lesions/OCD as the possibility of detecting such problems is reduced. Bone strength and likeliness of fracture is related to aBMD (Vahle *et al.*, 2015). Surprisingly, in this study the average aBMD in fracture pigs was higher than in non-fracture pigs. As the fracture pigs only account for 5% of trial pigs, it is a matter for future research. Indeed there is also an association between OCD and physical injuries such as joint lesions and bone fractures (Jensen *et al.*, 2007; Jensen and Toft, 2009). Certainly earlier identification could allow for gilts that portray signs of leg weakness to be removed from the replacement herd (de Koning *et al.*, 2012). The lack of an association is in accordance with unpublished work by Quinn where there was no association between lameness (locomotion scoring) and OCD or aBMD (Quinn, 2014). Quinn *et al.* (2015) found there was no correlation between locomotion and aBMD. However, there tended to be a correlation between lesions on the HC and locomotion scores.

Numerous studies report no association between OCD and lameness/leg weakness (Brennan and Aherne, 1986; Jørgensen, 1995; Jørgensen *et al.*, 1995; Stern *et al.*, 1995; Arnbjerg, 2007) and the majority of studies supporting the association involved sows that were specifically culled for lameness (D'Allaire *et al.*, 1987; Kirk *et al.*, 2005; Engblom *et al.*, 2007;

Engblom *et al.*, 2008; Jensen *et al.*, 2010). Given that treatment effects were detected in bone mineral density and cartilage degradation scores, the findings regarding locomotory ability suggest that either the locomotion scoring system was not sensitive enough to detect any difference, or differences in aBMD and OCD are not reflected in locomotory ability. Indeed the relationship between lameness and OCD is poorly understood (Dewey *et al.*, 1993; Heinonen *et al.*, 2006).

Nevertheless, it is possible that a more sensitive lameness/locomotory scoring system could help identify gilts suffering from OCD. Although locomotion scoring is of limited reliability (Main *et al.*, 2000; Channon *et al.*, 2009; D'Eath, 2012; Nalon *et al.*, 2013) if it was possible to identify features of locomotion which portray a long-term locomotory ability and assess different locomotory patterns that are associated with lameness a more objective, reliable and accurate scoring system could be created (Stavrakakis, 2014). The scoring system employed in the current study attempts to identify an affected limb (Main *et al.*, 2000). However, as OCD occurs bilaterally in the elbow joints (Grondalen and Vangen, 1974; Jørgensen *et al.*, 1995; Ytrehus *et al.*, 2007; Kirk *et al.*, 2008; Jensen and Toft, 2009) the pain is likely equalised across both limbs and therefore it might be very difficult for a pig to reduce weight on one of them. A method of locomotion scoring which takes into account different aspects of limb and body movements may be more useful in detecting subtle differences associated with limb or joint pain. The development of a new locomotion scoring protocol for different age groups would also be very useful.

3.5 Conclusion

This study found benefit to replacement gilts of feeding an increased level of the trace minerals Cu, Zn and Mn over those recommended for finisher pigs, on several measures of limb condition. This was the case even though these differences were not detectable using a locomotion scoring system. The benefits of supplementing these minerals could lead to potential improvements in the lifetime performance of replacement gilts and the longevity of sows. Separating replacement gilts from finishing stock, particularly entire males, can significantly reduce incidences and severity of joint lesions and OCD. There is thus clear indication that female-only pens and a mineral supplemented diet for replacement gilts can have benefits for limb heath and their welfare. However, it is important to note that there was reduced aBMD in female-only reared gilts.

Chapter 4 - Investigating the effects of group composition and mineral supplementation on health and welfare of replacement sows in early parities and on lifetime performance

4 Investigating the effects of group composition and mineral supplementation on health and welfare of replacement sows in early parities and on lifetime performance

4.1 Introduction

Replacement sows are the most valuable animals in the herd. Approximately 40 to 50% of sows are culled before their third or fourth parity (D'Allaire *et al.*, 1987; Boyle *et al.*, 1998; Schenck *et al.*, 2008). However, their replacement costs have not been covered by this time (Stalder *et al.*, 2003; Schenck *et al.*, 2008). Rearing conditions for replacement sows can affect their development and skeletal system which can affect their health in later life (Dewey, 2006). Many studies show that the majority of skeletal development occurs in young animals, which indicates that the most important time for preventing muscular-skeletal issues is during early development (Loitz and Zernicke, 1992; Umemura *et al.*, 1995; Iwamoto *et al.*, 2000). Nutrients and management factors affect issues of structural integrity (Nakano *et al.*, 1987; Crenshaw, 2006; Ytrehus *et al.*, 2007; Crenshaw *et al.*, 2013) and sow mortality is associated with lameness due to structural complications (Kirk *et al.*, 2005; Anil *et al.*, 2009; Mote *et al.*, 2009). Indeed Dijkhuizen *et al.* (1989) suggested that the greatest economic losses related to culling were due to locomotory problems.

Trace minerals Cu, Zn and Mn are important for maintaining fertility and claw integrity (Ballantine *et al.*, 2002) and they also have roles in immune function

(Tomlinson *et al.*, 2004). Several studies demonstrate the benefits of mineral supplementation to sows during gestation (Anil *et al.*, 2009; Ferket *et al.*, 2009; Nair and Anil, 2011). While there is less work investigating how supplementation during rearing alone could have an impact on sow welfare, performance and longevity a gilt rearing diet should clearly be fortified with specific minerals to aid reproduction stimulation and to establish strong bones and legs for a lengthy breeding life (Close and Cole 2000).

The way in which gilts are housed during rearing could also have long-term negative implications for their longevity. In many countries, replacement gilts are reared in the same pens as finisher pigs, and kept in mixed-sex groups up to breeding age (Boyle and Bjorklund, 2007; Quinn, 2014). This means that in countries that do not practice castration (e.g., Ireland) replacement gilts are housed with entire male pigs which perform high levels of aggressive and mounting behaviour (O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014). These behaviours increase the risk of injuries, such as limb and cartilage damage; Hartnett *et al.* (2019c), which are associated with lameness.

Osteochondrosis (OC) is a chronic degenerative joint disease (Dewey, 2006; Nalon *et al.*, 2013) and thus if it is present in early life is likely to reduce sow longevity. However, OC lesions do not always produce clinical signs of pain and discomfort in animals, this is seen also in horses even with severe pathological damage (Jeffcott, 1996). There is evidence to suggest that by breeding age there is already significant structural damage present in the joints of gilts, as we found 100% cartilage lesion prevalence and a 5% level of fractures, in 102 gilts culled at breeding age (Hartnett *et al.*, 2019c).

Nevertheless, we did not find any association between the level of damage observed, and the locomotory ability of these young animals.

At the same time, it is known OC is associated with pain due to changes caused in the tissues and subsequently the underlying bone (Frantz, 2006), thus it can be assumed that progressive damage to the joints could ultimately progress to lameness in later life, and potentially chronic stress. Prolonged stress and associated elevated cortisol levels can disrupt reproduction in female pigs (Turner *et al.*, 2005).

A stressful prenatal environment can lead to permanent postnatal changes in offspring metabolism (Armitage *et al.*, 2004; Wu *et al.*, 2006; Hill *et al.*, 2010). Both Haussmann *et al.* (2000) and Otten *et al.* (2000) indicated that prenatally stressed piglets tended to be lighter than piglets from gilts not exposed to Adrenocorticotropic hormone (ACTH). Prenatal stress is also associated with low birth weights in rodents and humans (Welberg and Seckl, 2001). With regard to lameness specifically, (Parada *et al.*, 2017) found that this disorder in pregnant gilts had negative effects on their offspring's growth and behaviour.

The aim of this study was to investigate the carry-over effects of rearing replacement sows in female-only groups and on a trace mineral supplemented diet, on aspects of their welfare in their early parities, and on their lifetime performance. We hypothesised that rearing gilts in female-only groups and on a mineral supplemented diet with supplementary levels of copper (Cu), zinc (Zn) and manganese (Mn) would improve their welfare during the first pregnancy and would have long term benefits for longevity and reproductive performance. A secondary hypothesis was that their offspring would have higher birth weights.

4.2 Materials and methods

4.2.1 Care and use of animals

This study was carried out in the 200 sow unit at the Pig Development Department in Moorepark, Fermoy, Co. Cork, Ireland between December 2016 and August 2017. The experimental work was authorized by the Teagasc animal ethics committee (Approval no: TAEC136-2016) and licensed by the Health Products Regulatory Authority (License no: AE19132-P057).

4.2.2 Experimental Design

Details of the experimental design including the treatments that were applied to the gilts during the rearing stage are outlined in Chapter 2. In brief, a two by two factorial arrangement was applied to 288 gilts. The first factor consisted of group composition, whereby half of the gilts were reared in female-only groups of 12 animals (FEM) from weaning onwards, and the other half were reared in mixed-sex groups (MIX). The second factor consisted of mineral supplementation; half of each of the group composition treatments was fed a standard finisher diet until breeding age (CON) and the other half were fed the same diet, but with supplementary levels of Cu, Mn, and Zn applied (SUPP). Gilts were reared across three replicates, born three weeks apart.

4.2.3 Gestation Management

Twenty gilts from each rearing treatment group were kept for breeding (n = 80). They were served twice using artificial insemination by a trained technician firstly at the onset of standing oestrus and then 24 h later using terminal line semen (Hermitage Genetics, Ireland). Hereafter they are referred to as sows. Each batch of sows was served 3 weeks apart.

After service, sows were group housed in a dynamic group of approximately 120 sows. The building had fully slatted concrete passageways between insulated solid concrete floored lying bays and the temperature was maintained at 18-20˚C by mechanical ventilation. The sows had access to two electronic sow feeders (ESF; Schauer Feeding System, Prambachkirchen, Austria) that recognised each animal by a transponder tag programmed to her individual daily feed allowance. Sows had access to ad libitum water from single-bite drinkers in the ESFs and from five separate drinker bowls.

Approximately two weeks prior to farrowing pregnant sows were moved to a smaller pen for management purposes for one week – for the purpose of close inspection at this late stage in pregnancy and ease of movement to farrowing rooms. The feed system, water access, heating and ventilation were the same as the larger area described above. One week prior to farrowing sows were moved to the farrowing accommodation where they were penned in individual farrowing crates, in rooms housing either 7 or 14 sows. In the farrowing room the temperature was approximately 24°C at the time of farrowing thereafter, gradually reducing to 21°C by weaning age (day 27 postpartum). Heating was generated by hot water pipes (controlled via computer). In all houses there was artificial lighting (on from 0700-1700h). There was also natural lighting through the windows. Light to darkness cycle was approximately 12:12 (hrs) to allow for normal circadian rhythm.

Sows were provided with wooden chew posts in the gestation housing and a length of synthetic rope in the farrowing crate. Sows were inspected daily and sick/injured animals were treated immediately; all antibiotic treatments were recorded.

4.2.4 Live-weight and back-fat thickness

All animals were weighed individually on day 7 and day 108 of gestation and at weaning using an electronic sow scales (EziWeigh 7i, O'Donovan Engineering, Co. Cork, Ireland). Back-fat thickness was measured on day 7, day 108 of gestation and on day of weaning using a Renco Lean Meter back-fat scanner on the P2 spot (last rib, 65 mm down the dorsal middle line) on both sides of the body. Both recordings were averaged to provide a single back-fat measurement for each animal at each time point.

4.2.5 Locomotory ability

Sows were locomotion scored approximately every 3 weeks (22.8 ± 8.43 days) during the 1st gestation, and at weaning (day 27 postpartum) after their first litter. All sows had 5 inspections (days 52, 71, 92, 108 of gestation and weaning day 143). Locomotory ability was scored while the sows walked on solid concrete for a distance of approximately 30 meters, from the front, rear and side of the animal. All observations were carried out by one trained observer. Locomotion was assessed using the scoring system of (Hartnett *et al.*, 2019c), which was adapted from Calderon Diaz *et al.* (2013), and ranged from 0 (perfect locomotion) to 5 (unable to move).

4.2.6 Salivary cortisol

Saliva samples were collected on four occasions during gestation (on days 51, 71, 93 and 107) between 0930h and 1000h on the morning of collection. A fifth sample was also collected the morning after entering the farrowing house.

Sows were not removed from the home pen for saliva sampling. They were encouraged to chew on a large cotton bud (Salivette, Sarstedt, Wexford, Ireland) until it was thoroughly moistened (approximately 30-60 seconds). The samples were placed in tubes and centrifuged for 15 min at 2500 x g, then stored at -20◦C until analysis.

Saliva samples were analysed using a commercially available cortisol assay kit (Salivary Cortisol kit, Salimetrics Europe Ltd., Suffolk, UK). Cortisol was detected at a minimum concentration of < 0.003 µg/dl. Inter-assay CV (n=24) = 4.69%. Intra-assay CV (n=794) = 7.68%.

4.2.7 Hoof lesion scoring and bursitis

Hoof lesion scoring was carried out by visual inspection on three occasions on day 70 and day 109 of gestation and on the day of weaning of the first litter. On day 109 of gestation the sows' hooves were scored while they were lying down in the farrowing crates. For all other inspections the sows were raised 0.75 meters above the ground using a hydraulic chute (FeetFirst Sow Chute; Zinpro Performance Minerals, Eden Prairie, Minnesota, USA). The medial and lateral toes, medial and lateral dew claws, sole and heels of both hind feet were inspected and severity of the following lesions scored; heel erosion, heel-sole separation, white line separation, dew claw length, dew claw cracks, toe length and vertical and horizontal toe cracks. The scoring system was a modified version of the FeetFirst claw lesion scoring guide from Zinpro Corporation. Details of the scoring system are described in Chapter 1.

While the animals were raised in the crate on day 70 of gestation and on the day of weaning, the hind legs were palpated and bursa scored using the following scoring system: 0 = none, 1= hazelnut, 2 = walnut, 3 = hens egg (Mouttotou et al., 1998). If more than one bursa was present each was scored separately.

4.2.8 Sow performance at first farrowing

Sows that became pregnant at first service were monitored at farrowing (day 114.6 ± 1.1 of gestation). Data collected included numbers of piglets born alive, still born and mummified. At birth, the weight and sex of each piglet was recorded and all received an ear tag for identification. Piglets were weighed again at weaning age (27 days old). Cross fostering was kept to a minimum, and piglets were cross-fostered within treatment.

4.2.9 Lifetime performance

All performance data from the time of first service until the fifth farrowing were recorded and managed using PigChamp software (PigChamp Inc., Ames, Iowa, USA). Recorded data included dates of service, farrowing, weaning, and the number of piglets born alive and dead at each farrowing. At each parity sows that did not become pregnant after two services were excluded from the dataset, so as to mimic practices on a commercial pig unit. The reason for and age at culling was also recorded.

4.2.10 Offspring performance

At weaning, piglets were assigned to pens of 12 animals within treatment. Pigs were assigned to pens on the basis of sex and weight, and so that there was no less than two piglets from any one sow in each pen. As much as possible, equal numbers of male and female pigs were assigned to each pen (5.36 ± 1.03 males per pen across all treatments). The average weaning weight for pigs included in this part of the experiment was 7.36 ± 1.5kg. At weaning, there were a total of 9 pens of piglets from sows which had been reared in female-only pens with a standard finisher diet, and 8 pens each from sows reared on the other three treatment combinations. Weaner pens were 2.4 m × 2.6 m with a fully-slatted plastic floor. The temperature was maintained by automatic heating and negative pressure mechanical ventilation at 28°C in the weaner house immediately post-weaning. It was lowered by 2°C every 2 weeks thereafter.

After approximately 7 weeks (45.9 ± 2.6 days) in the weaner accommodation, pigs were moved in their groups to the finisher accommodation (concrete slatted floors 4 m × 2.4 m). In the finisher house the temperature was maintained at 20°C with the same ventilation system as in the weaner house, only without heating. All rooms were naturally illuminated by the windows. There was also artificial lighting (150 lux in weaner house and 130 lux in the finisher house) for 10-12 hours/day. Pigs remained in the finisher accommodation for approximately another 11 weeks (79.0 ± 0.6 days), before being sent to slaughter at a target weight of ~110 kg. Only pens that had at least 11 pigs remaining by the time of slaughter were retained in the analysis to control for the effect of space allowance on performance.

Pigs were individually weighed (not fasted) at the move from the weaner house to the finisher house and on the day before slaughter. Throughout the production cycle, the pigs were fed ad libitum by a single-spaced wet-dry feeder with dry pelleted feed (one nipple drinker inside the feeder), with another nipple drinker providing water (i.e. two drinkers in the pen in total). Feed delivered to each pen in the weaner and finisher stage was recorded daily from the feed system, and downloaded twice per week. These data were used to calculate the average daily feed intake (ADFI) at pen level. Combined with pen weights at weaning, the move to the finisher house and at the slaughter date, average daily gain (ADG) and feed conversion efficiency (FCR; ADFI / ADG) for both weaner and finisher stages were calculated.

4.2.11 Statistical analysis

Data were analysed using SAS v 9.4. Results were deemed statistically significant when α level was below 0.05, and a tendency was considered when α level was between 0.05 and 0.1**.** The Tukey-Kramer adjustment was used for multiple comparisons where least squares means (LSmeans) were determined and P-values were adjusted. Degrees of freedom were estimated using Kenwood-Rogers adjustment. Data are presented as LSmeans and standard errors. PROC UNIVARIATE was used initially for evaluating data distribution. For all models, fixed effects included group composition (MIX v's FEM), dietary treatment (CON v's SUPP) and the interaction, as well as replicate. Inspection day was included as a fixed effect for sow LW, back-fat, cortisol and hoof score analysis, and farrowing number for analysis of the number of piglets born alive. For cortisol analysis plate was included in the analysis as a random effect.

In all analysis where the mixed procedure was used, residuals were examined to verify normality and homogeneity of variances. General linear mixed models (PROC MIXED) were used to analyse LW, back-fat and cortisol data. Generalised linear mixed models (PROC GLIMMIX) were used for locomotion, and bursitis data, and individual hoof disorders (PROC GENMOD), as the data were ordinal in nature.

For locomotion scores a multinomial distribution was specified in the model. The model could not converge when data from each inspection day were included as repeated measures, and as such each inspection day was analysed separately, and p-values adjusted post-hoc using a bonferroni adjustment.

For analysis of salivary cortisol, we considered initially the four sampling points while in the group pen in a single analysis, followed by a separate analysis of levels the morning after entering the farrowing crates. For analysis of salivary cortisol level in the farrowing crates, the level of cortisol the morning before (i.e. the last sampling point in the group pen) was considered a covariate. We used a log transformation to normalise the data. Only sows that became pregnant on their first service were included in the analysis.

A total hoof score for each sow was generated by summing the score for all disorders identified. These were analysed using linear mixed models (PROC MIXED). The individual hoof disorders were also analysed individually using generalised linear models (PROC GENMOD) as the data were ordinal, using a similar model to that used for locomotion scores. The maximum score present for each sow on each inspection day was the value included in analysis.

The total number of piglets born, and the number born alive were analysed across 5 parities, including all sows even those that did not farrow and using a mixed model (PROC MIXED) as before. The number of piglets born dead was analysed using a generalised mixed model (PROC GLIMMIX) with farrowing number as a random effect. Due to the low number of gilts from each treatment selected at breeding age (n = 20 per treatment combination), the numbers surviving through each parity were not statistically analysed, but raw data were summarised.

4.3 Results

4.3.1 Live-weight and back-fat thickness

There was no effect of either diet or group composition on sow LW (FEM CON, 179.67 ± 25.83 kg; FEM SUPP, 179.60 ± 30.30 kg; MIX CON 182.80 ± 25.36 kg; MIX SUPP, 175.36± 27.81 kg). Neither was there an effect of diet or group composition on back-fat thickness (FEM CON, 15.72 ± 2.29 mm; FEM SUPP, 16.62 ± 2.79 mm; MIX CON 16.05 ± 2.50 mm; MIX SUPP, 15.86 ± 3.29 mm). Naturally LW ($P < 0.001$) and back-fat thickness ($P < 0.001$) increased over time. There was a significant difference between back-fat measurement at each time point (day 7 of gest, 16.24 ± 0.27 mm; day 108 of gestation, 17.31 ± 0.34 mm; weaning, 14.08 ± 0.37 mm; $P < 0.001$ for all comparisons).

4.3.2 Locomotory ability

There was no effect of either group composition or diet on locomotion scores of sows during pregnancy. The median locomotion score was 2 (IQR 1 – 2). In general the sows showed good locomotion with only 13 incidences (n = 12 sows) of score 3 over the whole experimental period. No sow received a scored or 4 or 5 throughout the experimental period. However, if a pig received a score >3 they would be removed from the trial.

4.3.3 Salivary cortisol

There was an interaction between group composition and diet ($P < 0.05$; Figure 4-1) on salivary cortisol levels with sows reared in mixed-sex groups and on a mineral supplemented diet having the highest cortisol levels during pregnancy. Although there was an effect of sample point on cortisol levels, there was no discernible pattern over time ($P < 0.01$). There was a tendency for SUPP sows to have higher cortisol levels than CON ($P < 0.1$).

On the first morning after moving from group housing to the farrowing house we found no effect of either group composition (FEM, 0.175 ± 0.14 µg/Dl *vs.* MIX, 0.171 ± 0.16 µg/Dl) or diet (CON, 0.175 ± 0.15 µg/Dl *vs.* SUPP, 0.171 ± 0.16 µg/Dl) during rearing on salivary cortisol levels.

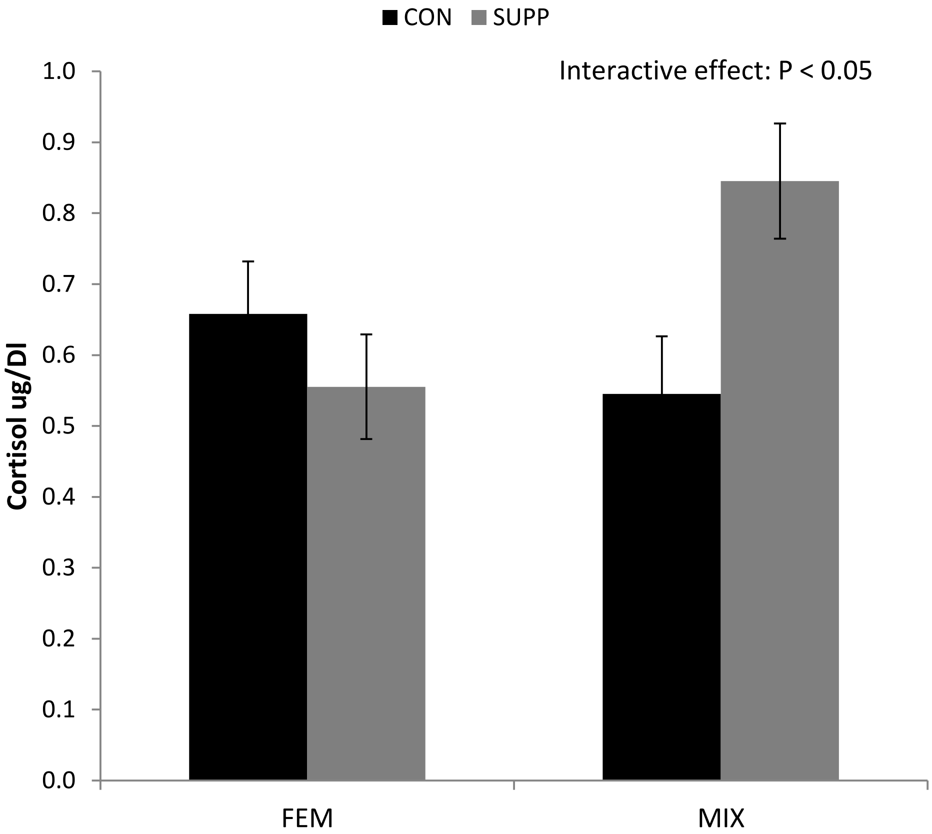

Figure 4-1 Salivary cortisol levels during gestation in sows reared either in female-only (FEM) or mixed-sex (MIX) pens, and fed a control finisher diet (CON) or the same diet supplemented with Cu, Mn and Zn (SUPP). Cortisol levels were log transformed prior to analysis. Data are presented using back transformed means and standard errors.

4.3.4 Hoof lesion scores

Total hoof scores were higher (i.e. worse) in the MIX (22.20 ± 0.61) sows than in the FEM (19.72 ± 0.59) sows ($P < 0.005$) across the study period. There was also an effect of inspection day, with hoof scores increasing over time (mid 1st gestation, 15.01 ± 0.53; farrowing 2nd gestation, 17.30 ± 0.49; weaning of 2nd farrowing, 30.58 ± 0.92; $P < 0.0001$). When comparing treatment groups, we found that within the CON treatment there was a significant increase in total hoof scores in sows reared in MIX groups ($P < 0.01$; Figure 4-2). In the SUPP group we found no difference in hoof scores when comparing between MIX and FEM groups ($P > 0.6$). Sows reared on the control diet tended to have higher horizontal crack scores than mineral supplemented sows ($P < 0.1$). Sows reared in female-only groups had lower heel erosion scores compared to sows reared in mixed-sex groups ($P < 0.05$). There were no other effects of mineral supplementation or group composition on individual hoof disorders (Table 4-1).

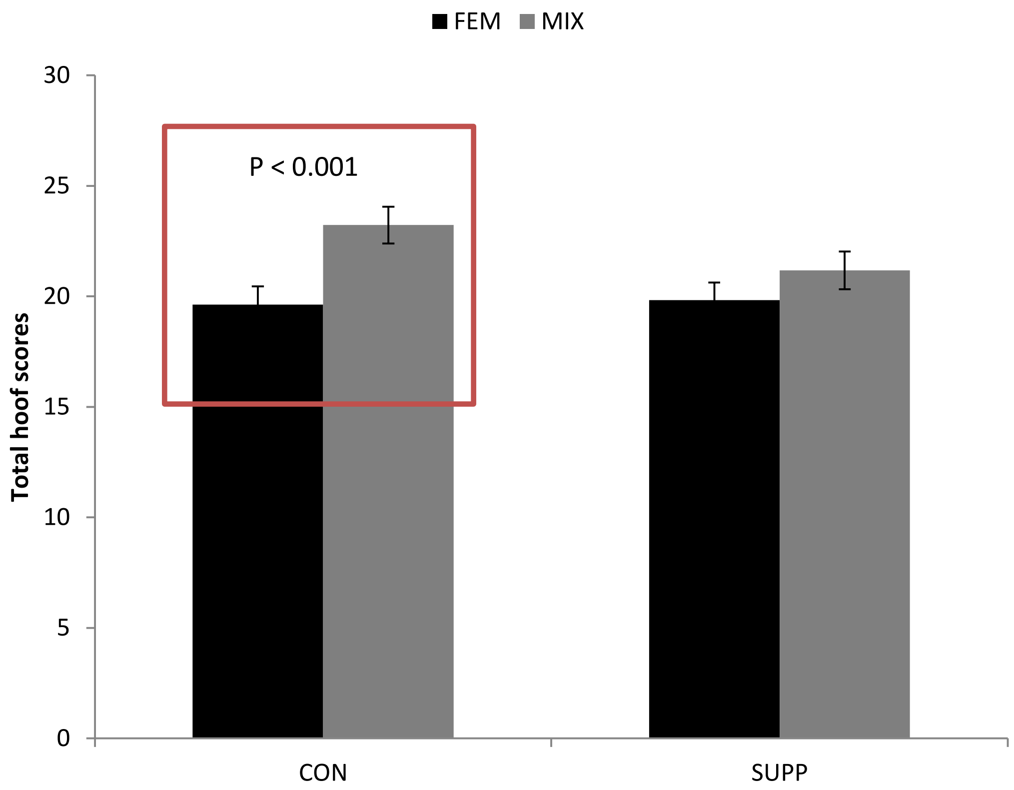

Figure 4-2 Total hoof scores in sows showing an effect of group composition (FEM, female-only; MIX, mixed-sex) when reared on the CON diet (control diet) compared to no effect of group composition when reared on the SUPP diet (mineral supplemented diet).

Table 4-1 Individual hoof disorders of gestating sows reared in either mixed-sex (MIX) or female-only (FEM) groups and on a standard finisher diet (CON) or on a CON diet supplemented with minerals (SUPP).

	Diet			Group composition		
	CON	**SUPP**	**P-value**	**MIX[1]**	**FEM**	**P-value**
Heel erosion[2]	1 (1-2)	2 (1-2)	0.92	2 (2-3)	2 (1-2)	0.05
Heel sole separation[2]	1 (0-2)	1 (0-2)	0.97	1 (0-2)	1 (0-2)	0.93
White line separartion[2]	2 (1-3)	2 (1-2)	0.17	2 (1-2)	2 (1-2)	0.43
Vertical cracks[2]	1 (0-1)	0 (0-1)	0.25	1 (0-1)	0 (0-1)	0.15
Horizontal cracks[2]	0 (0-1)	0 (0-0)	0.06	0 (0-0)	0 (0-0)	1.00
Dew claw length[2]	1 (1-2)	1 (1-2)	0.41	1 (1-2)	1 (1-2)	0.54
Dew claw cracks[2]	1 (0-1)	1 (0-1)	0.83	1 (0-2)	0 (0-1)	0.15
Toe length[2]	1 (1-1)	1 (1-1)	NS	1 (1-1)	1 (1-1)	NS

[1] MIX = sows raised in groups composed of 6 males and 6 females per group

[2] Data are provided as median and interquartile ranges.

4.3.5 Bursitis

There was no effect of group composition or mineral supplementation on bursitis scores (FEM, 2.03 ± 0.05; MIX, 1.96 ± 0.05; CON 2.02 ± 0.05; SUPP, 1.97 ± 0.05).

4.3.6 Sow performance

A summary of the total number of sows that farrowed in each parity, and the total number of piglets born to sows in each of the treatments at each parity is provided in Table 4-2.

There was no effect of group composition or diet on the lifetime born alive number when the numbers born alive at each parity were summed for each sow (Table 4-3). Neither was there an effect on the number of piglets born alive per farrowing, or the total number of piglets born at each farrowing (table 4-3). However, there was an effect of group composition on the numbers of stillborn piglets, with MIX gilts having the highest number of stillborn piglets across all five parities ($P < 0.05$; Table 4-3).

Table 4-2 The number of breeding females which remained in the herd at each parity (a), as well as the total number of piglets born alive (BA) to breeding females within each treatment (b). Data are presented as raw figures without statistical analysis

(a)

	CON	SUPP	MIX	FEM
Sows farrowed (no.)*				
Parity 1	31	34	32	33
Parity 2	23	26	27	22
Parity 3	16	19	18	17
Parity 4	14	16	16	14
Parity 5	13	13	14	12

*A total of 40 breeding females were selected for service within each individual treatment at breeding age. If a sow did not become pregnant after being served twice at any parity, she was removed from the data set.

(b)

	CON		SUPP		MIX		FEM	
	Total	Per sow	Total	Per sow	Total	Per sow	Total	Per sow
Piglets BA[1]								
Parity 1	368	11.9	391	11.5	365	11.4	394	11.9
Parity 2	292	12.7	362	13.9	369	13.7	285	13.0
Parity 3	216	13.5	281	14.8	249	13.8	248	14.6
Parity 4	210	15.0	243	15.2	239	14.9	214	15.3
Parity 5	207	15.9	206	15.8	227	16.2	186	15.5
All parities	1293		1483		1449		1327	

Table 4-3 Sow performance data from breeding females showing lifetime performance over 5 parities and also average performance by farrowing.

	Diet			Group composition		
	CON	SUPP	P value	MIX	FEM	P value
Lifetime BA[1]	34.34 ± 5.03	40.53 ± 5.03	0.39	39.81 ± 5.03	35.06 ± 5.03	0.51
Stillborn[2]	78	103	0.45	119	62	0.02
By farrowing						
Born alive	13.81 ± 0.39	14.01 ± 0.38	0.71	13.80 ± 0.38	14.01 ± 0.39	0.69
Total born	14.98 ± 0.40	15.60 ± 0.39	0.26	15.44 ± 0.39	15.14 ± 0.40	0.58

[1] The average number of piglets born alive (BA) to a sow within each treatment. Data were collected over 5 parities

[2] Data represent the total number of stillborn piglets from all sows within each treatment, over 5 parities. Data were analysed on a per sow basis, including parity as a random effect, to generate p-values

4.3.7 Offspring growth performance

There was no effect of group composition or mineral supplementation during maternal rearing on offspring weight at any production stage, ADG (from birth to weaning, weaning stage and finisher stage), ADFI (weaning and finisher stage), or FCE (weaner and finisher stage (Table 4-4).

Table 4-4 **Pre and post-weaning growth performance of the offspring of sows reared in either mixed-sex (MIX) or female-only (FEM) groups and on a standard finisher diet (CON) or on a CON diet supplemented with minerals (SUPP).**

	Diet			Group Composition		
	CON	**SUPP**	**P-value**	**MIX**	**FEM**	**P-value**
Weight (kg)*						
Total born	1.193 ± 0.062	1.195 ± 0.062	0.97	1.197 ± 0.062	1.192 ± 0.062	0.93
Live born	1.347 ± 0.045	1.396 ± 0.042	0.43	1.382 ± 0.046	1.361 ± 0.041	0.75
ADG	0.212 ± 0.010	0.219 ± 0.009	0.59	0.216 ± 0.011	0.215 ± 0.008	0.93
Weaning[1]	7.490 ± 0.245	7.303 ± 0.229	0.59	7.573 ± 0.250	7.220 ± 0.212	0.29
ADG (kg/day)*						
Weaner	0.572 ± 0.015	0.582 ± 0.014	0.95	0.572 ± 0.016	0.582 ± 0.014	0.97
Finisher	0.901 ± 0.015	0.912 ± 0.014	0.95	0.915 ± 0.016	0.899 ± 0.014	0.87
ADFI (Kg/day)*						
Weaner	0.863 ± 0.029	0.917 ± 0.028	0.54	0.878 ± 0.031	0.903 ± 0.027	0.93
Finisher	1.993 ± 0.029	2.018 ± 0.027	0.92	2.012 ± 0.030	1.999 ± 0.027	0.99
FCR (g/g)						
Weaner	1.513 ± .027	1.570 ± 0.026	0.54	1.529 ± 0.029	1.554 ±0.024	0.94
Finisher	2.215 ± 0.027	2.218 ± 0.024	0.99	2.210 ± 0.028	2.224 ± 0.024	0.99

*Data are provided as means and standard errors.

[1] Weights of all piglets weaned

4.4 Discussion

There is evidence that improvements to the management and housing of replacement gilt during rearing could help to improve sow longevity, primarily through reduced culling for lameness (Dewey, 2006) with associated benefits to animal health and welfare. As such, this study investigated the effect of two specific improvements to gilt nutrition and management (supplementation with minerals, and rearing in female-only groups) during the rearing period, having already demonstrated that these strategies had welfare benefits for gilts during the rearing period (Hartnett *et al.*, 2019c). However, in general, there were few effects of either strategy found on indicators measured during gestation or on sow lifetime performance.

Overall, locomotion scores were good during the study period, with very few incidences of clinical lameness (sows scoring 3 or greater) and no incidences of scores 4 and 5. Indeed there was little lameness in the animals during rearing (Hartnett *et al.* (2019c); Chapter 3), although the median score did increase from 1 in the former study, to 2 in the current study. This demonstrates that there was a slight impairment to locomotion overall, relative to the rearing period. This may be due to added weight and growth during gestation, as well as potentially to the accumulation of injuries.

Hoof lesion scores in mid gestation were considerably higher compared to the scores recorded in the same gilts during rearing (Chapter 2). Furthermore, they continued to increase until the last inspection point when sows farrowed for the second time. The increase in hoof scores over time was likely due to general wear and tear with age, as the hoofs must support more weight as pigs increase in size. This is further complicated by housing on fully slatted floors, which can result in injuries due to claws becoming caught or trapped in the slats (Gjein and Larssen, 1995a; Pluym *et al.*, 2011). Hoof lesions can be associated with pain and irritation as the lesions on the surface of the hoof can affect sensitive tissues, and secondary infections can occur as a result (Zoric *et al.*, 2004). Nevertheless, none of the hoof disorders we observed were severe, and this could be why we did not observe differences in locomotion score. In line with findings on the same animals during rearing (Chapter 2) total hoof lesion scores were higher in the sows reared in mixed-sex groups compared with sows reared in female-only groups. Thus the claw horn damage resulting from more activity (mounting, aggression, and play) on the concrete slatted floors during rearing persisted (Gjein and Larssen, 1995a; Pluym *et al.*, 2011; Quinn *et al.*, 2015). This is not surprising as concrete floors offer little opportunity for claw horn lesions to resolve (Heinonen *et al.*, 2013b).

When considering only sows that were reared on the control diet, we found that those in mixed-sex groups had higher total hoof lesion scores than those reared in the female-only groups, whereas this was not the case for sows reared on the mineral supplemented diet. This suggests that the mineral supplemented diet had a long term protective effect on hoof health. Thus if space or other constraints do not permit separate sex rearing, supplementation with the minerals used in the current study could have long term benefits for hoof health in sows.

In other studies the heel and horn wall region were the most affected areas of the hoof (Gjein and Larssen, 1995a; Kirk *et al.*, 2005; Anil *et al.*, 2007; Pluym *et al.*, 2011) and indeed these are the two areas where we saw the greatest effect of the treatments imposed; sows reared on the control diet tended to have higher horizontal crack scores than mineral supplemented sows, and sows reared in female-only groups had lower heel erosion scores compared to sows reared in mixed-sex groups. Studies with pregnant sows found that the addition of the trace minerals we used in the current study improved claw health (Nair and Anil, 2011) and reduced the prevalence of lameness and leg abnormalities (Ferket *et al.*, 2009). Nair and Anil (2011) found that the addition of trace minerals Cu, Zn and Mn to the diet of the breeding sow herd resulted in the reduction of heel erosion and white line lesions. However, similar to Quinn *et al.* (2015) we found that there was a low prevalence of severe lesions and a high prevalence of mild lesions. Mild lesions do not appear to influence locomotary ability (Gjein and Larssen, 1995b; Anil *et al.*, 2007; Quinn *et al.*, 2015) which could explain the lack of effect of treatment on the locomotion scores in the current study.

Surprisingly, we found that sows reared in supplemented groups tended to have higher cortisol levels than those reared on a standard finisher diet. Hoyt *et al.* (2016) found that increases in cortisol levels were associated with rises in the subject's activity levels, alertness, and relaxation, which suggests that a rise in cortisol levels could indicate a positive welfare state as well as a negative one, and thus cortisol levels should be interpreted in the context of other measures of welfare. de Jong *et al.* (1998) found that pigs housed in an enriched environment had higher baseline salivary cortisol levels compared to pigs reared in barren conditions during light hours. Indeed, (Kinane, 2019) found that sows in loose lactation crates had higher cortisol levels than those in standard crates, even though other measures (e.g. ability to move, interaction with piglets) pointed towards improved welfare in these sows. In that study, the authors hypothesised that the increased activity levels of the sows led to the higher cortisol levels. It is possible that around the time of sampling, the sows which were reared with the supplement were most active; nevertheless, it is important to note that the difference was only a tendency, and thus the result must be interpreted with caution. When measuring the effect of chronic stress (e.g. due to lameness, social stress etc.) patterns of cortisol secretion throughout the day, or the ability of the animal to increase cortisol secretion in response to a stressor may be more informative than simply sampling the animal at one sampling point a day.

Although we could not statistically analyse the data pertaining to sow longevity and total piglet output at each farrowing, we can see from the raw data that sows reared with the optimal diet (i.e. the mineral supplement) numerically, seemed to stay in the herd longer than those on the control. The opposite was the case for the grouping strategies; sows reared with males seemed to remain longer. Thus overall, the gilts reared with the mineral supplement had a greater number of piglets born alive over the five parities than those on the control, whereas the gilts reared in the mixed groups had a greater number than those in the female-only groups. What contributed to the greater number for gilts reared with the minerals was that on average, numerically they had more piglets born alive in the 2nd, 3rd and 4th parities than those on the control diet, although this was not statistically significant. The pattern was not so clear for the grouping strategies; gilts reared with males alternated between having more or fewer born alive piglets than those with females, and thus the numerical total number born was not as great for these animals. Moreover, gilts reared with the males, had significantly more piglets born dead over the five parities. This could help explain why even though numerically they had a greater number of total piglets born, at the same time numerically they had fewer born alive, when the data were analysed statistically. We know from the literature that good hoof structure and locomotion is associated with increased litter size, more piglets born alive, and fewer stillborn piglets (Pluym *et al.*, 2013b). Thus the increased hoof damage which we observed both during rearing (Chapter 2) and in the current study, as well as the higher levels of damage to the cartilage (Chapter3 ; Hartnett *et al.* (2019c)) in gilts reared in the mixed-sex treatment, and without supplementary minerals, likely contributed to reduced reproductive performance. Nevertheless, these data are preliminary and should be considered pilot data which could inform a much larger future study.

There is evidence in the literature that maternal stress during pregnancy can negatively affect offspring growth. For instance, models of social stress in gestating pigs found that weight gain post-weaning can be negatively affected (Rutherford *et al.*, 2012). Moreover, feed-restricted gilts had higher salivary cortisol levels during gestation, indicating greater levels of physiological stress, and subsequently these gilts gave birth to lighter piglets (Amdi *et al.*, 2013). On the other hand, (Otten *et al.*, 2007) found that when ACTH was administered to gestating gilts in late gestation, piglet birth weight was increased, and this was sustained until 21 days of age. Thus the type and timing of a pre-natal stressor could affect the outcome for the piglets. As we confirmed that both hoof health and cartilage condition was negatively impacted by both rearing with males and the standard finisher diet, we expected that sows from these treatments would have experienced pain and stress during gestation, even if there were no overt signs of lameness. However, we found no effect of either treatment on offspring performance either pre or post weaning. This could be a reflection of the fact that there was no difference in salivary cortisol levels during gestation; however, as stated above, the method of sampling may not have been ideal in detecting chronic stress. In fact, in a separate study to this thesis, we found a range of behavioural differences in offspring born to the gilts in the current study (Hartnett *et al.*, 2019a). Piglets from gilts reared with males screamed more in an open arena test than those from the female-only groups, and piglets from gilts supplemented with the minerals explored more, stood still less, and grunted less, than those on the control diet. Thus, although there was no effect on physical performance, there is yet the possibility that piglets were affected by maternal rearing (Hartnett *et al.*, 2019a).

4.5 Conclusions

During gestation, hoof lesions were worst in sows which were fed a standard finisher diet without supplementation and reared in mixed-sex pens compared to all other treatment combinations; therefore if space is limited, supplementary minerals may help ameliorate some of the negative effects of rearing gilts with entire male pigs. However, supplementing gilts with minerals during rearing resulted in minor albeit positive effects on the severity of hoof cracks during pregnancy. Rearing sows in female-only groups as gilts showed positive effects during gestation and the reproductive period, therefore decreasing the likelihood of culling for lameness.

Chapter 5 - The effect of group composition and mineral supplementation during rearing on serum markers of osteochondrosis in replacement gilts

5 The effect of group composition and mineral supplementation during rearing on serum markers of osteochondrosis in replacement gilts

5.1 Introduction

Osteochondrosis (OC) is an irregularity of endochondral ossification whereby cartilage necrosis occurs and results in thickened, abnormal cartilage (Ytrehus *et al.*, 2007) which is vulnerable to injury (Mow, 1990). Trauma to the abnormal cartilage results in cartilage fractures, thus forming a cartilage flap (separation of a segment of cartilage from the subchondral bone) known as osteochondrosis dissecans (OCD) (Kinzel *et al.*, 1976; Kincaid and Van Sickle, 1981; Nakano and Aherne, 1988). It develops during rearing in young growing gilts (de Koning *et al.*, 2016) and is one of the most important causes of leg weakness leading to premature culling in breeding sows (Dewey, 1999; Dewey, 2006; de Koning *et al.*, 2013).

The majority of skeletal development occurs in young animals, and it can be affected by rearing conditions (Dewey, 2006). Ytrehus *et al.* (2004a) suggested that the sensitive phase for porcine OC development occurs between 7 and 13 weeks of age, which indicates that there are age-dependent effects on its development. If the rate of OC development depends on the age of an animal, then it is possible that prevalence and/or severity of OC could thus be reduced by preventative methods at targeted ages. Indeed the most important time for preventing muscular-skeletal issues is during early development (Loitz and Zernicke, 1992; Umemura *et al.*, 1995; Iwamoto *et al.*, 2000). Much of the culling of sows is due to lameness (Lucia *et al.*, 2000), which can arise due to damage to the skeletal system. Hence strategies to reduce OC development during development could significantly reduce sow culling rates (approximately 40 to 50% are culled before their third or fourth parity; (D'Allaire *et al.*, 1987; Boyle *et al.*, 1998; Schenck *et al.*, 2008).

Clinical signs of OC (i.e. lameness) are not obvious until the lesions have progressed to the final stage of development (Mccoy *et al.*, 2013). To diagnose these lesions, a post mortem examination is necessary (Kirk *et al.*, 2008). Studies focusing on multiple species during the growing phase provide evidence that lesions developed at predilection sites at an earlier age than clinical signs are apparent (Ytrehus *et al.*, 2004b; Olstad *et al.*, 2007; Mccoy *et al.*, 2013). Without clinical signs, is it difficult to detect animals that may be in pain, and in the case of developing gilts, affected animals should not be bred.

Indeed, in general, locomotion scoring of pigs has a low level of reliability (Main *et al.*, 2000; Channon *et al.*, 2009; D'Eath, 2012; Nalon *et al.*, 2013), and is thus of limited usefulness in identifying animals affected by OC. Thus, it is possible that a more accurate way of measuring OC is by means of biomarker analysis. Biomarkers (biological-markers) are metabolic products resulting from synthesis or degradation in certain tissues which are delivered into the blood stream, maintained in the tissue of interest, or excreted in bio-fluids such as urine (Garnero *et al.*, 2000). Biomarker levels are altered when there is an imbalance between the synthesis and degradation processes (Garnero *et al.*, 2000; Garnero *et al.*, 2001). Therefore, biomarker levels could potentially correlate with the metabolic actions happening in the joint and underlying bone (Frantz, 2006).

Frantz *et al.* (2010) stated that biomarker identification may aid in the selection process of breeding animals against OC and help reduce the economic and animal welfare consequences of this disease. However, there are only a few biomarkers of bone or cartilage turnover that have been evaluated in pigs (Carter *et al.*, 1996; Weiler *et al.*, 2001; Shaw *et al.*, 2006). Frantz *et al.* (2010) evaluated a variety of metabolic biomarkers in the categories of synthesis (C-propeptide of type II (CPII) and Chondroitin sulphate epitope 846(CS846)), degradation (collagen type II cleavage (C2C), Carboxy-terminal cross-linked telopeptide (CTXII) and Cartilage oligomeric matrix protein(COMP)), bone turnover (Carboxy-terminal cross-linked telopeptide of type I collagen (ICTP), Amino-terminal telopeptide of type I Collagen (NTX) and Pyridinoline cross-links collagen (PYD)) and bone formation (Bone-specific alkaline phosphatase fragment of type II collagen (BAP) and Osteocalcin). This work found that CPII and C2C were significantly related to OC progression, when levels were evaluated relative to histological indicators of early OC development.

CPII increases in horses with OC lesions compared to those without (Black *et al.*, 1989; Kollerup *et al.*, 1994; Lindqvist *et al.*, 2005).Thus CPII has potential as an indicator of OC in swine, as the disease process is similar to OC in other species. In an experimental model, elevated levels of C2C in both serum and urine were related to progressive arthritis (Song *et al.*, 1999). Thus these metabolites have potential to aid in OC detection in replacement gilts.

Structural bodily integrity is influenced by dietary nutrient content and management factors (Nakano *et al.*, 1987; Crenshaw, 2006; Ytrehus *et al.*, 2007; Crenshaw *et al.*, 2013), and indeed sow mortality is linked to lameness resulting from structural complications (Kirk *et al.*, 2005; Anil *et al.*, 2009; Mote *et al.*, 2009) Separating replacement gilts from finishing stock, particularly entire males, could significantly reduce the incidence and severity of joint lesions and OC dissecans (OCD); in mixed-sex groups, females are subjected to potential trauma caused by the higher levels of sexual mounting and aggression performed by entire males, compared with females (Boyle and Bjorklund, 2007; O'Driscoll *et al.*, 2013; Teixeira and Boyle, 2014; Hartnett *et al.*, 2019c). Epidemiological and clinical results show that degenerative joint disease such as osteoarthritis is primarily caused by mechanical means with genetic and acquired factors further contributing to its severity (Felson *et al.*, 1990; Sharma *et al.*, 2013). Although the consequences of trauma associated with handling and transport may cause lameness (Grondalen, 1976; Nakano and Aherne, 1988) there is limited research to date on the effect of trauma due to sexual mounting and exposure to aggression, on female pigs. Nevertheless, in Chapter 3, we described the reduction in incidence of cartilage lesions and OCD in gilts which were reared in all female groups, compared with those reared in mixed-sex groups.

Moreover, we also described in Chapter 3 the positive effects of addition of the trace minerals copper (Cu), zinc (Zn) and manganese (Mn) to the diet on both increased aBMD (areal bone mineral density) in the humerus, and on cartilage lesion and OCD development (Hartnett *et al.*, 2019c). As we found that by the time animals get to breeding age there was evidence of cartilage damage in all animals, it is evident that there are likely high levels of cartilage degradation and synthesis occurring. As OC is degenerative, differences in both gross lesions and levels of biomarkers are likely to increase, and may become even more apparent later in life.

The aim of this study was to validate the analytes C2C and CPII as biomarkers of OC. We initially compared serum C2C and CPII concentrations with joint lesions and OCD scores on gilts which were culled at breeding age, before moving on to investigate levels of C2C and CPII concentrations in similar animals, but at three time points from breeding until second farrowing. We hypothesised that the effect of rearing gilts in a female-only group and on a diet with supplementary levels of Cu, Zn, and Mn would have a positive effect on bone cartilage turnover and OC status in the sows. The ultimate aim of this study was to investigate a method of identifying animals with varying levels of severity of cartilage degradation by means of blood sampling live animals, and to determine whether rearing conditions affected the indicators of cartilage turnover.

5.2 Materials and methods

5.2.1 Care and use of animals

This study was carried out in the 200 sow unit at the Pig Development Department in Moorepark, Fermoy, Co. Cork, Ireland between December 2016 and August 2017. The experimental work was authorized by the Teagasc animal ethics committee (Approval no: TAEC136-2016) and licensed by the Health Products Regulatory Authority (License no: AE19132-P057).

5.2.2 Animals and Management

Details of the housing and management of the animals included in this experiment are outlined in Chapters 2 and 4. In brief, 288 gilts were assigned to either mixed-sex (MIX; 6 males and 6 females) or female-only (FEM; 12 females) groups at weaning. They were all fed the same standard weaner, then finisher diet unit 117 days of age, at which point half of each of the treatment groups was assigned a new diet which contained a mineral supplement containing increased levels of Cu, Zn and Mn. At finishing age (day 148 of age) all male pigs and half of the female pigs were sent for slaughter. The remaining gilts stayed in their groups until breeding age, at which point 102 gilts were slaughtered and had their right forelimb removed for cartilage lesion and aBMD (areal bone mineral density) examination (Chapter 3). The remaining 80 gilts (n = 20 per treatment combination) were bred and retained in the Moorepark research herd as breeding animals until the end of their productive lives (Chapter 4).

5.2.3 Joint lesion and locomotion scoring

Dissection of the limbs which were removed from the gilts culled at breeding age was carried out at the elbow joint to expose the humeral condyle (HC). This is made up of the capitulum, the trochlea and the trochlear notch (TN) surface. Two types of scoring methodology were used. First, the HC and TN were examined and given the total number of abnormalities summed (Joint lesion Count)). The following abnormalities which were present were included; invagination, thinning, and overgrowth of the cartilage. In addition, we also scored the joints was as per Quinn et al. (2015), with scores ranging from 1 (normal) to 4 (severe abnormality; Joint Lesion Score (JLS)).

In addition to these two methodologies, the number of areas of OCD (identified by detachment of the cartilage from the underlying bone) that were present were also counted (OCD prevalence).

These animals were also locomotion scored using an adapted version of (Calderon Diaz *et al.*, 2013) (Chapter 2) ranging from 0 (perfect) to 5 (unable to move). Locomotory ability was scored while the gilts walked on solid concrete for a distance of approximately 30 m, from the front, rear and side of the animal. All the observations were carried out by one trained observer. Observations were carried out 6 times during the rearing stage; observation days were 81, 96, 110, 138, 152 and 166 days of age.

5.2.4 Blood sampling

5.2.4.1 Experiment 1

Blood was collected at a single time point, approximately 2 days prior to slaughter (day 194 of age) from the 102 gilts that were slaughtered at breeding age.

5.2.4.2 Experiment 2

The second experiment involved taking blood from the 80 gilts that were retained in the herd for breeding. Blood samples were taken at 3 time points (n = 181 samples); at breeding age (day 194 of age) mid gestation of their 1st pregnancy (day 70.5 ± 2.4 of the first gestation) and just prior to the gilts' 2nd farrowing (day 107.3 ± 0.5 of the second gestation).

5.2.4.3 Sampling method

Blood samples were collected by jugular venepuncture using Vacuette 9ml serum clot activator tubes. Blood sampling was carried out by a trained and authorised individual in accordance with as standard operating procedure developed by the Teagasc Pig Development Department. The area of the pigs' neck was cleaned and sterilised, and a fresh needle was used to draw the blood. Once all blood samples were collected, they were centrifuged at 4 °C at 3,000rpm for 15 minutes, then serum extracted and stored in a −20 °C freezer until analysis. The frozen serum samples were transported to SRUC Edinburgh on dry ice for analysis.

5.2.5 Laboratory analysis

5.2.5.1 C2C

The C2C ELISA kit (IBEX Pharmaceuticals Inc. Montreal, Canada) was used for the assay analysis. The analysis was carried out in a 96-well plate format using a mouse monoclonal antibody and a synthetic peptide which represents the neoepitope (Poole *et al.*, 2004) which is combined with a protein and spread onto the C2C ELISA plate as the assay is a competitive immunoassay. Firstly the C2C standards and unknown samples from the serum samples were added to a polypropylene mixing plate, followed by specific mouse IgG antibody (C2C antibody). The samples were pre-incubated in the mixing plate to ensure that the antibody bound to the free C2C peptide. The samples were transferred from the mixing plate onto the C2C ELISA plate and incubated so the antibody bound to the immobilized peptide in the wells, to the C2C standards or to the endogenous neoepitope in the serum samples.

The C2C ELISA plate was washed and the conjugated goat anti-mouse horseradish peroxidase (GAMHRP) was added to the C2C plate. The GAMPHRP bound to any mouse antibody present on the C2C plate. The C2C plate was washed again and Tetramethylbenzidine substrate (TMB) was added to every well. TMB reacted with GAMHRP which created a blue colour substance. A stopping solution was added to stop the reaction and the signal was amplified by the acid present in the stopping solution, this reaction turned the blue colour to yellow colour which was quantified at 450 nm. The amount of neoepitope present in the sample is inversely proportional to the optical density (OD) at 450 nm.

5.2.5.2 CPII

For CPII analysis, a CPII ELISA kit (IBEX Pharmaceuticals Inc. Montreal, Canada) was used. Similar to the C2C assay, the CPII assay is a competitive 96 well immunoassay using bovine CPII antigen spread onto the CPII ELISA plate. Firstly a mixing plate is used for bovine CPII standards and the unknown serum samples then a rabbit polyclonal antiserum specific for CPII is added to the mixing plate. The mixing plate containing the mixture is incubated to allow the antibody to bind to the free CPII antigen. The samples from the mixing plate are then transferred to the CPII ELISA plate and incubated so the antibody bound to the immobilized CPII in the wells, to the CPII standards or to the endogenous epitope in the serum samples.

The CPII ELISA plate was washed the conjugated goat anti-rabbit horseradish peroxidase (GARHRP) was added to the CPII plate. The GAMPHRP bound to any rabbit antibody present on the CPII plate. The CPII plate was washed again and Tetramethylbenzidine substrate (TMB) was added to every well. TMB reacted with GARHRP which created a blue colour substance. A stopping solution was added to stop the reaction and the signal was amplified by the acid present in the stopping solution, this reaction turned the blue colour to yellow colour which was quantified at 450 nm. The amount of neoepitope present in the sample is inversely proportional to the optical density (OD) at 450 nm.

5.2.6 Statistical analysis

Data were analysed using SAS v 9.4. The treatment group was the experimental unit for analysis. Results were deemed statistically significant when α level was below 0.05, and a significant tendency was considered when

α level was between 0.05 and 0.1. The Tukey-Kramer adjustment was used for multiple comparisons where least squares means (LSmeans) were determined, and P-values were adjusted. Degrees of freedom were estimated using Kenwood-Rogers adjustment. Data are presented as LSmeans and standard errors.

The distribution of all data was initially evaluated graphically (PROC UNIVARIATE), and in all analyses where mixed models were applied, residuals were examined to verify normality and homogeneity of variances. Data are presented as LSmeans and standard errors. Any samples with a coefficient of variation (CV%) greater than 20% were removed from the analysis. When analysing the ratio between C2C and CPII, plate was removed as a random effect.

5.2.6.1 Experiment 1

Correlations between the two analytes, their ratio, the JLS, the individual abnormality counts, and locomotion scores were calculated (PROC CORR). Pearsons correlations are reported where both sets of data were continuous and normally distributed, and otherwise Spearmans Rank correlations are reported.

We then used linear mixed models to investigate whether serum levels of C2C and CPII varied between gilts in each JLS category. As there were only 4 gilts assigned a score of 1, we merged these animals with those of score 2, and categorised these as mild. Likewise, there were only 6 gilts with a score of 4, so these were merged with gilts with a score of 3, and categorised these as moderate. The presence of OCD (a score of > 5) remained as it was, and we categorised these as severe. The model had a single fixed effect of JLS, as well as the random effect of ELISA plate. We also carried out a similar analysis to investigate whether serum levels of C2C and CPII differed between gilts of different locomotion scores.

Finally, to investigate the effect of rearing treatment, both CPII and C2C and their ratio were analysed using linear mixed models (PROC MIXED) with fixed effects being group composition, diet, the interaction between the two, and replicate. Where the interaction was not significant it was removed from the final model. Rearing pen and the ELISA plate were included in the analysis as random effects.

5.2.6.2 Experiment 2

This analysis had a similar model to that described for the investigation of treatment effects (Experiment 1), but with the addition of sampling time as a repeated effect. Pen was not used as a random effect as all sows are housed together during this experimental period. When carrying out mixed models we used the slice function to obtain an estimate of treatment differences at time point A so that we could compare the results to those from Experiment 1 (the time that blood samples were taken in Experiment 1 was the same as the time of the first blood sampling time Experiment 2).

5.3 Results

5.3.1 Experiment 1

5.3.1.1 Correlations

There was a tendency for a negative, but weak correlation between serum levels of CPII and C2C (r = -0.191; P = 0.08).

We saw a tendency for a negative correlation between C2C and the total count of lesions on the joint surface (r = -0.197; P = 0.06). When this was broken down into the separate disorders (thinning, invaginations and overgrowth), there was only a significant, and negative correlation between C2C and the number of thinnings (r = -0.217; P < 0.05). There was no correlation between C2C and locomotion score.

With regard to CPII there was no correlation with any of the cartilage measurements, and only a tendency for a negative correlation with locomotion score (r_s =-0.19; P = 0.09).

The ratio between C2C and CPII was positively correlated with both the total joint lesion score (r = 0.299; P < 0.05) and with the number of thinnings present (r = 0.280; P = 0.01).

5.3.1.2 Differences in analyte levels across JLS categories

We found no significant difference between levels of C2C (figure5-1) or CPII (figure 5-1) across the three severity categories of cartilage damage.

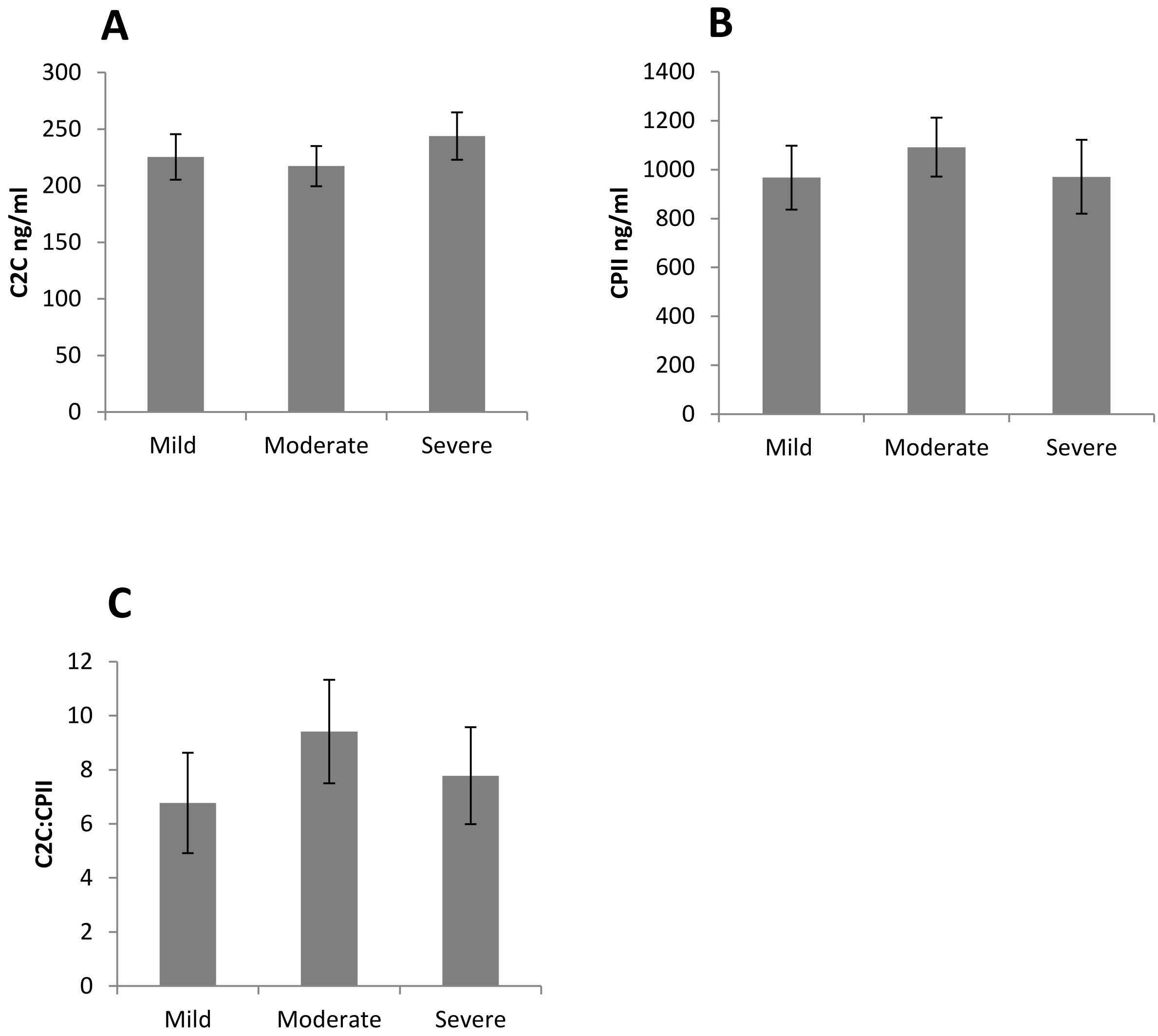

Figure 5-1 Levels of (A) C2C, (B) CPII and (C) the ratio of the two analytes in gilts which had mild Joint Lesion Scores (JLS; Joint Lesion Scores of 1(normal) and 2 (mild abnormality)), moderate JLS (representing cartilage lesion scores 3 (moderate abnormality) and 4 (severe abnormality), and severe damage (Osteochondrosis dissecans present, which was damage to the cartilage to the point where it has separated from the underlying bone).

5.3.1.3 Differences in analyte level across Locomotion scores

We found no difference between C2C, CPII and the ratio of the two analytes when compared across gilts of locomotion score 0, 1 or 2. Numerically, C2C rose and CPII decreased with locomotion score, although this was not statistically significant. The ratio between C2C and CPII also numerically decreased with the locomotion score (figure 5-2).

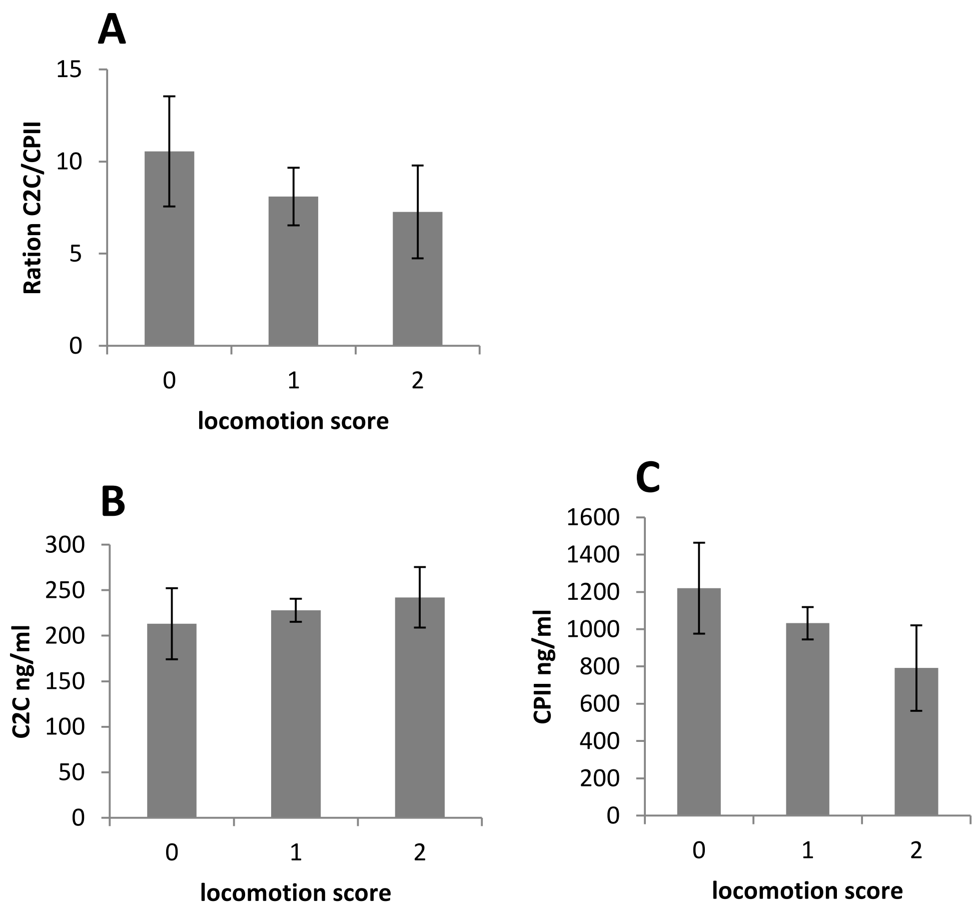

Figure 5-2 Levels of (A) C2C and (B) CPII and the ratio between the two (C) when compared against locomotion scores.

5.3.1.4 Effect of rearing treatment

There was no effect of group composition or mineral supplementation on serum CPII, C2C or the ratio between the two (Table 5-1). However, with regard to the ratio, there was a tendency (P <0.1) for an interaction between group composition and mineral supplementation (Figure 5-3).

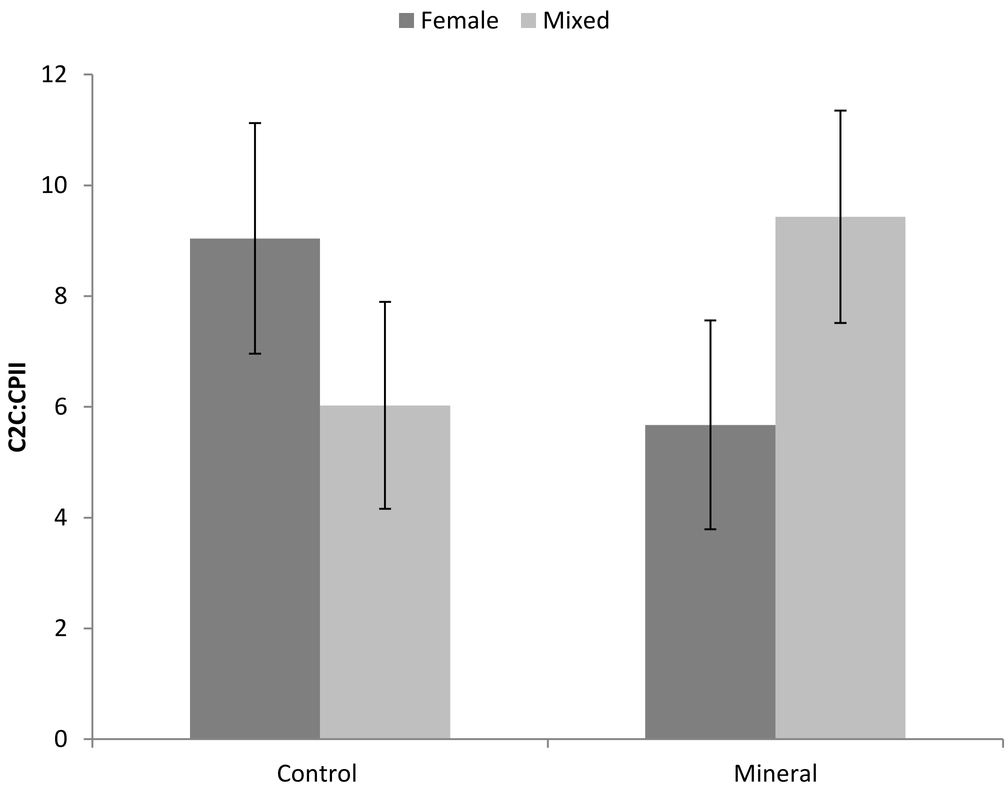

Figure 5-3 The ratio between the two analytes C2C and CPII in the serum of gilts at breeding age. Gilts were either reared in Female-only or Mixed-sex groups from weaning, and fed either a standard finisher control diet (Control) or the same diet supplemented with Cu, Mg and Zn (Mineral) From day 117 of life until breeding age.

Table 5-1.Serum levels of C2C, CPII and the ratio between the two, in gilts culled at 196 days of age. Gilts had been reared in either female-only (FEM) or mixed-sex (MIX) groups, and on either a standard finisher diet (CON), or the same diet supplemented with supplementary Zn, Cu and Mn (SUPP) from day 117 of life until slaughter.

	Diet			Group composition			
	CON	SUPP	P value	FEM	MIX	P value	Interaction
C2C ng/ml	219.44 ± 23.30	239.25 ± 22.12	0.42	234.77 ± 23.89	223.92 ± 21.76	0.67	NS
CPII ng/ml	582.41 ± 127.95	588.21 ± 129.73	0.97	589.04 ± 128.99	581.58 ± 127.12	0.96	NS
Ratio C2C:CPII	7.53 ± 1.53	7.55 ± 1.46	0.99	7.36 ± 1.53	7.73 ± 1.45	0.83	0.07

5.3.2 Experiment 2

5.3.2.1 Effect of rearing treatment

There was no effect of group composition or mineral supplementation on serum C2C, CPII or the ratio between the two overall, nor was there an interaction between either of the treatments and time. However, for both C2C and CPII there was an effect of sample time point, with both analytes decreasing with time. When considering only the first sampling time (breeding age), gilts which were supplemented with the minerals had higher C2C levels than those fed a standard finisher diet ($P < 0.05$; Tables 5-2 and 5-3).

Table 5-2 Levels of C2C, CPII and the ratio between the two analytes for gilts reared in mixed-sex groups (MIX) or female-only groups (FEM) at three sampling times

	Breeding age[1]	Mid first gestation[2]	2nd Farrowing[3]	P-value[4]
C2C ng/ml*				
MIX	195.4 ± 21.8	173.9 ± 24.2	146.1 ± 26.1	
FEM	198.4 ± 23.3	165.9 ± 24.8	134.4 ± 26.8	0.58
CPII ng/ml*				
MIX	2130.7 ± 148.1	1936.5 ± 152.7	1459.2 ± 184.1	
FEM	1893.0 ± 125.5	1935.4 ± 148.3	1174.6 ± 178.1	0.17
Ratio*				
MIX	8.9 ± 1.2	8.7 ± 1.2	8.7 ± 1.4	
FEM	7.8 ± 1.0	10.5 ± 1.1	6.8 ± 1.4	0.71

*Data presented as means and standard errors.

[1]Breeding age was at day 194 of age

[2]Approx day 71 of the first gestation

[3]Day 107 of the second gestation

[4]P-values represent the overall effect of group composition over all time points

[a, b] indicate a significant difference within sampling time

Table 5-3 Levels of C2C, CPII and the ratio between the two analytes for gilts fed either a standard finisher control diet (CON) or the same diet supplemented with Cu, Zn and Mn (SUPP) at three blood sampling times

	Breeding age[1]	Mid first gestation[2]	2nd Farrowing[3]	P-value[4]
C2C ng/ml*				
CON	182.7 ± 22.2[a]	172.2 ± 25.2	134.7 ± 27.5	
SUPP	211.1 ± 22.9[a]	167.5 ± 23.8	145.8 ± 25.4	0.25
CPII ng/ml*				
CON	2029.6 ± 139.4	1925.1 ± 156.4	1234.4 ± 194.1	
SUPP	1994.1 ± 133.7	1946.7 ± 143.8	1399.4 ± 169.1	0.69
Ratio*				
CON	8.9 ± 1.1	8.8 ± 1.2	6.6 ± 1.5	
SUPP	7.8 ± 1.1	10.5 ± 1.1	8.9 ± 1.3	0.35

*Data presented as means and standard errors.

[1]Breeding age day 194 of age

[2]Approx day 71 of the first gestation

[3]Day 107 of the second gestation

[4]P-values represent the overall effect of diet over all time points

[a, b] indicate a significant difference within sampling time

[4] P-values represent the overall difference between treatments over all time periods

5.4 Discussion

Overall, we found that there was very little effect of our rearing treatments on either C2C or CPII levels, or their ratio, in either of the experiments. Surprisingly, there were also limited relationships between analytes and both of the measures of cartilage condition we recorded. Although we expected a rise in both serum CPII and C2C levels in association with increases in cartilage damage, and as a consequence in gilts reared in either mixed-sex groups or fed the standard finisher diet, this was not observed.

Cartilage is composed of a major structural protein Type II collagen, which accounts for approximately half of the extracellular cartilage matrix (Vilar *et al.*, 2016). C2C is involved in collagen degradation, and thus has been investigated as potential biomarker for degenerative cartilage pathologies, such as OCD (Garnero and Delmas, 2003). Indeed C2C concentration is used as a diagnostic tool for osteoarthritis in dogs, where serum levels of C2C are significantly increased in cases of OCD (Matyas *et al.*, 2004; Goranov, 2007; Vilar *et al.*, 2016). CPII, on the other hand, is associated with cartilage synthesis, so lower levels could be associated with a reduced level of cartilage repair. Cahue *et al.* (2007) researched knee osteoarthritis in humans, and proposed that because the levels of C2C increased in association with osteoarthritis, and CPII decreased, the ratio of both analytes could be used as a marker for cartilage quality, as well as the concentrations of each analyte individually. Indeed they found a greater C2C: CPII ratio in association with an increase in osteoarthritis progression in humans (Cahue *et al.*, 2007). This is in line with our finding that as the ratio between C2C and CPII increased, so did the number of cartilage lesions observed.

Somewhat contrary to research in humans and dogs, Frantz *et al.* (2010) found that serum CPII levels were higher, and C2C levels lower, in pigs with OCD than in healthy pigs. With regard to CPII, they theorised that this was because in animals with OCD, higher levels of CPII represented the animal mounting a response to increased levels of cartilage wear. However, when considering only pigs which had OCD, and not healthy animals, they found a somewhat opposing relationship, which was similar to research in other species; in this sub cohort, there was a negative correlation between CPII levels and the severity of OCD. They hypothesised that for animals already experiencing OCD, the various stages of progression of the disease could be represented by varying levels of the analyte. Pigs with lower OCD severity scores could have had lesions which were in either the early or late stage of development, and consequently high CPII levels, as the animal was beginning to mount a response, or had successfully done so. Low serum CPII concentrations then represented animals with more extensive lesions which the animal was unable to repair. With regard to C2C, there was a contrary pattern to CPII; again, within the cohort of animals with OCD, the correlation between C2C level and OCD severity was positive, as per research in other species. The authors were unable to provide a hypothesis as to why this was the case, but it could likely be due to variation in C2C levels at various stages of progression of the OCD lesions, similar to CPII. Thus the relationship between CPII, C2C and OCD development and severity is complex, which can explain the lack of significant associations in our study. It is possible that more detailed histological examination of the cartilage could have yielded more significant results.

Frantz *et al.* (2010) carried out detailed histological analysis of the cartilage, whereas we simply examined the surface using visual inspection.

Markers of both cartilage and bone metabolism were successfully correlated with osteoarthritis (Chu *et al.*, 2002; Hegemann *et al.*, 2002) and OC (Frisbie *et al.*, 1999; Billinghurst *et al.*, 2004) and are used to determine osteoarthritis treatment effectiveness (Ofluoglu and Ofluoglu, 2005). There was a tendency for a negative, but weak correlation between serum levels of CPII and C2C, which could indicate de-regulation of cartilage turnover when the level of one analyte is relatively high or low. We also found a tendency for a negative correlation between C2C and the total count of lesions on the joint surface, as well as the number of thinnings, which is somewhat in line with the results of Frantz *et al.* (2010), who reported lower levels of C2C in animals with OCD than without. Due to the difficulties in identifying progression of the disease, described by (Frantz, 2006), and the different methodologies in scoring of the lesions, it is difficult to draw concrete conclusions. Nevertheless, animals which had low joint lesion counts in our study did not necessarily have OCD (complete separation of cartilage and bone), and as such, we can consider our results to be in line with this previous work.

There was no correlation between C2C and locomotion score, and only a tendency for a negative correlation between CPII and locomotion score, indicating that there were higher CPII levels in the pigs with lower (good) locomotion scores. This is in line with the results in Chapter 3, where we found no relationship between locomotion score, and the severity of cartilage lesions. One reason for this is that there were very few animals with scores of 0 or 2, and none higher than 2. It is possible that any high scoring sows in experiment 2 may have been removed from the breeding herd by involuntary culling as 6 sows were culled due to lameness. Nevertheless, although there was no significant difference in locomotion scores, there appeared to be a numerical pattern, with C2C levels increasing, and CPII levels decreasing, with severity of locomotory disorders. This result was the opposite of what one would expect from previous studies in pigs, whereby higher C2C levels and lower CPII levels were found in pigs with OCD relative to those without. Again, this could be due to the inaccuracies in the scoring system, and due to the fact that our scoring system where we counted the number of lesions did not necessarily reflect actual OCD, rather the amount of superficial as well as severe damage to the cartilage. It is possible that gilts in our study were mounting an adequate response to damage to the cartilage by increasing levels of C2C and decreasing levels of CPII when minor levels of damage occurred. Moreover, numerous studies report no association between OCD and lameness/leg weakness (Jørgensen, 1995; Jørgensen *et al.*, 1995; Arnbjerg, 2007), and even though we previously found rearing treatment effects on both bone mineral density and cartilage degradation scores, the findings regarding locomotory ability suggest that either the locomotion scoring system was not sensitive enough to detect any difference, or differences in aBMD and OCD are not reflected in locomotory ability (Hartnett *et al.*, 2019c).

The range of concentrations in our C2C and CPII experiment 1 (C2C 37.8 – 458 ng/ml; CPII 259.1 - 2497.4 ng/ml) results is larger than the range (C2C 60.1- 202.9 ng/ml; CPII 537.5 - 1723.1 ng/ml) detected by Frantz et al. (2010). However Frantz *et al.* (2010) studied 71 gilts whereas we studied 102 gilts, so it is perhaps not surprising that we detected a larger range.

Our C2C and CPII concentration values decreased as the sows aged, which indicates less cartilage turnover as the animals aged. This was unexpected, as according to the literature, OCD is progressive and not reversible. It is probable that there is more cartilage synthesis in younger animals due to the growth and development that occurs when animals are younger. Bell *et al.* (2001) stated that younger animals have higher levels of bone biomarkers due to their rapid growth rate. Another limitation could be due to the natural variation in bone metabolism within the gilt population (Shaw *et al.*, 2006).

Although in experiment 1 we found much lower levels of CPII compared to time point A of the sow study, this was an artefact of the statistical analysis due to using the plate as a random effect. Values on two of the plates which were used to analyse CPII from experiment 1 were numerically much lower than all other plates in analysis, and this artificially lowered the estimated means. Nevertheless, numerically, the direction of the results for both analytes was the same for both samples from Experiment 1, and the first sampling time from Experiment 2, which represented animals at the same age. Indeed in Experiment 2, at this sampling time, gilts which were supplemented with the minerals had higher C2C levels than those fed a standard finisher diet; in Experiment 1, we detected the greatest numerical difference between these treatments for C2C. This suggests that mineral supplemented gilts were experiencing more cartilage degradation than those fed the standard finisher diet. This is somewhat in contradiction to the results from Chapter 3, where we found that these animals had improved cartilage condition. Thus, as stated above, the higher levels may simply represent an improved level of cartilage turnover.

A limitation to our study is that gilts selected for Experiment 2 were the ones we chose to be suitable for breeding; animals deemed unsuitable due to being much larger or smaller than the rest of the group, or experiencing any health disorder, were culled at breeding age (they formed the cohort of animals in Experiment 1). As such, results from Experiment 1 and Experiment 2 are not directly comparable, and this could explain why C2C levels were significantly difference at breeding age in Experiment 2, but not Experiment 1.

5.5 Conclusion

Overall, gilt rearing strategy did not affect serum C2C or CPII concentrations either at breeding age, or subsequently. Nor did we find many relationships between either analyte (C2C and CPII), and our measures of cartilage condition. However, we found that using the ratio of these two analytes C2C and CPII has potential as an indicator of cartilage damage, although further validation is needed to establish whether it could be effective in estimating whether an animal is suitable to enter the breeding herd.

Chapter 6 - General Discussion and Conclusions

6 General Discussion and Conclusions

6.1 Overview

This thesis is comprised of a longitudinal gilt rearing study, where all aspects of the gilts' lives were controlled from the service of their mothers, until the end of the 5th parity. This is the first of its kind to the best of my knowledge, in this research area. Chapter 1 is a review of the literature which outlines the effects and risk factors of rearing strategies on gilt development. From the literature it is evident that there is potential for, and a need to develop, a rearing strategy that minimises lameness and promotes longevity. Chapter 2 (rearing period) outlines the rearing period when our treatments were applied; gilts were either reared in mixed-sex pens or female-only pens, and fed a diet with or without mineral supplementation with increased levels of copper (Cu), zinc (Zn) and manganese (Mn). Chapter 3 (bones analysis) investigated the condition of the gilts' limbs at breeding age which can be considered the end of the rearing period. For this, our analysis was carried out post-mortem on 102 gilts, as we wanted to see the internal effects on the limbs. Chapter 4 (reproductive period) investigates the carryover effects of our rearing strategies, and we selected 80 gilts to serve, and follow through their reproductive life. For this part of the thesis, the animals are no longer on any treatment, and all managed in the same way. Finally, Chapter 5 (bloods analysis) investigated the effect of the rearing strategies on hypothesised markers of bone cartilage turnover and OC status in both gilts at breeding age (n = 182 gilts) and at two further time points for the 80 breeding gilts included in Chapter 4 (reproductive period).

In this chapter (Chapter 6) I will outline the key findings of this thesis, and recommendations this thesis proposes. I will also discuss limitations of these findings along with the areas which require further research. The ultimate aim of this study was to determine rearing strategies for gilts to improve their welfare and to future proof the breeding herd, by implementing a female-only group composition and the addition of a mineral supplement to improve limb health and the overall development of the gilts, thus enhancing their welfare and longevity.

6.2 Introduction

Replacement gilts are often reared with finisher pigs destined for slaughter, including entire males. This exposes them to high levels of mounting and aggression, which is stressful and injurious and may be causing damaging effects to the replacement gilts. This could cause a knock-on effect of increased involuntary culling for lameness, which will consequently increase replacement rate. In relation to nutrition, previous studies have focused on reducing energy levels in gilts with the intention to reduce the effect of rapid weight gain such as their counterparts for the meat industry (Harper *et al.*, 2002; Quinn *et al.*, 2015). However, changing the composition of the diet may deter farmers from purchasing another specific gilt developer feed. Therefore this study wanted to research the potential benefits of just adding a trace mineral supplement to a standard finisher diet.

6.3 Discussion

For this thesis we employed a two by two factorial arrangement of treatments, which provided information on four rearing strategies; a rearing strategy was applied in mid-finisher stage (Chapter 2; rearing period) where we had 4 treatment combinations, mixed-sex groups with or without supplementation (with additional Cu, Zn and Mn) and female-only groups with or without mineral supplementation. We then investigated the effect of these strategies on welfare of the replacement gilts. Surprisingly, in general, few effects of mineral supplementation were found. However rearing gilts in female-only groups reduced their exposure to aggression and sexual behaviour with associated benefits to their health (reduced injuries to the body and hooves). As expected, the amount of aggression observed was greater in the mixed-sex groups than in the female-only group. Indeed we found that the mixed-sex groups were more active in general, as there was also more play behaviour in these pens. This was also reflected in body lesion scores; both female-only and mixed-sex groups had similar levels at the first inspection day, but as the animals aged, lesion scores in gilts reared in mixed-sex groups increased and remained higher than gilts reared in female-only groups for the rest of the experiment. This is likely due to on-going integument damage due not only to aggression, but mounting and play.

We hypothesised that gilts reared with males would have higher levels of cortisol either due to firstly the stress of being mounted and exposure to aggression, or secondly, due to underlying pain associated with hoof and leg injuries resulting from these behaviours. We also expected that these effects could be modulated by the dietary treatments, as any benefits to limb health through mineral supplementation could have reduced the impact of rearing with males. However in the rearing period, although the behaviour observations confirmed that gilts were exposed to higher levels of mounting and aggression when reared with male pigs than when in female-only groups, we did not observe any corresponding increase in cortisol. Indeed this was the case even though our hypothesis that these animals would have more internal damage to the bones was confirmed in Chapter 3. It could be the case that any damage to the cartilage or bones was not sufficient during rearing to result in an elevated level of salivary cortisol. Surprisingly, in the reproductive period we found that sows which had been reared in supplemented groups tended to have higher cortisol levels than those reared using a standard finisher diet. It is well understood that cortisol levels do not always represent a negative affective state, and could sometimes indicate a positive welfare state, rather than a negative one; thus cortisol levels should be interpreted in the context of other measures of welfare. Indeed, in recent work carried out in the same research centre, sows which were managed in farrowing crates which could be opened or closed during lactation had higher cortisol levels overall, and particularly on the day after the crate had been opened, than sows in conventional closed crates (Kinane, 2019). The hypothesis in that study was that the surge in cortisol could be due to feelings of excitement when the crate was opened, or due to increased activity (de Jong *et al.*, 1998; Hoyt *et al.*, 2016) as the sows utilised the extra space to turn around and change positions. It is possible that if gilts reared with the minerals had healthier limbs, they may have moved around more in the group pen, which could have contributed to this tendency. Nevertheless, in both the rearing and the reproductive phases, there was either no, or only a tendency for any, difference in cortisol level.

In the rearing period locomotory ability was generally very good across all treatments and no lameness was detected in any of the trial animals. Thus it is not surprising that locomotion score was not affected by treatment. In Chapter 3 where we examined the bones of the animals, we observed no beneficial effect of mineral supplementation on locomotion scores. Clearly, our findings indicate that supplementation of a finisher diet with minerals associated with bone/limb health alone is not enough to influence locomotory ability at this stage of development. Given that treatment effects were detected in bone mineral density and cartilage degradation scores, the findings regarding locomotory ability suggest that either the locomotion scoring system was not sensitive enough to detect any difference, or differences in aBMD and OCD are not reflected in locomotory ability, at least not until the damage is severe. Indeed the relationship between lameness and OCD is poorly understood (Dewey *et al.*, 1993; Heinonen *et al.*, 2006). In the reproductive period, having already demonstrated that these strategies had welfare benefits for gilts during the rearing period when it came to their bone condition, there were yet few effects on locomotory ability of either rearing strategy. However, the median score did increase from 1 in the rearing period, to 2 in the reproductive period which demonstrates that there was a slight impairment to locomotion overall, relative to the rearing period. This may be due to added weight and growth during gestation, as well as potentially to the accumulation of injuries.

During rearing, hoof scores were higher in mixed-sex groups, which are likely due to the higher levels of activity (mounting, aggression, and play) in pigs in these groups on the concrete slatted flooring. In line with this total hoof scores were higher in the sows reared in mixed-sex groups compared with sows reared in female-only groups during reproductive period. Thus the damage that occurred as a result of higher levels of exposure to activity (mounting, aggression, and play) during the rearing period did not resolve once the gilts were removed from the males in the reproductive period. Both during rearing and during the subsequent examinations hoof scores increased over time; hoof scores were increased by the inspection day in mid gestation, compared to during rearing, and continued to increase until the last sampling point when sows farrowed for the second time. This is likely due to general wear and tear with age, as the hooves must support more weight as pigs increase in size. It also demonstrates the necessity for excellent hoof condition early in life, as it is likely to dis-improve over time, regardless of the rearing conditions.

In the reproductive period we found that when considering only sows that were reared on the control diet, animals reared the mixed-sex groups had higher total hoof scores than those in the female-only. However, this was not the case for sows reared on the mineral supplemented diet. This suggests that the mineral supplemented diet had a long term protective effect on hoof condition, when the rearing conditions exposed sows to more physical activity. Thus if space or other conditions do not permit separate sex rearing, supplementation with the minerals used in the current study could have long term benefits for hoof health. Sows reared on the control diet tended to have higher horizontal crack scores than mineral supplemented sows, and sows reared in female-only groups had lower heel erosion scores compared to sows reared in mixed-sex groups. In both the rearing and the reproductive periods we found that there was a low prevalence of severe lesions and a high prevalence of mild lesions. Mild lesions do not appear to influence locomotary ability, and as during both life stages (rearing and reproductive period) none of the hoof disorders we observed were severe; this could be why we did not observe differences in locomotion score.

In Chapter 3 where we analysed the bones, we found that when we implemented the strategy of supplementing the gilts' diet during rearing with the trace minerals Cu, Zn and Mn, gilts had increased bone mineral density. Moreover, female-only rearing and mineral supplementation combined resulted in a beneficial effect on bone mineral density of the humerus. Rearing gilts in female-only pens reduced the number of joint lesions associated with cartilage degradation, with numerically fewer incidences of fractures in the humeral condyle (HC) joint. Thus both strategies employed appeared to improve limb health,

As we had over 100 bones to examine which were taken from gilts at breeding age, we decided to aim to develop a new, improved method of scoring joint lesions (counting all lesions), and to compare this system to the one used in previous studies (joint lesion scores, (JLS); Quinn *et al.* (2015)). We found that both systems of scoring joint lesions produced results which were comparable to those from the study by Quinn *et al.* (2015), with the same direction and magnitude of differences between the scoring systems. The newer scoring system was developed in an attempt to improve the objectivity of the scoring system, and also so that we could determine whether any differences between treatments were due to particular pathologies; upon initially examining the bones with the previously published version, we found that the definitions for each level of the scoring system were quite vague. Data from both systems corresponded with one another, in that as the JLS score increased, so did the number of lesions on the cartilage. However, counting the lesions enabled us to discern differences between treatment groups in several measures, whereas the JLS system did not. Thus overall, we recommend use of this newer method of scoring cartilage in studies such as the one described in this thesis, as it appears it may be more sensitive to detecting treatment differences than the previously published version.

We found that female-only rearing had a beneficial effect on the number of joint lesions observed. Indeed four of the five gilts with fractures in the humeral condyle were reared with entire males in mixed-sex groups. This equates to 5% of all the gilts on trial which raises concern for gilt welfare.

In the reproductive period, the aim was to investigate the rearing strategies we employed on aspects of sow welfare in their early parities, and on their lifetime performance. We hypothesised that rearing gilts in female-only groups and on a mineral supplemented diet with supplementary levels of Cu, Zn and Mn would improve their welfare during the first pregnancy and would have long term benefits for longevity and reproductive performance. A secondary hypothesis was that their offspring would have higher birth weights. Although we could not statistically analyse the data pertaining to sow longevity and total piglet output at each farrowing, we can see from the raw data that sows reared with the optimal diet (i.e. the mineral supplement) numerically, seemed to stay in the herd longer than those on the control. The opposite was the case for the grouping strategies; sows which had been reared with males seemed to remain longer, although the pattern was not as clear as that of mineral supplementation. Thus overall, the 'herd' of gilts reared with the minerals had a greater number of piglets born alive over the five parities included in the study than those on the control, whereas the gilts reared in the mixed groups had a greater number than those in the female-only groups. What contributed to the greater number for gilts reared with the minerals was that on average, numerically they had more piglets born alive in the 2nd, 3rd and 4th parities than those on the control diet, although this was not statistically significant. The pattern was not so clear for the grouping strategies; gilts which had been reared with males alternated between having more or fewer born alive piglets than those with females for each parity, and thus the numerical total number born was not as great for these animals. Moreover, gilts reared with the males, had significantly more piglets born dead over the five parities. This could help explain why even though numerically they had a greater number of total piglets born, at the same time numerically they had fewer born alive. Although both of the rearing strategies investigated showed benefits for sow welfare, they did not affect the weights, ADG and ADFI of offspring from birth until slaughter. Previous studies have found an effect of pre-natal stress on offspring performance, both when it is artificially applied (e.g. by application of Adrenocorticotropic hormone (ACTH)) (Otten *et al.*, 2007; Otten *et al.*, 2015), or when animals are submitted to social stress (Rutherford *et al.*, 2012), or affected by an illness (e.g. lameness (Parada *et al.*, 2017)). As there is a response across a range of stressor types, it is possible that any stress caused by our rearing treatments was not enough to induce a response in the offspring of the sows when it comes to physical growth. However, in work that was carried out outside of this thesis, we found differences in the pre-weaning behavioural response to open field and human approach tests, in piglets born from sows reared using our strategies. Thus we cannot conclusively state that there were no pre-natal effects, as it is possible that the stress response axis of the offspring was impacted.

In Chapter 5 where we analysed the bloods, we aimed to validate the analytes C2C and CPII as biomarkers of OC. We initially compared serum C2C and CPII concentrations with joint lesions and OCD scores on 102 gilts which were culled at breeding age from Chapter 3 (where we analysed the bones), before moving on to investigate levels of C2C and CPII concentrations on the 80 gilts selected for breeding from Chapter 4 (reproductive period), but at three time points from breeding until second farrowing. The reason for these two investigations was so we could understand the relationship between these biomarkers and the amount of internal damage of gilts at breeding age, to see if the levels of these analytes taken later in life could provide an insight into the level of damage present in breeding sows. The gilts which entered the breeding herd were genetically similar and reared in the same way as those that were slaughtered. The ultimate aim of this study was to investigate a method of identifying animals with varying levels of severity of cartilage degradation by means of blood sampling live animals, and to determine whether rearing conditions affected the indicators of cartilage turnover.

C2C is involved in collagen degradation, and thus has been investigated as potential biomarker for degenerative cartilage pathologies, such as OCD (Garnero and Delmas, 2003). Indeed C2C concentration has been used as a diagnostic tool for osteoarthritis in dogs, where serum levels of C2C are significantly increased in cases of OCD (Matyas *et al.*, 2004; Goranov, 2007; Vilar *et al.*, 2016). CPII, on the other hand, is associated with cartilage synthesis, so lower levels could be associated with a reduced level of cartilage repair. Cahue *et al.* (2007) researched knee osteoarthritis in humans, and proposed that because the levels of C2C increased in association with osteoarthritis, and CPII decreased, the ratio of both analytes could be used as a marker for cartilage quality, as well as the concentrations of each analyte individually. Indeed they found a greater C2C: CPII ratio in association with an increase in osteoarthritis progression in humans (Cahue *et al.*, 2007). This is in line with our finding that as the ratio between C2C and CPII increased, so did the number of cartilage lesions observed.

Our C2C and CPII concentration values decreased as the sows aged, which indicates less cartilage turnover as the animals aged. This was unexpected, as according to the literature, OCD is progressive and not reversible. It is probable that there is more cartilage synthesis in younger animals due to the growth and development that occurs when animals are younger. Bell *et al.* (2001) stated that younger animals have higher levels of bone biomarkers due to their rapid growth rate. There was no correlation between C2C and locomotion score, and only a tendency for a negative correlation between CPII and locomotion score, indicating that there were higher CPII levels in the pigs with lower (good) locomotion scores. This is somewhat in line with the results in the rearing period and bone analysis study, where we found no relationship between locomotion score, and the severity of cartilage lesions. One reason for this is that there were very few animals with scores of 0 or 2, and none higher than 2. It is possible that any high scoring sows in experiment 2 may have been removed from the breeding herd by involuntary culling, as 6 sows were culled due to lameness. Nevertheless, although there was no significant difference with locomotion scores there appeared to be a numerical pattern, with C2C levels increasing, and CPII levels decreasing, with severity of locomotory disorders. This result was the opposite of what one would expect from previous studies in pigs, whereby higher C2C levels and lower CPII levels were found in pigs with OCD relative to those without.

Due to the difficulties in identifying progression of the disease, described by (Frantz, 2006), and the different methodologies in scoring of the lesions, it is difficult to draw concrete conclusions. Nevertheless, animals which had low joint lesion counts in our study did not necessarily have OCD (complete separation of cartilage and bone), and as such, we can consider our results to be in line with this previous work. Overall, we found that there was very little effect of our rearing treatments on either C2C or CPII levels, or their ratio, in either of the experiments. Surprisingly, there were also limited relationships between analytes and both the measures of cartilage condition we recorded. Although we expected a rise in both serum CPII and C2C levels in association with increases in cartilage damage, and as a consequence in gilts reared in either mixed-sex groups or fed the standard finisher diet, this was not observed.

6.4 Conclusions

Rearing gilts in female-only groups resulted in;

- Reduced exposure to aggression and sexual mounting and as a consequence they had fewer, body lesion scores and less hoof damage during rearing.
- Reduced incidence and severity of joint lesions and OCD.
- Overall, a positive effect on gilt welfare during rearing and gestation.
- Although our study was not large enough to analyse the data statistically, it can be hypothesised that ultimately rearing gilts in female-only groups could decrease the likelihood of culling for lameness, thus increasing sow welfare and longevity.

Supplementation with trace minerals Cu, Zn and Mn resulted in;

- A reduction in heel erosion during rearing stage.
- Positive effects on the severity of hoof cracks during pregnancy.
- Increased areal bone mineral density.

Overall, the benefits of supplementing these minerals and rearing gilts separate to males could lead to potential improvements in the lifetime performance of replacement gilts and the longevity of sows. However, it is important to note that locomotory ability, live-weight, bursitis and salivary cortisol levels were not affected by rearing in female only groups or supplementing gilt diets with Cu, Zn and Mn.

Additionally, we found that using the ratio of the two analytes C2C and CPII has potential as an indicator of cartilage damage, however further validation is needed to establish whether it could be effective in estimating whether an animal is suitable to enter the breeding herd.

6.5 Recommendations

The rearing strategies applied in the thesis are highly applicable to the pig industry. A pig producer can optimally rear their own replacement gilts, so as to maintain a closed herd and minimise biosecurity risk. However, if a pig producer desires to reduce lameness and to improve sow longevity, from the findings of this thesis it is advisable to invest in a mineral supplement for intended breeding gilts during the rearing stage. Currently finisher and sow gestating diet recommendations by the NRC do not meet developing gilts nutritional requirements. A pig producer wanting to increase these minerals levels can do so without changing the feed completely, as in this study the minerals were added to a standard finisher diet. Trace mineral investment can improve the structural quality of the breeding herd as this study highlights significant findings of greater bone mineral density which is related to bone strength and soundness. Many producers may lack space and may not have a unit designated for rearing gilts, however, by keeping gilts separate from male pigs internal joint damage and hoof injuries can be reduced, both of which are associated with lameness. Furthermore, if space or other conditions do not permit separate sex rearing, supplementation with the minerals used in the current study could have long term benefits for hoof health. These strategies can help a pig producer by increasing welfare status of his breeding herd, thus overall herd health. The ultimate aim of applying these strategies is to reduce lameness prevalence on farms, thus reducing the sow replacement rate and improving sows welfare and longevity.

6.6 Implications for future research

It is possible that the development of a more sensitive lameness/locomotory scoring system could help identify gilts suffering from OCD. Methods of locomotion scoring which currently being used are of limited reliability (Main *et al.*, 2000; Channon *et al.*, 2009; D'Eath, 2012; Nalon *et al.*, 2013), and thus if it was possible to identify features of locomotion which portray a long-term locomotory ability and assess different locomotory patterns that are associated with lameness (Stavrakakis, 2014), future researchers may be more capable of detecting treatment differences in studies such as ours. The development of a new locomotion scoring protocol for different age groups would also be very useful. A more sensitive system developed for young animals could lead to a more accurate assessment which could consider subtle changes in gait that may indicate any underlying joint conditions.

Bone strength and likeliness of fracture is related to aBMD (Vahle *et al.*, 2015). Surprisingly, in this study the average aBMD in fracture pigs was higher than in non-fracture pigs. As a matter for future research it would be interesting to investigate the strength of bones by carrying out a 3-point bending test (Nielsen *et al.*, 2007) or breaking test (Griggio, 2010), to determine bone strength and fracture likelihood of each animal.

We carried out a study that coincides with this thesis for future analysis. The work focused on evaluating the effects of the gilt rearing strategies on the welfare and behaviour of the offspring born to the gilts in this study. Maternal stress and health status during pregnancy can have life lasting effects on the resulting progeny's growth, efficiency, metabolic and reproductive performance (Rutherford *et al.*, 2012). Physiological effects of prenatal stress include reduced body weight, reduced ACTH response, and increased heart-rate and alterations to immune function. Ultimately, prenatal stress results in a phenotype with impaired coping ability (Welberg and Seckl, 2001). Many of the traits that can be affected by early life experiences, such as stress reactivity and immune function, are highly relevant to the ability of animals to exist under human management conditions (Rutherford *et al.*, 2012). This is particularly the case for pigs, due to the vulnerability of the newly weaned piglet with regard to immune status, and digestive capability. Thus is important commercially to reduce negative prenatal effects on production parameters. Recent work in Teagasc showed that feed-restricted gilts had higher salivary cortisol levels during gestation, indicating greater levels of physiological stress, and subsequently these gilts gave birth to lighter piglets (Amdi *et al.*, 2013). Investigation of how typically occurring circumstances in agricultural systems affect not only gestating females, but also their offspring, is warranted. Post-natal offspring measurements carried out included viability scoring at birth to assess vitality (Schmitt *et al.*, 2019), response to tail docking to assess response to an acute injury, as per Sutherland *et al.* (2008) and glucose levels to validate a stress response to an acute stressor (Hicks *et al.*, 1998) and thus we intend to use this as an estimate of the physiological stress response caused by tail docking. We also carried out pre-weaning measurements such as a back test to assess a piglet's coping style, tympanic ear temperatures to assess stress levels, punch-hole biopsy to assess immune response and open arena testing to assess behavioural response. We found that there were some indications from the piglet behaviour that there may have been a pre-natal effect.

Chronic stress may be assessed in a number of ways which is necessary to validate cortisol results. Hair cortisol and stress tests could be applied to further research to get a better indication of the stress levels in the animals and also to be able to distinguish between acute stress and chronic stress. As a separate study, from an on-farm observation, I recorded gilt behaviour to establish a link to the piglet and to determine if there is an association between the mothers coping style compared to her offspring's coping style. Behavioural measurements recorded in gilts were; behaviour when entering and occupying the hydraulic-chute for hoof scoring inspection where I recorded ease of movement into crate, and vocalisations and kicking during inspection. I also recorded gilt behaviour entering the farrowing crates, behavioural response to a forced human approach (FHA) and a forced human touch (FHT) test while wearing routine navy overalls and repeated wearing obscure white overalls. The association of gilt and piglet coping/behavioural responses requires further analysis. There is a vast amount of data which requires further analysis, however there is scope to use the findings of this extra study in future publications.

The diets in the rearing stage could be applied at an earlier age. The age we applied the diets at was arbitrary. For this study we had intended to apply diets at the start of the finisher stage. However, due to human error these diets were applied five weeks into the finisher stage. A similar study carried out by Quinn *et al.* (2015) applied diets at this age, thus our study was comparable.

A future implication could involve retrieving the right forelimb from all animals culled from the herd, whether voluntary or involuntary, to investigate the long term effect on bone cartilage. A much larger study is needed, at epidemiological scale, to confirm whether the longevity of gilts is actually affected by the rearing strategies. To gain a deeper understanding of the effect rearing strategies on the lifetime performance of a sow, it would be desirable to get more detailed measurements of both the sows and offspring beyond first parity.

Validation of OC in animals can be assisted by the analysis of inflammation markers (cytokines) present in the plasma. Cytokines mediate cellular interactions and regulate differentiation, proliferation and survival of immune cells by triggering the production of other cytokines, which can increase (proinflammatory) or reduce (anti-inflammatory) immune response. Therefore, cytokines are biomarkers for inflammatory conditions and potential diagnostic references for diseases. The benefit of analysing both OC biomarkers and inflammation markers (cytokines) is that the biomarkers and cytokines have the potential to correspond with one another, thus establishing a better perception on the internal condition of the animal. We analysed plasma samples for cytokines IL-1B and CRP which coincide with the serum samples we analysed for C2C and CPII. However, due to the complexity of the analysis and the new development of the method our data could not be extrapolated and used in this thesis. However, with the help of a statistician these results may be recovered and potentially published in the future.

6.7 Research limitations

- The relationship between CPII, C2C and OCD development and severity is complex, which can explain the lack of significant associations in our study. It is possible that more detailed histological examination of the cartilage could have yielded more significant results. (Frantz *et al.*, 2010) carried out detailed histological analysis of the cartilage, whereas we simply examined the surface using visual inspection. It would have been preferable to do a histological examination on the bones and joint for a more in depth analysis.
- In relation to lameness it is possible that any potentially high scoring animals may have been removed from the breeding herd by involuntary culling, as there were 6 sows culled due to lameness. Furthermore, these animals would not have been scored at any time points post removal from the herd and leading to good results from the remaining pigs. This could have artificially 'raised' the overall level of the scores, and not been representative of all animals.
- A limitation to our study is that gilts selected for Experiment 2 were the ones we chose to be suitable for breeding; animals deemed unsuitable due to being much larger or smaller than the rest of the group, or experiencing any health disorder, were culled at breeding age (they formed the cohort of animals in Experiment

1). As such, results from Experiment 1 and Experiment 2 are not directly comparable, and this could explain why C2C levels were significantly different at breeding age in Experiment 2, but not Experiment 1.

- It would have been preferable to analyse more blood samples which I had collected to get a more in depth analysis of the pigs over a longer period of time. However, the short period of time that I was appointed to do this analysis in SRUC, Edinburgh prohibited this. The blood samples not used have been archived and may be used in a future study.
- Another limitation could be due to the natural variation in bone metabolism within the gilt population (Shaw *et al.*, 2006). If variation was greater than we anticipated, a greater sample size may have been needed to detect significant differences. Nevertheless, our ethical approval and experimental licence were based upon sample size calculations which we carried out using data in the existing literature. Our data could contribute to the body of data which can be used to estimate future animal and group numbers.
- There are a few limitations when collecting salivary cortisol. Salivary cortisol has lower cortisol levels than blood, so may not be sensitive for accurate analysis. Also it is known that chronically stressed animals don't necessarily have elevated cortisol, they have a suppressed circadian pattern. Cortisol levels are affected by feeding patterns due to sows being housed in a dynamic group

housing system with an electronic sow feeder (ESF) thus; there is no control over when the sows last ate. Furthermore, due to a time restriction on collection for consistency saliva samples were taken between 0930 and 1000 hours on collection days, therefore we also have no control of their activities during this time and cortisol fluctuate with activities such as sleeping, exploring or interacting with other animals.

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