Linking early life conditions to osteochondrosis prevalence in gilts

Osteochondrosis (OC) involves the development of defects in growth of bones. Growth of bones in young pigs is accomplished by growth cartilage that is situated at the ends of long bones, termed the epiphysis, that is involved in joint movement. More specifically, growth cartilage resides, amongst others, just below the surface of an epiphysis between the articular cartilage (overlying cartilage) and the subchondral bone (underlying bone). Chondrocytes (cartilage cells) proliferate (divide) and undergo hypertrophy (includes enlargement of the cell) while producing an extracellular matrix that provides strength to the entire structure. The extracellular matrix is eventually converted into bone tissue (ossification) resulting in growth of bone. Osteochondrosis involves the formation of necrotic growth cartilage due to vascular disruption that can take place due to loading of joints. Necrotic growth cartilage is not converted into bone tissue (ossification) and remains as a weak site in the underlying bone. This necrotic cartilage may fracture due to loading of joints, causing irregularities at the surface of a joint. Such joint surface irregularities may impair normal joint movement, causing lameness in the animals. Considering that breeding gilts will have to last several parities to produce fattening piglets, these animals may be at risk to develop OC induced lameness, decreasing animal welfare and increasing the risk for premature culling. Important to note is that OC develops at a very young age in gilts at around 10 weeks of age. Thus, if one wants to take measures to reduce OC prevalence in gilts, one needs to start early. Additionally, the early time frame of OC development might indicate that measures undertaken have time dependent effects on OC prevalence. The aim of this study was 1) to assess possible time dependent effects on OC prevalence of early life environmental conditions consisting of either dietary restriction (Chapter 2) or floor type (Chapter 3); 2) to assess whether early in vivo characteristics are associated with OC prevalence at a later age and consisted of either conformation and locomotive characteristics (Chapter 4) or natural (auto-) antibodies (Chapter 5); and 3) to assess whether OC prevalence is affected by dietary composition consisting of a difference in dietary carbohydrate and arginine level (Chapter 6). In Chapter 2 we assessed time dependent effects of dietary restriction on OC prevalence in 211 Topigs 20 (Dutch Large White x Dutch Landrace) breeding gilts acquired after weaning (4 weeks of age). The underlying mechanism behind dietary restriction affecting OC prevalence was that dietary restriction affects body weight development and, in turn, affects loading on the joints. Gilts received 1 of 4 of the following treatments: ad libitum feeding continuously from weaning until slaughter (AA); restricted feeding continuously from weaning until slaughter (RR); ad libitum feeding from weaning until 10 weeks of age, after which gilts were switched to restricted feeding (AR); or restricted feeding from weaning until 10 weeks of age, after which gilts were switched to ad libitum feeding (RA). Restricted feeding was calculated as 80% of the ad libitum uptake of the preceding week. At 26 weeks of age, gilts were slaughtered and the elbow, hock, and knee joint were dissected and macroscopically assessed for irregularities of the joint surface indicative of OC. Results indicated that the gilts responded as expected in body weight gain to the treatments. Gilts in the AA treatment were overall heavier than the other treatments, gilts in the RR treatment were overall less heavier than the other treatments, and gilts in the RA and AR treatments showed a higher and lower increase in body weight, respectively, after 10 weeks of age compared to before 10 weeks of age. Differences in OC prevalence were found between treatments at the animal level (all joints combined) and indicated that gilts in the RA treatment had significantly ($P < 0.05$) higher odds of being affected by OC than gilts in the RR and AR treatments (OR = 2.5 and OR = 1.9, respectively). We speculated that these results are due to a rapid increase in body weight after 10 weeks of age when gilts are switched from restricted to ad libitum feeding and thereby increases loading experienced on the joint. Possibly, the growth cartilage within the joint is sensitive for this rapid increase in body weight and might be damaged, leading to an increased risk for OC development. In Chapter 3 we assessed time dependent effects of floor type on OC prevalence in 212 Topigs 20 (Dutch Large White x Dutch Landrace) breeding gilts acquired after weaning (4 weeks of age). The underlying mechanism behind floor type affecting OC prevalence was that the type of floor may affect impact loading or slipping of the gilts that could result in higher impact loading or traumatic events experienced on the joints. Gilts received 1 of 4 of the following treatments: a conventional floor from weaning until slaughter (CC); wood shavings as bedding from weaning until slaughter (WW); a conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding (CW); or wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a conventional floor (WC). Indications of behavioral activity of the gilts were assessed at 8, 10 (2 days after the treatment switch) and 17 weeks of age. At 24 weeks of age, gilts were slaughtered and the elbow, hock, and knee joint were dissected and macroscopically assessed for irregularities of the joint surface indicative of OC. Results indicated that gilts kept on wood shavings after 10 weeks of age showed a higher degree of play behaviors as compared to gilts kept on a conventional floor. No effect of treatments on overall prevalence of OC was found. At the animal level, however, gilts had greater odds to have severe OC lesions ($P \leq 0.05$) in the CW treatment (OR = 2.3), WC treatment (OR = 2.6), and WW treatment (OR = 3.7) compared with gilts in the CC treatment. We speculated that the negative effect of a deep litter type system using wood shavings may be mediated through differences in play behaviors recorded between treatments that place more stress on the joints to cope with the irregular loading patterns likely associated with play behaviors. A higher degree of play behavior could then increase the risk to aggravate the existing minor lesions to severe lesions of OC. Considering that OC is suggested to affect, amongst others, lameness of the animals, we assessed whether conformation and locomotive characteristics (CLC) throughout the rearing period of a gilt have associations with OC at slaughter in Chapter 4. If strong and persistent associations exist, then CLC may be viable indicators of OC in vivo. The CLC were measured in the gilts used in the experiments described in Chapters 2 and 3. Gilts were subjectively assessed for CLC at, on average, 4, 9, 11, 16, and 22 (Chapter 3) or 24 (Chapter 2) weeks of age. Assessment of CLC included 10 conformation and 2 locomotive characteristics using a 9-point grading scale by 2 observers. The CLC included stiff or slow gait, swaying hindquarters, X- or O-shaped front and hind legs, size of the inner and outer claw of the front and hind legs, steep or weak pasterns of the front and hind legs, sickled or buckled front legs, straight or sickled hock, and straight or bowed hind legs. Several CLC were associated with OC at several ages. The CLC most frequently associated with OC ($P < 0.05$) were O shape or X shape of the hind legs, straight or bowed hind legs, and straight or sickled hock. However, associations were not consistently present throughout every assessment within an entire experiment or were not consistently present between experiments. For a large part, these inconsistencies are likely due to the fact that a gilt was not consistently affected by the same CLC at each assessment. Considering that both OC and CLC are dynamic over time, where a condition can appear but also disappear, the viability of using CLC to predict risk for OC is, at this point, limited. In another attempt to associate an in vivo measured characteristic in the rearing period of gilts with OC at slaughter, we focused on natural (auto-) antibodies (N[A]Ab) in Chapter 5. The function of N(A)Ab revolves around the maintenance of homeostasis and prevention of disease by clearance of damaged cells, intracellular components, and cell waste products. Considering that OC involves the formation of necrotic cartilage, one could envision that N(A)Ab would be able to recognize waste products resulting from this necrotic cartilage formation. To assess if such an association exists, blood samples were collected from the gilts used in the experiment described in Chapter 3 at 6, 10, and 24 weeks of age and were analyzed for N(A)Ab titers against 11 (auto-) antigens using ELISA. Results indicated that several N(A)Ab at 6 weeks of age were associated with OC at 24 weeks of age. In general, a higher odds ($P < 0.05$) to have OC at the animal level was associated with higher levels of IgM N(A)Ab measured at 6 weeks of age against chondroitin sulfate A (OR=1.5) and actin (OR=1.3), at 24 weeks of age with thyroglobulin (OR=1.3) and actin (OR=1.3), and of IgG N(A)Ab measured at 6 weeks of age against insulin (OR=1.4). Differences in OC affected animals and OC unaffected gilts were in general less than half a titer point and indicate that associations may not be very strong nor suitable for reliable prediction of risk to have OC. Further research will require the expansion of the antigen repertoire recognized by N(A)Ab that are possibly associated with OC to a higher degree. In Chapter 6 we assessed whether dietary composition has an effect on OC prevalence in 212 Topigs 20 (Dutch Large White x Dutch Landrace) breeding gilts acquired after weaning (4 weeks of age). Necrotic growth cartilage has been suggested to be associated with chondrocytes and vasculature proliferating towards the necrotic growth cartilage in reparative attempts. In an attempt to affect these processes, we fed gilts diets differing in carbohydrate and arginine content. Carbohydrates affect metabolic parameters such as glucose, insulin, and insulin-like growth factor-1 (IGF-1) which all can have an effect on chondrocyte functioning. Arginine can have an effect on vascularization under hypoxic conditions as can be expected during OC development. Considering that OC is associated with reparative attempts undertaken by blood vessels and chondrocytes, we hypothesized that carbohydrates and arginine may affect these processes. Gilts were subjected to a 2x2 factorial treatment design of dietary carbohydrate and arginine level. Carbohydrate level consisted of 12.5% cornstarch and 12.5% dextrose added to a basal diet (C+ treatment) versus an isocaloric diet in which cornstarch and dextrose were replaced with 8.9% soya bean oil (C- treatment). Arginine supplementation consisted of 0.8% arginine supplemented to a basal diet (A+ treatment) versus 1.64% alanine as the isonitrogenous control (A- treatment). At 24 weeks of age, blood samples of 34 randomly selected gilts around feeding were taken and assessed for insulin, glucose, IGF-1, and NO levels. After slaughter at 25 weeks of age, the elbow, hock, and knee joint were dissected and macroscopically assessed for irregularities of the joint surface indicative of OC. Arginine supplementation tended to decrease OC prevalence ($P = 0.07$) at the animal level (all joints combined) and the knee joint. Carbohydrate treatment affected prevalence of OC only in the knee joint ($P < 0.05$) in which gilts in the C- treatment had higher odds to have OC (OR = 2.05) than gilts in the C+ treatment. These effects of dietary treatment were likely mediated through the diets affecting body weight development. Gilts in the C+A+ treatment had a lower body weight than gilts in the other treatments. Possibly, the treatment effects on OC prevalence were influenced by this differences in body weight attained. Indeed, when assessing body weight at slaughter in statistical models, body weight was significant ($P < 0.05$) while treatment effects became non-significant. Through this experiment, we again have indications that loading of joints, mediated through body weight changes, can affect OC prevalence. Chapter 7 discussed, amongst others, the relationship between OC and growth rates. Additionally, new data on OC prevalence in the Dutch commercial husbandry was presented. The relationship between growth rates and OC in the gilts used in the experiments described in this thesis (Chapter 2, 3, and 6) was assessed to study whether growth rates have a strong association with OC. These associations were assessed within treatments for gilts that did not receive a switch in treatments. Results indicated limited associations with OC, which might be caused by insufficient contrasts of growth rates within a treatment. Considering that there were clear effects of dietary restriction on body weight (and therefore also growth rates) and OC prevalence (Chapter 2) between treatment groups, effects of growth rates on OC may only be apparent with large contrasts in growth rates. New data on OC prevalence from left hind legs in Dutch commercial husbandry sows send for premature culling indicated a 90% prevalence of OC. However, the majority of OC scores were minor lesions (OC score 1 and 2). Depending on the number of legs assessed, the prevalence of OC in the commercial sows compared to our experimental gilts was lower, similar, or higher. An increase in parity was not associated with a significant increase in OC and lameness as a culling reason was not the main factor associated with OC. This data was not able to clearly indicate welfare implications of OC. Considering that in our experiments no gilts were removed for severe lameness related to OC, one may wonder what the exact welfare implications of OC for sows are. A long term study in sows from weaning to culling will be necessary, so that individual sows can be monitored throughout life for OC and lameness incidence. Such a study will provide a better understanding of the welfare implications of OC. In conclusion, we found that dietary restriction and floor type during young age in the rearing period affect OC prevalence in Topigs 20 breeding gilts. Conformation and locomotive characteristics were associated with OC at several ages during the rearing period in the gilts, but were not consistently associated with OC at every age or between ages. Natural (auto-) antibodies measured as early as 6 weeks of age were associated with OC, but results were, at this point, not strong enough to be viably used in practice. Finally, a high dietary carbohydrate level decreased the prevalence of OC, but this effect was likely mediated through body weight changes resulting from the dietary treatments. We speculated that loading of joints through behavioral activity, such as play behavior, and body weight changes affect OC prevalence and it is therefore important not to stimulate a rapid high body weight gain during the rearing period. When studying OC, one must realize that OC development is time dependent and, therefore, (environmental) factors may affect OC prevalence differently at different ages during the rearing period of breeding gilts.

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Linking early life conditions to osteochondrosis prevalence in gilts

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Linking early life conditions to osteochondrosis prevalence in gilts

D.B. de Koning

Thesis

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ABSTRACT

Osteochondrosis (OC) involves the development of necrotic growth cartilage near the surface of a joint and is suggested to be associated with lameness in sows. Development of OC in pigs occurs at young age in a relatively short time frame of several weeks around 10 weeks of age. Due to this time dependency, one may wonder whether there are time dependent effects of factors or conditions associated with OC. The aim of this thesis was to assess whether OC prevalence is associated with conditions encountered in early life such as dietary restriction, floor type, conformation and locomotive characteristics (CLC), natural (auto-) antibodies (N[A]Ab), and carbohydrate levels. Indications for time dependent effects were found for dietary restriction on OC prevalence. This indicated that gilts receiving restricted feeding from 4 to 10 weeks of age and switched to ad libitum feeding until 26 weeks of age had a significantly higher prevalence of OC when compared to gilts receiving restricted feeding after 10 weeks of age. Time dependent effects of floor type were not clearly present, but gilts housed on a deep litter type system using wood shavings after weaning had a higher prevalence of severe OC when compared to gilts kept on a concrete partially slatted floor. Feed with a lower carbohydrate level increased OC prevalence compared to feed with a higher carbohydrate level. We hypothesized that the effects of dietary restriction, floor type, and dietary carbohydrate levels were mediated through loading of the joints either by, respectively, a short rapid increase in weight gain, higher incidence of play behaviors, or by an overall increased body weight. Although OC has been suggested to be associated with various CLC such as lameness, a consistent association of CLC at young age with OC at slaughter could not be found. This is likely due to CLC and OC both varying over time, making associations between the 2 entities complicated. There were indications that a component of the immune system is associated with OC as N(A)Ab against several antigens were found after weaning in association with OC. However, as with the CLC, associations with OC were not consistent over time, making it difficult to discern the exact associations between N(A)Ab and OC. To conclude, several early life conditions were found to affect or be associated with OC prevalence in gilts. This indicates that if one wants to reduce OC prevalence, one needs to start early after weaning. However, the exact implications of OC on CLC such as lameness and, therefore, welfare remain uncertain and require further studies into the long term welfare effects of OC.

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CHAPTER 1

General introduction

1. INTRODUCTION

Osteochondrosis (OC) results from abnormalities in endochondral ossification and has been studied for over half a century in various animal species (reviewed by Ytrehus et al., 2007; McCoy et al., 2013). The only certainty is that OC is a multifactorial problem. Factors such as housing and dietary restriction (van Grevenhof et al., 2011), growth (Stern et al., 1995; Busch and Wachmann, 2011; van Grevenhof et al., 2012), hormones (Sloet van Oldruitenborgh-Oosterbaan et al., 1999; Billinghurst et al., 2004; Gangl et al., 2007; de Grauw et al., 2011), behavioural activity (van Weeren and Barneveld, 1999; Lepeule et al., 2013; Praud et al., 2013), and genetics (Ytrehus et al., 2004c; Jørgensen and Nielsen, 2005) have been associated with the occurrence of OC. However, contradictory results persist with regards to some of the associated factors, which perhaps lies in the fact that contributing factors to OC development are still not completely understood.

In order to understand the development of defects in endochondral ossification resulting from OC, the process of endochondral ossification will be briefly described after which OC development is discussed. Lastly, several factors that are associated with OC will be discussed, leading to the research that has been performed as part of this thesis.

1.1 ENDOCHONDRAL OSSIFICATION

Bone growth is accomplished by a process involving endochondral ossification in young growing animals in specialized growth cartilage present in, among others, long bones and is confined to 2 areas (reviewed by van Weeren, 2006; Mackie et al., 2008; Ytrehus et al., 2007). One area is present just above the metaphysis (termed here as the metaphyseal growth cartilage), while the other one lies further into the epiphysis just below the epiphyseal / articular surface (termed here as the epiphyseal growth cartilage). Although differences exist in the structures between the 2 growth cartilages, they share a similar function, i.e. accomplishing bone growth. The epiphyseal growth cartilage is the focus of this study, as defects in this cartilage have implications for joint surface deformities that could impair movement in a joint and lead to lameness. The chondrocytes (cartilage cells) present within the growth cartilage are arranged in several layers (Figure 1.1). The first layer is the reserve zone (containing precursors for the proliferative zone), followed by the proliferative zone and the hypertrophic zone

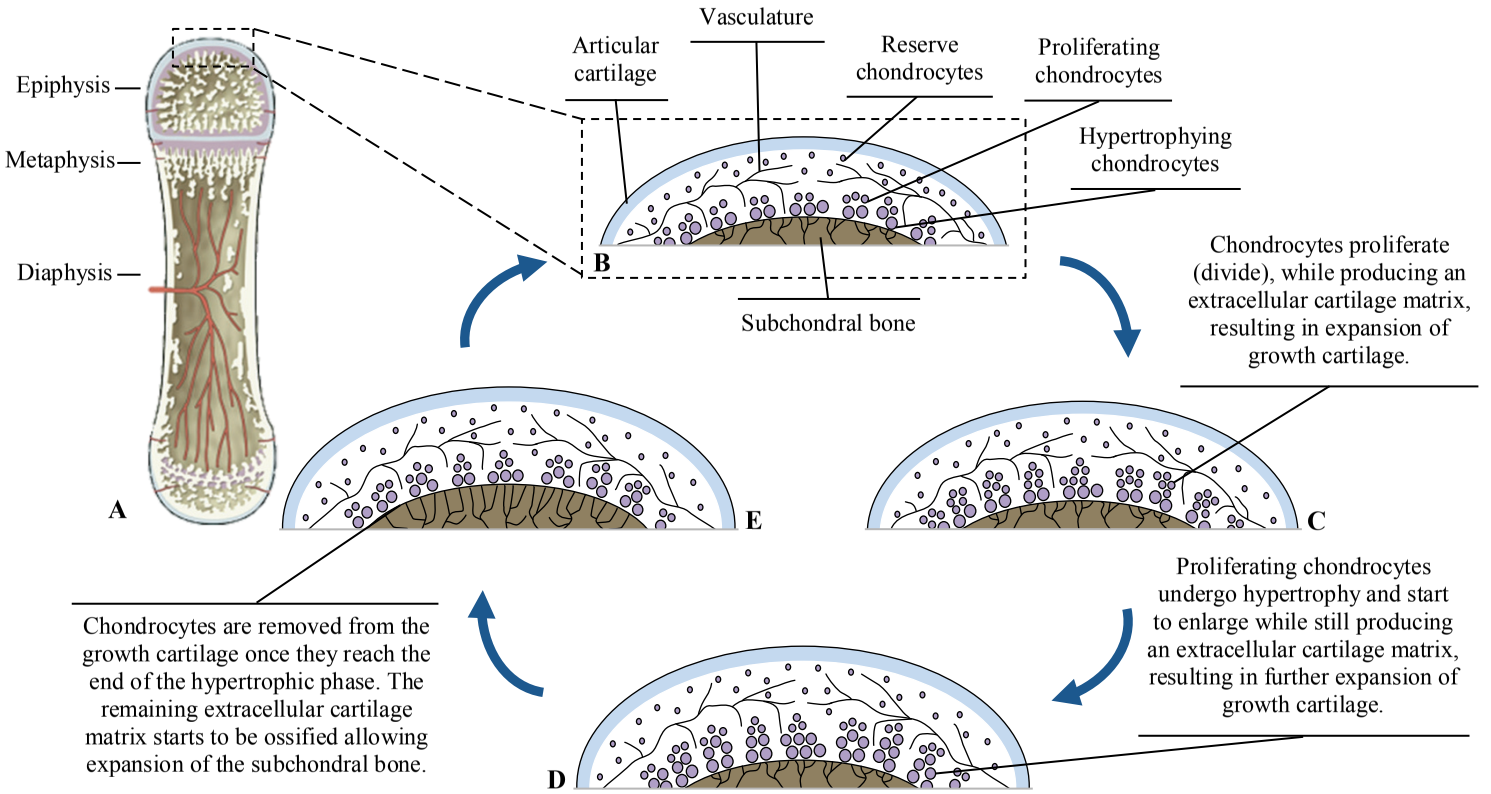

Figure 1.1. Overview of endochondral ossification (not depicted to scale) in the epiphyseal growth cartilage residing just below the epiphyseal / articular surface in long bones (A). Epiphyseal growth cartilage consists of chondrocytes residing in the reserve, proliferative, and hypertrophic zone that are nourished by vasculature residing in cartilage canals (B). Growth is accomplished by proliferation (C) and hypertrophy (D) of chondrocytes while they produce an extracellular cartilage matrix consisting of various components such as collagen an proteoglycan (not shown in the figures). At the end of the hypertrophic zone, chondrocytes are removed and the remaining extracellular matrix is eventually ossified (E). This process repeats itself in young growing animals. (Figure A modified from van Weeren, 2006).

that allow for growth of the bone by proliferation (division) and consequently hypertrophy (including enlargement) of chondrocytes, allowing the growth cartilage to expand (Carlson et al., 1985; Ekman et al., 1990; Henson et al., 1997b; and reviewed by Hunziker, 1994; Mackie et al., 2008; Ytrehus et al., 2007). During proliferation and hypertrophy, the chondrocytes produce an extracellular matrix containing, amongst others, collagen and proteoglycan that provide the growth cartilage with strength to withstand compression and tensile forces. (reviewed by Martel-Pelletier et al., 2008; Kheir and Shaw, 2009; Garcia-Carvajal et al., 2013; Gao et al., 2014). The proliferative and hypertrophic chondrocytes are nourished by vascular elements contained in so called cartilage canals that run through the entire epiphyseal growth cartilage (Ytrehus et al., 2004ab). The hypertrophied chondrocytes produce a matrix that is able to be calcified and when they have reached the last stage of hypertrophy, they are removed from the growth cartilage leaving the extracellular matrix behind (Carlson et al., 1985; Ekman et al., 1990; Henson et al., 1997b; and reviewed by Hunziker, 1994; Mackie et al., 2008; Ytrehus et al., 2007). Consequently, in a process involving vascular invasion from the subchondral bone, partial breakdown of extracellular matrix and deposition of bone tissue on the extracellular matrix by amongst others chondroclasts, osteoclasts and osteoblasts, the cartilage tissue is converted into bone tissue (ossification) at the ossification front (Carlson et al., 1985; Ekman et al., 1990; Henson et al., 1997b; and reviewed by Hunziker, 1994; Mackie et al., 2008; Ytrehus et al., 2007). The progression of the ossification front proceeds at a rate faster than that of the production of new cartilage tissue and eventually leads to the complete conversion of the growth cartilage to bone tissue, leaving only the articular cartilage at the surface of the epiphysis (reviewed by Ytrehus et al., 2007; Laverty and Girard, 2013). There is a multitude of factors present that regulate this process of endochondral ossification, which is beyond the scope of this introduction. However, endochondral ossification can be disturbed by a condition termed osteochondrosis.

1.2 OSTEOCHONDROSIS DEVELOPMENT

Osteochondrosis is defined as a restricted area of necrotic growth cartilage that is not ossified (Carlson et al., 1986; Woodard et al., 1987b; Woodard et al., 1987a; Carlson et al., 1989; Ekman et al., 1990; Carlson et al., 1991; Wegener and

Heje, 1992; Carlson et al., 1995; Thorp et al., 1995; Henson et al., 1997b; Ytrehus et al., 2004abd; Olstad et al., 2007; 2008bc, 2011). Over the past decades, a clear characteristic of OC has been the association of necrotic cartilage with necrotic cartilage canals. This implies that a disruption in vasculature results in chondrocytes that are not able to sustain themselves any longer and consequently become necrotic (reviewed by Ytrehus et al., 2007; Laverty and Girard, 2013; McCoy et al., 2013; Olstad et al., 2015). In contrast, cartilage canals can undergo normal regression (chondrification), which has not been associated with the necrotic growth cartilage that is characteristic of OC (Woodard et al., 1987a; Carlson et al., 1989; Ekman et al., 1990; Carlson et al., 1991, 1995; Ytrehus et al., 2004abd). Indeed, relatively recent histological studies performed in pigs (Ytrehus et al., 2004abd; and reviewed by Ytrehus et al., 2007) and horses (Olstad et al., 2007; 2008bc, 2011; and reviewed by Olstad et al., 2015) have shown a clear association between vascular disruption and OC. These studies indicate that vascular disruption, resulting in necrotic growth cartilage, takes place at the ossification front where vessels within the growth cartilage can form anastomoses with vessels of the subchondral bone once the ossification front encounters a cartilage canal (Figure 1.2). Several factors may contribute to the risk of vascular disruption including fragility of the anastomosis structures, fragility of the dynamic structure at the ossification front (degradation of extracellular matrix and deposition of immature bone) where anastomoses occur (Ytrehus et al., 2004b; Olstad et al., 2008c; and reviewed by Ytrehus et al., 2007; Laverty and Girard, 2013; McCoy et al., 2013), and different collagen structures near the ossification front that may not provide optimal support (Wardale and Duance, 1994; Lecocq et al., 2008; and reviewed by Laverty and Girard, 2013). These studies suggest that these factors create an environment in which there is insufficient structural support for the vasculature at the ossification front and creates an environment that would make vascular elements prone to disruption, leading to necrotic growth cartilage. A (large) area of necrotic growth cartilage (OC latens) will not be ossified due to an inappropriate extracellular matrix and chondrocyte signalling once the ossification front encounters the defect (OC manifesta), and may fracture under loading of the joint leading to joint surface abnormalities (OC dissecans) as shown in Figure 1.3 (Ytrehus et al., 2004a; Ytrehus et al., 2004b; and reviewed by Ytrehus et al., 2007).

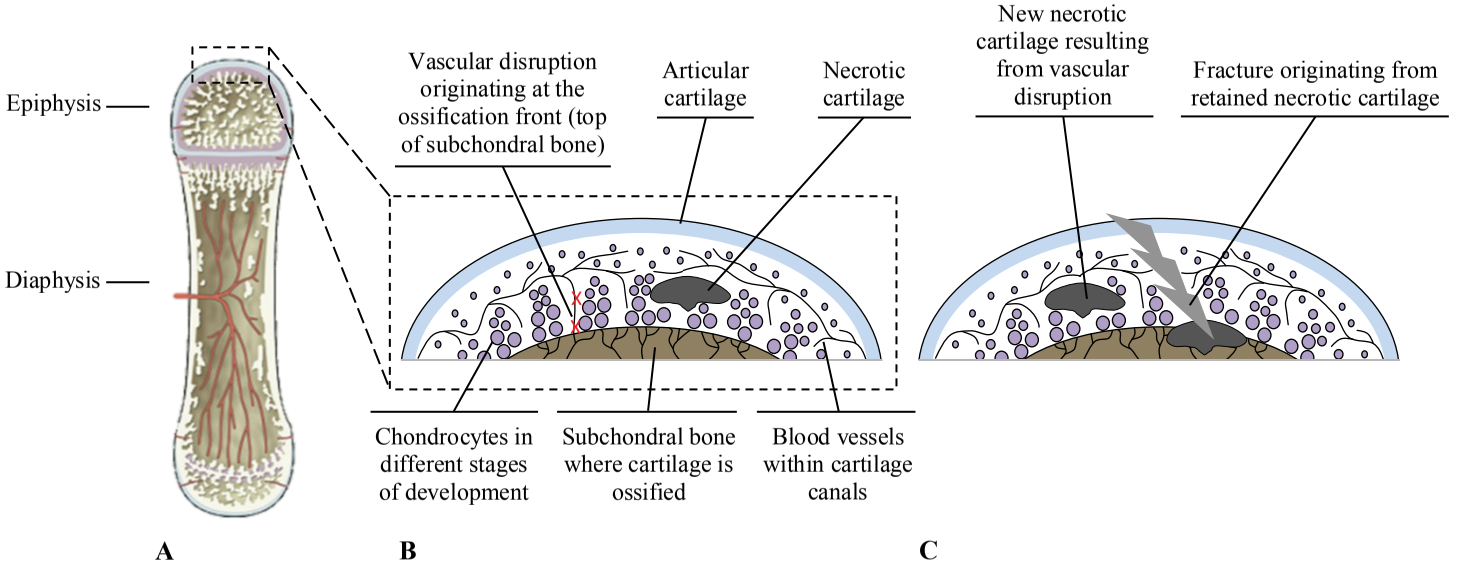

Figure 1.2. Overview of the development of osteochondrosis (OC; not depicted to scale) as described by Ytrehus et al. (2007). Osteochondrosis occurs in, amongst others, long bones in the epiphysis in the growth cartilage (A). In the epiphyseal growth cartilage, chondrocytes reside that from top to bottom consist of reserve zone chondrocytes, and proliferating and hypertrophying chondrocytes that are nourished through blood vessels residing in cartilage canals (B). The chondrocytes produce an extracellular matrix deposited around them. At the ossification front vascular disruption may take place when blood vessels from the growth cartilage anastomose with blood vessels from the subchondral bone (B). The chondrocytes distal from the disruption may not sustain themselves and become necrotic (OC latens; C). Necrotic cartilage is not ossified due to an inappropriate extracellular matrix structure and signalling, and is retained in subchondral bone (OC manifesta), which may fracture and extend to the surface of the joint leading to joint surface deformities (OC dissecans; C).

Figure A modified from van Weeren, 2006.

However, the ensuing formation of necrotic growth cartilage from vascular disruption does not necessarily have to be a detrimental process. Clusters of viable chondrocytes and vasculature surrounding necrotic cartilage are reported to proliferate towards the defect and have been suggested as reparative attempts of the necrotic cartilage (Carlson et al., 1986; Woodard et al., 1987b; Ekman et al., 1990; Wegener and Heje, 1992; Henson et al., 1997b; Ytrehus et al., 2004bd; Olstad et al., 2007; 2008ac; and reviewed by Ytrehus et al., 2007; Olstad et al., 2015). Studies have indicated that the presence of blood vessels in the epiphyseal growth cartilage is transient as an animal grows and suggests a short time frame for OC development to take place in pigs (Carlson et al., 1991, 1995; Ytrehus et al., 2004ab). Ytrehus et al. (2004ab) have shown that vasculature were seen to be abundant at 7 weeks of age in knee joints harvested from piglets, but gradually declined until vasculature was hardly present at approximately 15 weeks of age. Others have also indicated that necrotic cartilage is nearly absent in pigs weighing less than 21 kg of body weight (Carlson et al., 1991) or younger than 10 weeks of age (Ekman et al., 1990) and that pigs of around 70 kg show an almost complete absence of vasculature (Carlson et al., 1991). Ytrehus et al. (2004a) expressed level of vasculature as the length of blood vessels within the growth cartilage (lateral and

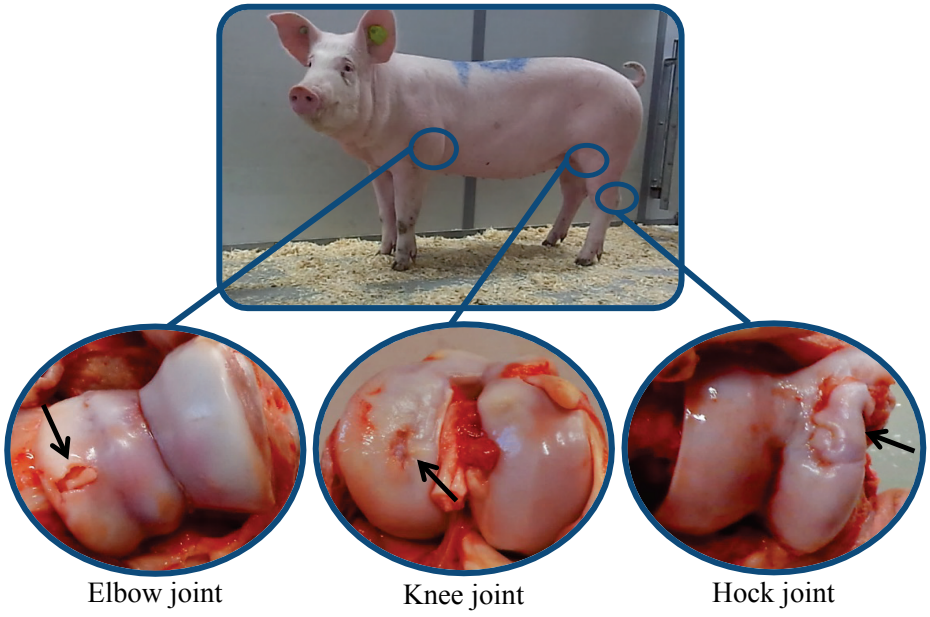

Figure 1.3. Severe lesions of osteochondrosis in the elbow, knee, and hock joint.

medial femoral condyles) of pigs divided by the surface of the condyle (vascular index). They were unable to find significant correlations between the vascular index and OC lesions, even though the vascular index was lower in older animals that had a higher OC prevalence. Thus, the authors indicated that this should be interpreted as an apparent increase in vascular disruptions with age. The studies by Ytrehus et al. (2004abd) indicate that a major part of OC development will lie between 7 and 16 weeks of age. Studies by Olstad et al. (2014ab) indicate a similar timeframe but show that OC lesions may still develop up to 22 weeks of age at a lower rate than at younger age. Therefore, the window of susceptibility for OC to develop reduces with age and measures to reduce OC development have a short time frame to have effect. The exact or ideal moment to start such measures are, however, unknown.

1.3 OSTEOCHONDROSIS IMPACT

When OC becomes severe enough to result in joint surface abnormalities, it may impair movement in the joint that could result in lameness and implications on animal welfare. Studies with relatively large numbers of pigs (> 1000 pigs) in the Scandinavian region report prevalence of up to 90%, including OC manifesta and dissecans lesions (Lundeheim, 1987; Stern et al., 1995; Ytrehus et al., 2004c; Busch and Wachmann, 2011). However, the majority of OC lesions are not severe OC dissecans lesions. The exact prevalence of OC in the Netherlands is not known, but a current study (van Grevenhof et al., unpublished data) has attempted to quantify prevalence of OC in the Netherlands. Preliminary results indicate an OC prevalence of up to 90%, which is similar as in the Scandinavian studies.

Although OC has been associated with higher degrees of lameness (Lundeheim, 1987; Goedegebuure et al., 1988) and reduced longevity (Yazdi et al., 2000), it remains difficult to indicate what the exact implications are of OC on animal welfare and longevity as not all OC affected pigs are prematurely culled due to lameness (van Grevenhof et al., unpublished data). Jensen et al. (2012) quantified the impact of OC on profitability in Scandinavia through simulation studies using opinions from scientists in the field of animal behaviour and welfare with experience in leg disorders, and veterinarians with experience and interest in leg health. Based on expected veterinary costs, average daily gain, and feed conversion, Jensen et al. (2012) estimated that the effects of OC would lead to a 25

to 30 euro loss per finisher pig of which the likely highest factor resulting in loss of profitability was a reduced average daily gain. Additionally, OC was considered by the scientists and veterinarians as the second highest cause of pain from lameness. Current studies in the Netherlands are being performed in an attempt to quantify the prevalence of OC and its impact on welfare in practice, and is further elaborated on in Chapter 7.

To determine if OC has an impact on welfare it is important to know whether it is strongly associated with lameness. Over the years, OC has been associated with various 'abnormal' conformation and locomotive characteristics (CLC) of which an important component is lameness (Jørgensen, 1995, 2000; Jørgensen et al., 1995; Stern et al., 1995; Jørgensen and Andersen, 2000; Luther et al., 2007; Kirk et al., 2008; de Koning et al., 2012). These studies indicate that severe OC is, amongst others, associated with an increase in lameness and stiff gait of the animals. The associations of OC with CLC have not been consistently reported to be in the same direction or magnitude (Jørgensen, 1995, 2000; Jørgensen et al., 1995; Stern et al., 1995; Jørgensen and Andersen, 2000; Luther et al., 2007; Kirk et al., 2008; de Koning et al., 2012). For example, Jørgensen and Andersen (2000) indicated that associations between stiff gait characteristics and OC can be opposite between Yorkshire and Landrace boars. However, if OC is associated with CLC such as lameness even to a mild or moderate degree, it could pose as an animal welfare threat. Indeed, lameness is suggested to be an important factor for premature culling in sows that can range up to 30% of total culling (Dagorn and Aumaitre, 1979; D'Allaire et al., 1987; Stein et al., 1990; Jørgensen and Sørensen, 1998; Scott et al., 2006, 2007). Osteochondrosis has also been shown to increase the risk for premature culling (Yazdi et al., 2000) and 34% of pigs culled for lameness have been reported to be attributed to OC (Dewey et al., 1993), suggesting that some associations between CLC and OC exist. The relationship of CLC with OC is elaborated upon further in Chapter 4.

A problem with assessing the impact of OC on animal welfare is the difficulty to assess OC in vivo. Attempts have been made to try and associate OC development in vivo (at young age) using possible biomarkers predicting risk of OC. Previous studies showed associations of OC with cartilage breakdown products related to collagen and proteoglycan turnover to assess total cartilage turnover (Semevolos et al., 2001; Billinghurst et al., 2003; Billinghurst et al., 2004;

Gangl et al., 2007; Frantz et al., 2010; de Grauw et al., 2011). Additionally, a variety of hormones and plasma components (glucose, insulin like-growth factor-1, growth- and, parathyroid hormones, calcium, etc.) have been associated with OC (Ralston, 1996; Sloet van Oldruitenborgh-Oosterbaan et al., 1999; Pagan, 2001; Semevolos et al., 2001; Verwilghen et al., 2009). However, a reliable biomarker of OC has yet to be found to identify animals at risk for developing OC that could help in reducing the impact OC might have on an animal. A novel mechanism may exist that has not been assessed before and constitutes a part of the immune system consisting of natural (auto-) antibodies (N[A]Ab). One of the functions of N(A)Ab revolves around maintaining homeostasis of the body by clearing debris from damaged cells (reviewed by Lutz et al., 2009; Binder, 2012; Elkon and Silverman, 2012). Their possible association with OC and practical use is further elaborated upon in Chapter 5. Recently computed tomography methods have been proposed as a screening tool for OC status in vivo in young pigs (Olstad et al., 2014a) and could provide opportunities to identify OC at young age.

Considering that breeding gilts will have to last several parities and that the development of OC is suggested to increase the risk to develop lameness with all its negative consequences, it merits studying the development of OC and to investigate preventive measures against it or possible biomarkers to detect it. When OC can be reduced in practice through preventive measures or due to selection during the rearing period of gilts, it could be possible to develop a more robust breeding sow.

1.4 ENVIRONMENTAL MEASURES

One method of reducing OC prevalence in sows in practice may lie in taking measures in the 'environment' encountered by gilts. As stated at the beginning of the introduction, many factors have been associated with OC. Environmental factors that may influence OC prevalence are concerning diet and floor type, which are the main focus of this thesis.

1.4.1 Dietary Restriction

Dietary restriction in pigs in relation to OC has been studied on several occasions in the past (Carlson et al., 1988; Jørgensen, 1995; van Grevenhof et al., 2011; Quinn et al., 2015). One of the mechanisms underlying the influence of

dietary restriction on OC prevalence is the difference achieved in joint loading between pigs restricted in feed and pigs that receive a higher feeding level. A higher bodyweight would naturally lead to higher load bearing on the joints. Joint loading and trauma have previously been suggested as a risk factor to develop OC either through an increase in vascular disruption or an increase in fracturing necrotic growth cartilage (Nakano and Aherne, 1988; Carlson et al., 1991; Ytrehus et al., 2004bc). Additionally, pressure applied on a joint is likely also placing compressive stresses on the chondrocytes that can result in altered chondrocyte functioning (Alberty et al., 1993; Quinn et al., 1999; Davisson et al., 2002; Stokes et al., 2007) thereby affecting survivability of chondrocytes or reparative responses undertaken by the chondrocytes (Ytrehus et al., 2004d). Additionally, feeding levels applied will also affect growth rates of the animals that can place higher compressive stresses on the chondrocytes to which they may not be adapted to (Carlson et al., 1989; Ekman et al., 1990; Carlson et al., 1991; and reviewed by Laverty and Girard, 2013). In horses, higher growth rates have been associated with a higher OC prevalence (van Weeren et al., 1999; Donabédian et al., 2006; Lepeule et al., 2009). Several studies have found that higher feeding levels applied to pigs can increase OC prevalence, which have been suggested to occur due to increased growth rates (Carlson et al., 1988; van Grevenhof et al., 2011, 2012; Quinn et al., 2015), while others cannot find effects of feeding levels (Jørgensen, 1995; Håkansson et al., 2000). Whether an association exists between dietary restriction and OC prevalence and possible underlying mechanisms will be further elaborated upon in chapter 2.

1.4.2 Dietary Carbohydrates and Arginine

Another factor implied in dietary measures are the metabolic hormones and products that can be affected by dietary composition. In general, insulin and insulin like growth factor-1 (IGF-1) have been shown to increase proliferation and survivability of chondrocytes (Böhme et al., 1992; Ballock and Reddi, 1994; Hunziker et al., 1994; Alini et al., 1996; Henson et al., 1997a). Higher glucose, insulin, and IGF-1 levels have been associated with a higher prevalence of OC in horses fed on similar feed levels (Ralston, 1996; Pagan, 2001; Semevolos et al., 2001; Verwilghen et al., 2009). Lower levels of IGF-1, in contrast, have also been associated with more OC in horses (Sloet van Oldruitenborgh-Oosterbaan et al.,

1999) and chondrocytes in OC lesions exhibit a loss in IGF-1 staining (Thorp et al., 1995). Therefore, one could envision that chondrocytes may be affected by dietary composition such as dietary carbohydrates that are known to affect glucose, insulin, and IGF-1 responses (van den Brand et al., 1998, 2000, 2001; Wientjes et al., 2012, 2013).

An overlooked aspect of OC and dietary contents seems to concern vascularization within the growth cartilage that could lead to either less disruption of vascular elements or increase reparative vascular responses of necrotic growth cartilage. For example, arginine supplementation has been reported to be an angiogenesis factor under hypoxic conditions (Schwarzacher et al., 1997; Murohara et al., 1998; Duan et al., 2000a; Duan et al., 2000b; Dulak et al., 2000; Hazeleger et al., 2007), as expected during OC development. Therefore, one could envision that supplementing diets with arginine may increase reparative responses undertaken by vasculature once necrotic cartilage has formed. The possibility of dietary carbohydrates and arginine to have an effect on OC prevalence will be further elaborated on in chapter 6.

In conclusion, there is some evidence that OC can be affected through feeding strategies or dietary composition. However, due to the contradictory results found of dietary treatments, the usefulness of influencing OC prevalence through dietary measures remain uncertain. If dietary restriction or composition are effective in reducing OC prevalence, feeding strategy may serve as a relatively simple tool to reduce OC prevalence in practice.

1.4.3 Housing System / Floor Type

Aside from dietary measures, another environmental factor is the housing that the animals are kept in with respect to floor type. The type of floor or floor bedding that the animals are kept on can affect chances for traumatic events to occur and have an influence on joint loading, which were implicated in OC development as discussed above. For example, if one would compare a concrete (partially) slatted floor to a floor that has some degree of bedding, the concrete floor could be more slippery and result in higher impact loading during movement of the animals. These factors could increase the risk of traumatic events and loading of the joints. Additionally, considering that the way pigs are housed will influence their behavior (Lay Jr. et al., 2000; Bolhuis et al., 2005, 2006), it is

possible that the way pigs are housed could affect OC development as well. Pigs that are very active may be placing more stress on the joint to cope with by applying irregular (high) loading patterns during movement. The increased irregular stress on the joint may put too much strain on the vascular elements and chondrocytes. In horses it has been found that the type and intensity of exercise (tends to) affect severity of OC lesions and locations affected with OC lesions (van Weeren and Barneveld, 1999; Lepeule et al., 2013; Praud et al., 2013). Deep litter type systems, which use a high level of straw and wood shavings as floor bedding, are reported to increase activity levels of pigs (Morgan et al., 1998; Lay Jr. et al., 2000; Bolhuis et al., 2005, 2006; Scott et al., 2006; van Grevenhof et al., 2011). Floor type could, therefore, have an effect on OC prevalence through activity (exercise) as well. However, literature reports little on the effect of floor type and OC prevalence in pigs, where some are able to find effects of floor type on OC prevalence (van Grevenhof et al., 2011; Etterlin et al., 2014) and others are not (Jørgensen, 2003; Scott et al., 2006). As a consequence, the usefulness of applying housing strategies with respect to floor type to reduce OC prevalence in practice are uncertain. If the way pigs are kept affect OC prevalence, it can serve as an additional tool to reduce OC prevalence in practice. The possible associations and mechanisms of the effects of floor type on OC are elaborated upon in chapter 3.

1.5 FINAL REMARKS AND OUTLINE THESIS

Osteochondrosis may have consequences for animal welfare and longevity of pigs, which is especially important for sows that have to last several parities. Osteochondrosis lesions are likely to develop and persist if the adaptive capacity of the chondrocytes is surpassed (Carlson et al., 1989; Ekman et al., 1990; Carlson et al., 1991; Ytrehus et al., 2004b; Ytrehus et al., 2004d). Adaptive capacity of chondrocytes is likely surpassed by factors such as loading of the joints. Loading places a certain stress on the chondrocytes that might surpass their capacity to cope / survive with a vascular disruption, and to which the chondrocytes are additionally unable to elicit a proper reparative response (Carlson et al., 1989; Ekman et al., 1990; Carlson et al., 1991; Ytrehus et al., 2004bd). In order to reduce prevalence of OC in sows in practice, one needs to know if environmental factors are placing too much stress on the growth cartilage to cope with during the rearing period of the gilts, as signified by the prevalence of OC lesions. Once possible factors have been

established as risk factors for OC development, one may wonder about time dependence of those factors. As mentioned, vasculature within the growth cartilage is transient and as OC development is dependent on presence of vasculature, OC development will take place in a short time frame that likely lies around 10 weeks of age for at least the knee joint in pigs (Ytrehus et al., 2004abd). Possibly, an (environmental) factor or condition will have a different association with OC prevalence when gilts are exposed to them at different periods during rearing and, therefore, during different periods of OC development before and after 10 weeks of age. Therefore, studies would need to take into account that a factor or a condition encountered before or after 10 weeks of age, may have a different outcome in OC prevalence.

To the best knowledge of the author, no studies have been performed in gilts that evaluated time dependent effects of environmental factors and conformation and locomotive characteristics at young age (around 10 weeks of age) on OC development, nor have dietary components been assessed that possibly affect chondrocyte and vascular functioning on OC prevalence in gilts. Therefore, the purpose of this thesis is to assess several factors that have been associated with OC in the past and study whether some of the associations are time dependent. In chapter 2 and 3, the possible time dependent effects of, respectively, dietary restriction and floor type on OC prevalence are described. In chapter 2, gilts were fed ad libitum or restricted (80% of ad libitum uptake) after weaning (4 weeks of age) and were kept either on the same feed level after 10 weeks of age or were switched from restricted to ad libitum or vice versa. In chapter 3, gilts were kept on a partially concrete slatted floor or deep litter type system after weaning (4 weeks of age) and were kept either on the same floor type after 10 weeks of age or were switched from concrete to deep litter or vice versa. In the experiments described in chapter 2 and 3, conformation and locomotive characteristics (CLC) were assessed at several ages in the gilts used. The possible time dependent associations of these CLC with OC are described in chapter 4. In chapter 5, a novel in vivo biomarker consisting of naturally occurring antibodies at young age is presented in association with OC from the gilts used in the experiment described in chapter 3. Chapter 6 presents the effects of a high dietary carbohydrate level (12.5% corn starch + 12.5 % dextrose added to a basal diet) versus a low dietary carbohydrate level (an iso-caloric 8.9% soybean oil added to a basal diet) that is either supplemented with

0.8% arginine or not supplemented with arginine. Finally, chapter 7 discusses the results of these studies all together and discusses, amongst others, associations of OC with growth rates and behaviour, and presents new preliminary data of OC prevalence in sows from Dutch commercial husbandry.

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CHAPTER 2

The influence of dietary restriction before and after 10 weeks of age on osteochondrosis in growing gilts[1]

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2.1 ABSTRACT

Osteochondrosis (OC) is one of the main causes of leg weakness causing premature culling in breeding sows and develops in a short time frame in young growing gilts. Dietary restriction may have different effects on OC prevalence depending on the age of the gilts. The aim of this study is to investigate age-dependent effects of dietary restriction, ad libitum vs. restricted (80% of ad libitum), on the occurrence of OC in gilts at slaughter (26 weeks of age). At weaning (4 weeks of age), 211 gilts were subjected to one of 4 treatments of a feeding regime. Gilts were administered either ad libitum feeding from weaning until slaughter (AA); restricted feeding from weaning until slaughter (RR); ad libitum feeding from weaning until 10 weeks of age, after which gilts were switched to restricted feeding (AR); or restricted feeding from weaning until 10 weeks of age, after which gilts were switched to ad libitum feeding (RA). At slaughter, the elbow, hock, and knee joints were harvested. Joints were scored macroscopically for articular surface deformations indicative of OC. Gilts in the RA treatment had significantly higher odds of being affected by OC than gilts in the RR and AR treatments in the hock joint (OR = 3.3, P= 0.04 and OR = 8.5, P = 0.002, respectively) and at animal level (OR = 2.5, P= 0.001 and OR = 1.9, P= 0.01, respectively). Gilts in the AA treatment had higher odds of being affected by OC than gilts in the AR treatment in the hock joint (OR = 5.3, P= 0.01). The results indicate a possible pathway to reduce the prevalence of OC in breeding gilts that will have to last several parities. Switching from restricted feeding to ad libitum feeding after 10 weeks of age increases OC prevalence as opposed to restricted feeding after 10 weeks of age.

Keywords: age, body weight, dietary restriction, gilts, osteochondrosis

2.2 INTRODUCTION

Osteochondrosis (**OC**) in the epiphyseal growth cartilage involves a disturbance in the ossification process of growing pigs, which can cause leg weakness (reviewed by Ytrehus et al., 2007) and reduced longevity in sows (Yazdi et al., 2000). As breeding gilts will have to last several parities, it is important that OC incidence is reduced to optimize health and welfare, and thereby longevity and economic value of these gilts. A factor involved in reducing OC may be dietary restriction during rearing in gilts.

Several studies have found evidence of a positive relationship between dietary restriction and OC incidence (Glade and Belling Jr., 1986; Carlson et al., 1988; van Grevenhof et al., 2011). However, other studies were unable to find similar relationships (Jørgensen, 1995; Donabédian et al., 2006). Variable results may be related to the duration and timing at which restrictions were administered. There are indications that the sensitive time period for porcine OC development lies between 7 and 13 weeks of age (Ytrehus et al., 2004ab), which hence implies age-dependent effects of dietary restriction on OC development. However, the age-dependent effects of dietary restriction on OC in breeding gilts are yet to be established. The presence of age-dependent effects would imply that OC incidence could be reduced by taking preventative measures (adjustment of the amount of feed administered) at young age.

The aim of this study was to investigate whether age-dependent effects of dietary restriction (ad libitum versus 80% of ad libitum feeding) on OC incidence in breeding gilts at 26 weeks of age can be identified, taking into account the abovementioned period of sensitivity for OC development. This will help to elucidate the effect of dietary restriction on OC prevalence and to assist in the development of feeding strategies that can be used in practice to reduce OC prevalence in breeding gilts.

2.3 MATERIALS AND METHODS

2.3.1 Ethical Note

Osteochondrosis can cause articular surface irregularities, possibly resulting in lameness that might affect the welfare of the gilts. Assessments to detect serious impairments of welfare in the gilts were therefore performed daily.

Severely lame or wounded gilts were taken out of the experiment and euthanized. The experiment and all measurements were approved by the Animal Welfare Committee of Wageningen University and Research center in compliance with the Dutch law on animal experimentation.

2.3.2 Animals

The experiment was performed using 211 Topigs 20 (Dutch Large White x Dutch Landrace) gilts acquired from a commercial breeding company (Van Beek SPF Varkens BV, Lelystad, The Netherlands). This crossbred line represents a large part of the commercially kept sows in The Netherlands that will produce finishing pigs. The gilts entered the experiment after weaning at, on average, 26 ± 1.5 (SD) days of age. Gilts were group housed in 8.37 m^2 pens consisting of 60% slatted floor (slatted synthetic-coated expanded metal) and 40% solid floor (epoxy-coated solid concrete floor). Gilts had access to various enrichment items (such as biting chains, burlap sacks, solid plastic balls, rubber mats) at all times, which were switched every 3 to 4 days. Gilts had ad libitum access to water at all times through a drinking nipple. Gilts were weighed at birth, 19 days of age, at weaning, and once every 2 weeks after weaning until slaughter at an average of 189 ± 3.9 days of age.

2.3.3 Treatments

Gilts were mixed and housed after weaning in 32 pens of 6 to 7 individuals and divided in 4 treatment groups with respect to dietary restriction on the basis of an equal distribution of littermates and body weight (**BW**) measured at 19 days of age (1 week before the start of the experiment). The 32 pens were divided over 4 departments (8 pens per department) with an equal distribution of the treatments per department. Treatments consisted of 4 feeding regimes administered to pens to assess age-dependent effects of dietary restriction. Two of the treatments entailed a switch in the amount of feed administered at 10 weeks of age (73 days of age). The switch was set at 10 weeks of age so that the piglets were subjected to different dietary restrictions within the first and second halves of the possible sensitive time period of OC development between 7 and 13 weeks of age (Ytrehus et al., 2004ab). The experimental setup resulted in 8 pens per treatment. Treatments consisted of: ad libitum feeding continuously from weaning until slaughter (**AA**); restricted feeding continuously from weaning until slaughter (**RR**); ad libitum feeding from

weaning until 10 weeks of age, after which gilts were switched to restricted feeding (**AR**); or restricted feeding from weaning until 10 weeks of age, after which gilts were switched to ad libitum feeding (**RA**). Restricted feeding was calculated as 80% of the ad libitum uptake of the preceding week. Up to 10 weeks of age, restricted feeding calculations were based on the 16 pens that received ad libitum feeding up to that age (AA and AR treatments). After 10 weeks of age, restricted feeding was calculated using the 8 pens that were exposed to only ad libitum feeding (AA treatment) to ensure that calculations were based on a steady ad libitum feed intake. Ad libitum-fed gilts received feed using a dry feeder unit. The feed-restricted gilts received feed in 2 portions per day (0800 and 1600 h) in 2 troughs with ample feeding space and with metal bars separating individual feeding places.

After weaning, gilts received first a pelleted weaning diet (9.49 MJ NE/kg; 158 gr/kg crude protein; 10.8 gr/kg digestible lysine). Subsequently, gilts changed to 3 successive diets, adapted to their feed uptake and age. Diets were switched gradually during 2 days in which diets were offered as a 50% mixture of the old and new diets. The successive diets included a standard pelleted grower diet (9.84 MJ NE/kg; 172 gr/kg crude protein; 9.9 gr/kg digestible lysine), to which the gilts were switched at approximately 43 days of age. Thereafter, the diet was changed to standard pelleted finishing diet 1 (9.58 MJ NE/kg; 162 gr/kg crude protein; 8.9 gr/kg digestible lysine) at approximately 75 days of age. Last, at approximately 115 days of age, gilts were switched to standard pelleted finishing diet 2 (9.23 MJ NE/kg; 145 gr/kg crude protein; 6.9 gr/kg digestible lysine).

2.3.4 Osteochondrosis Assessment

At the end of the experiment, gilts were slaughtered at a local slaughterhouse and several joints were harvested for macroscopical evaluation of the irregularities of the articular surfaces, which are indicative of OC (Jørgensen and Nielsen, 2005; Busch and Wachmann, 2011). Slaughter was performed over a 1-week period. Half of the population (equal distribution of treatments and pens) was slaughtered within 2 days at an average of 185 days of age. The other half of the population was slaughtered within 2 days at an average of 192 days of age. After slaughter, carcasses were stored according to standard slaughtering practices for 1 day at 4 °C. All 4 legs from each gilt were collected through dissection of the

shoulder and hip joints. After collection, the legs were stored again at 4 °C for a maximum of 2 d, after which dissection of joints was performed.

The elbow, knee, and hock joints were dissected for macroscopical assessment for presence and severity of OC on a total of 22 locations (Table 2.1). Joints were scored on a 5-point grading scale from 0 to 4 (as described by van Weeren and Barneveld, 1999), with a score of 0 indicating no abnormalities and 4 indicating severe abnormalities (for pictures of the different classifications see van Grevenhof et al., 2011). Osteochondrosis was scored by a veterinarian specializing in orthopedics, who was unaware of the treatments, and experienced in judging OC.

Table 2.1. The locations within joints assessed for osteochondrosis

Joint	Joint location
Elbow joint	Medial humeral condyle
	Lateral humeral condyle
	Anconeal process
	Proximal edge of radius
	Synovial fossa of radius
	Other locations
Knee joint	Lateral femoral trochlea
	Medial femoral trochlea
	Sulcus distal femur
	Patella
	Lateral femoral condyle
	Medial femoral condyle
	Other locations
Hock joint	Sagittal ridge of distal tibia
	Lateral trochlea of talus
	Medial trochlea of talus
	Lateral malleolus of tibia
	Medial malleolus of tibia
	Base of talus
	Lateral tibial cochlea
	Medial tibial cochlea
	Other locations

2.3.5 Statistics

Body weight Statistical Model. Body weight progression per treatment and measurement was analyzed and is presented. Measurements were analyzed on pen level, which is the experimental unit. Several BW measurements were performed per gilt within an experimental unit pen, which cannot be viewed as independent observations. Therefore, a repeated measurement analysis was performed using PROC MIXED in SAS 9.2 (SAS Inst. Inc., Cary, NC). Homogeneous variance of BW in growing animals cannot be attained, and therefore, a heterogeneous covariance structure was applied (SAS, 2010). The statistical model used for BW is as follows:

$$Y_{ijkl} = \mu + Treat_i + Meas_j + (Treat \times Meas)_{ij} + Pen_{k(i)} + \varepsilon_{ijkl,}$$

in which Y_{ijkl} represents the ijklth BW observation. The mean is represented by μ. $Treat_i$ represents the fixed class effect of treatment administered (i = AA, AR, RA, RR). The time points at which BW were measured is represented by the fixed class effect $Meas_j$ (j = measurement 0, 1, …, 13). Interaction between treatments and measurements is represented by the fixed class effect $(Treat \times Meas)_{ij}$ to assess differences between treatments within each measurement. $Pen_{k(i)}$ represents the random effect of the experimental unit pen (k = pen 1, 2, …, 32) nested within treatment and is used to assess the treatment effects on pen level. As mentioned, a repeated measurement analysis was performed with gilts as the subject. A heterogeneous first-order autoregressive covariance structure was applied to this repeated structure, indicating that ε_{ijkl} is normally distributed, but where measurements are correlated over time with a heterogeneous covariance structure per gilt within an experimental unit pen. Addition of other components to account for environmental influence (such as dam from which the gilts descended and department in which gilts were housed) did not result in further improvement of the model. Results are displayed as the least squares (**LS**) means ± SE.

Osteochondrosis Affected Locations. The locations within joints in which no OC was found for all gilts were omitted from the analyses as these locations are uninformative. Indications of bilateral symmetry of OC lesions between joints of the bilateral homologues of a gilt were calculated using Spearman's rank correlations. These correlations were not calculated for the medial humeral condyle, sagittal ridge of the distal tibia, and lateral femoral condyle, as this was deemed inappropriate because the number of OC-affected locations was less than or equal to 5 observations for each location.

Grouping of Osteochondrosis Scores. Osteochondrosis was scored on an ordinal scale of 0 to 4. Although the possibility exists to take into account ordinal data using ordinal logistic regression (Stokes et al., 2000), this was deemed an inappropriate analysis for the current dataset. The required sufficient number of observations of each combination of treatment and OC score to apply ordinal logistic regression was not attained (Stokes et al., 2000) and provided some convergence problems. The statistical analysis that was appropriate in the current situation was binary logistic regression. To accommodate binary logistic regression, the OC scores from the macroscopic evaluation of the joints were grouped to a 0 and 1 variable, where 0 indicates no abnormalities (OC score of 0)

and 1 indicates an OC score higher than 0.

Osteochondrosis Statistical Model. The OC scores were analyzed at the pen level, which is the experimental unit. Osteochondrosis was scored on several locations per joint, and therefore, observations within a gilt within a pen are not independent. A repeated measurement analysis was performed using PROC GLIMMIX in SAS 9.2. The statistical model used to assess treatment effects on OC prevalence is as follows:

$$\text{Logit } P(y_{ijklm} = 1) = \alpha + \text{Treat}_i + \text{Dept}_j + Pen_{k(ij)} + Gilt(Dam)_l + \varepsilon_{ijklm},$$

in which the model estimates the probability (P) that the ijklmth observation of OC (y) for a joint or at the animal level (all joints combined) is 1 (OC score higher than 0). Logit is the link function used to model the mixed linear regression analysis. The α component represents the estimate of the log odds of being affected with OC disregarding the independent variables (baseline odds (Kleinbaum and Klein, 2010)). Treat_i represents the fixed class effect of treatments administered (i = AA, AR, RA, RR). Dept_j represents the fixed class effect of the department in which gilts were housed (j = department 1, 2, 3, 4) and is used to account for environmental variation. $Pen_{k(ij)}$ represent the random effect of the experimental unit pen (k = pen 1, 2, …, 32) nested within treatment and department and is used to assess OC scores at the pen level for the treatment effects. A random term was added that consists of gilts nested within dams from which the gilts originated to account for dam effects [$Gilt(Dam)_l$; l = dam 1, 2, …., 46]. As mentioned, a repeated measurement analysis was performed with gilts as the subject, indicating that the random residual term from a binary distribution (ε_{ijklm}) shows dependency of the joint locations assessed for OC within each gilt within an experimental unit pen. Addition of other components to account for environmental influence (such as birth weight and slaughter age) did not result in further improvement of the model. The prevalence of OC is presented on the ordinal scale and as the total number of OC-affected joint locations and gilts. When significant treatment effects are present, odds ratios (**OR**) are presented to indicate the extent of the effect.

2.4 RESULTS

Four out of the 211 gilts were euthanized before the end of the experiment because of health problems. The joints were assessed for OC status after the animals were euthanized and the result was taken into account in the analyses. Two gilts were taken out of the experiment because of a rectal prolapse. Two gilts were taken out of the experiment because of severe lameness. No joint surface irregularities indicative of OC were present at the assessment of the joints within these 4 gilts.

2.4.1 Body weight

The BW progression per treatment and measurement is displayed in Figure 2.1A, confirming that the treatments indeed produced the intended contrasts in BW. To clearly visualize the contrasts per treatment group, Figure 2.1B shows the same BW per treatment, but now compared with the population mean BW.

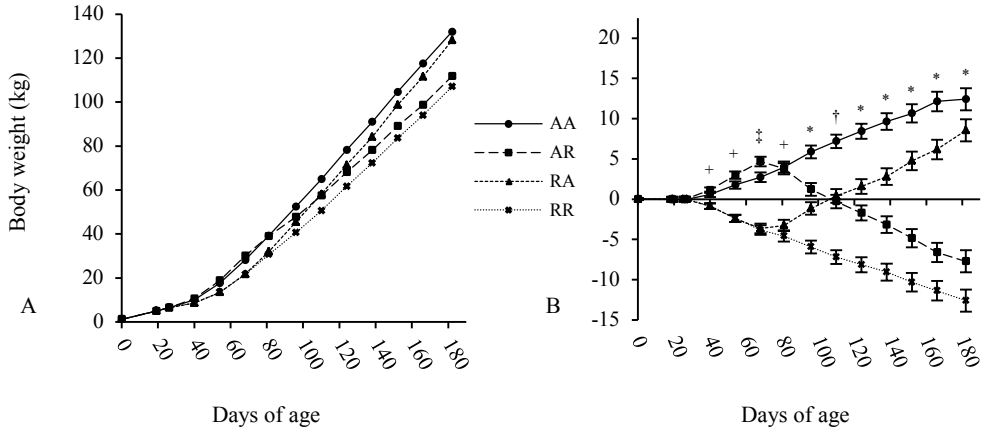

Figure 2.1AB. Body weight progression per treatment and measurement. (A) The least squares (LS) means of each treatment per measurement. (B) To visualize the contrasts between treatments more clearly, the same LS mean ± SE BW of each treatment per measurement compared with the population BW mean is presented. Treatments are: AA = ad libitum feeding from weaning until slaughter; AR = ad libitum feeding up to 10 weeks of age, after which feeding was switched to restricted (80% of ad libitum uptake); RA = restricted feeding up to 10 weeks of age, after which feeding was switched to ad libitum; RR = continuously restricted feeding from weaning until slaughter. Significant differences ($P < 0.05$) between treatments are indicated with a symbol for each measurement where significant differences were found. Symbols indicate the following significant differences, where no common superscript is found: + = AA^a, AR^a, RA^b, RR^b; ‡ = AA^a, AR^b, RA^c, RR^c; † = AA^a, AR^b, RA^b, RR^c; * = AA^a, AR^b, RA^c, RR^d

Significant differences ($P < 0.05$) in BW between the ad libitum-fed gilts (AA and AR treatments) and the feed-restricted gilts (RA and RR treatment) up to 10 weeks of age (73 days of age) were present from approximately 40 days of age onwards (approximately 14 days after the start of the experiment; Figure 2.1). After 10 weeks of age, the mean BW of the AR-treated gilts approached the mean BW of the RR-treated gilts, whereas the mean BW of the RA-treated gilts approached the mean BW of the AA-treated gilts. Significant differences ($P < 0.05$) in gilt BW between all treatments were present from 96 days of age onward (24 days after the switch at 10 weeks of age), except for the AR and RA treatments at 110 days of age, where both groups showed a similar mean BW, as at this moment in time the BW curves cross. The final average BW of gilts was 132 ± 1.4 kg for those receiving the AA treatment, 128 ± 1.4 kg for the RA treatment, 111 ± 1.4 kg for the AR treatment, and 107 ± 1.4 kg for the RR treatment.

2.4.2 Osteochondrosis Prevalence

The locations affected by OC in the elbow joint (total of 6 locations) were the medial humeral condyle (bilateral homologues), the lateral humeral condyle (bilateral homologues), and the anconeal process (bilateral homologues); in the hock joint (total of 5 locations) the sagittal ridge of the distal tibia of the right hind leg, lateral trochlea of talus (bilateral homologues), and the medial trochlea of talus (bilateral homologues); in the knee joint (total of 5 locations) the lateral femoral condyle (bilateral homologues), the medial femoral condyle (bilateral homologues), and a location on the lateral side of a lateral femoral condyle from the right hind leg. The majority of OC scores (data not shown) found in the elbow joint were on the lateral humeral condyle (54.8% of which 58.8% were scored as a 3 or 4); in the hock joint on the medial trochlea of the talus (65.5% of which 5% were scored as a 3 or 4); and in the knee joint on the medial femoral condyle (95.6% of which 20.8% were scored as a 3 or 4).

Significant positive correlations ($P < 0.001$ for all correlations) of OC prevalence and severity between the joint locations of the bilateral homologues were found for the lateral humeral condyle (correlation of 0.41), anconeal process (correlation of 0.85), lateral trochlea of talus (correlation of 0.38), medial trochlea of talus (correlation of 0.56), and medial femoral condyle (correlation of 0.56).

Joint Level Osteochondrosis Prevalence. The prevalence of OC per location assessed per joint and at the animal level is shown in Table 2.2. The knee joint displayed the highest total number of affected locations (OC score higher than 0), but not the highest number of locations affected with OC scores 3 and 4 (28 affected locations). The elbow joint had the lowest total number of affected locations but the highest number of locations affected with OC scores 3 and 4 (36 affected locations). The prevalence of OC scores 3 and 4 was generally lower compared with OC scores 1 and 2. The ratio of the prevalence of OC scores 3 and 4 to OC scores 1 and 2 was approximately 0.11 for the hock joint, 0.26 for the knee joint, and 0.37 at the animal level. Only in the elbow joint was the prevalence for OC scores 3 and 4 higher than that of OC scores 1 and 2 (ratio of 1.38).

Animal Osteochondrosis Prevalence. The number of locations affected with OC does not necessarily represent the number of gilts affected with OC, as a gilt may have multiple locations within a joint affected with OC. Therefore, Table 2.3 shows the prevalence of gilts affected with their maximum OC score. For example, if a gilt is scored on a joint at 6 locations with OC scores 0, 1, 2, 3, 3, 4, then that gilt is counted within the OC score 4 category (maximum OC score present within a joint or gilt). The total prevalence of gilts with OC scores 3 and 4 was generally lower compared to those with the OC scores 1 and 2. The ratio of the prevalence of OC scores 3 and 4 to OC scores 1 and 2 was approximately 0.16 for the hock joint, 0.35 in the knee joint, and 0.59 at the animal level. Only in the elbow joint was the prevalence of scores 3 and 4 higher than that of OC scores 1 and 2 (ratio of 1.71).

2.4.3 Treatment Effects

Gilts in the RA treatment showed the highest overall prevalence of OC in all joints and at the animal level (Table 2.2 and 2.3). Except for the hock joint, gilts in the RA treatment also displayed the highest prevalence of OC scores 3 and 4 compared with the other treatments. The lowest prevalence of OC-affected gilts was generally found in the RR and AR treatments. Looking at the animal level (Table 2.2 and 2.3), the order of treatments from high to low OC prevalence is RA, AA, AR, RR. Significant treatment effects at the pen level on the prevalence of OC were found for the hock joint and at the animal level and is indicated in Table 2.3.

Table 2.2. Prevalence (number and percentage) of osteochondrosis (OC)[1] on the locations within joints[2] assessed per treatment [3] and total prevalence.

	AA		AR		RA		RR		Total	
	n	%	n	%	n	%	n	%	n	%
Elbow										
0	302	95.0	309	97.2	290	91.2	303	97.1	1,204	95.1
1	0	0.0	1	0.3	3	0.9	3	1.0	7	0.6
2	5	1.6	4	1.3	8	2.5	2	0.6	19	1.5
3	8	2.5	3	0.9	10	3.1	2	0.6	23	1.8
4	3	0.9	1	0.3	7	2.2	2	0.6	13	1.0
Affected[4]	16	5.0	9	2.8	28	8.8	9	2.9	62	4.9
Hock										
0	246	92.8	259	97.7	238	89.8	251	96.5	994	94.2
1	9	3.4	3	1.1	15	5.7	5	1.9	32	3.0
2	6	2.3	3	1.1	12	4.5	2	0.8	23	2.2
3	2	0.8	0	0.0	0	0.0	2	0.8	4	0.4
4	2	0.8	0	0.0	0	0.0	0	0.0	2	0.2
Affected	19	7.2	6	2.3	27	10.2	9	3.5	61	5.8
Knee										
0	232	87.5	225	84.9	221	85.0	236	90.8	914	87.0
1	21	7.9	30	11.3	22	8.5	22	8.5	95	9.0
2	4	1.5	3	1.1	6	2.3	0	0.0	13	1.2
3	6	2.3	7	2.6	8	3.1	1	0.4	22	2.1
4	2	0.8	0	0.0	3	1.2	1	0.4	6	0.6
Affected	33	12.5	40	15.1	39	15.0	24	9.2	136	13.0
Animal										
0	780	92.0	793	93.5	749	88.9	790	95.0	3,112	92.3
1	30	3.5	34	4.0	40	4.7	30	3.6	134	4.0
2	15	1.8	10	1.2	26	3.1	4	0.5	55	1.6
3	16	1.9	10	1.2	18	2.1	5	0.6	49	1.5
4	7	0.8	1	0.1	10	1.2	3	0.4	21	0.6
Affected	68	8.0	55	6.5	94	11.2	42	5.0	259	7.7

[1] OC was assessed on a 5-point grading scale where 0 indicates no OC and 4 indicates the severest form of OC.

[2] The locations affected with OC were as follows: in the elbow joint (6 affected locations) the medial humeral condyle, the lateral humeral condyle, and the anconeal process; in the hock joint (5 affected locations) the sagittal ridge of the distal tibia, lateral trochlea of talus, and the medial trochlea of talus; in the knee joint (5 affected locations) the lateral femoral condyle, the medial femoral condyle, and the lateral side of a lateral femoral condyle.

[3] AA = ad libitum feeding from weaning until slaughter; AR = ad libitum feeding up to 10 weeks of age, after which feeding was switched to restricted (80% of ad libitum uptake); RA = restricted feeding up to 10 weeks of age, after which feeding was switched to ad libitum; RR = continuously restricted feeding from weaning until slaughter.

[4] Affected is the total number of locations with an OC score higher than 0.

Table 2.3. Prevalence (number and percentage) of a maximum osteochondrosis (OC)[1] score present within a gilt[2] per treatment[3] and total prevalence.

	AA		AR		RA		RR		Total	
	n	%	n	%	n	%	n	%	n	%
Elbow										
0	43	81.1	46	86.8	38	71.7	46	88.5	173	82.0
1	0	0.0	1	1.9	2	3.8	1	1.9	4	1.9
2	3	5.7	3	5.7	3	5.7	1	1.9	10	4.7
3	5	9.4	2	3.8	4	7.5	2	3.8	13	6.2
4	2	3.8	1	1.9	6	11.3	2	3.8	11	5.2
Affected[4]	10	18.9	7	13.2	15	28.3	6	11.5	38	18.0
Hock										
0	39	73.6	49	92.5	36	67.9	44	84.6	168	79.6
1	5	9.4	2	3.8	9	17.0	4	7.7	20	9.5
2	5	9.4	2	3.8	8	15.1	2	3.8	17	8.1
3	2	3.8	0	0.0	0	0.0	2	3.8	4	1.9
4	2	3.8	0	0.0	0	0.0	0	0.0	2	0.9
Affected	14^{ab}	26.4	4^{c}	7.5	17^{a}	32.1	8^{bc}	15.4	43	20.4
Knee										
0	32	60.4	30	56.6	27	51.9	36	69.2	125	59.5
1	13	24.5	17	32.1	13	25.0	14	26.9	57	27.1
2	1	1.9	1	1.9	4	7.7	0	0.0	6	2.9
3	5	9.4	5	9.4	6	11.5	1	1.9	17	8.1
4	2	3.8	0	0.0	2	3.8	1	1.9	5	2.4
Affected	21	39.6	23	43.4	25	48.1	16	30.8	85	40.5
Animal										
0	21	39.6	23	43.4	11	21.2	29	55.8	84	40.0
1	10	18.9	17	32.1	13	25.0	14	26.9	54	25.7
2	7	13.2	6	11.3	10	19.2	2	3.8	25	11.9
3	9	17.0	6	11.3	10	19.2	4	7.7	29	13.8
4	6	11.3	1	1.9	8	15.4	3	5.8	18	8.6
Affected	32^{ab}	60.4	30^{a}	56.6	41^{b}	78.8	23^{a}	44.2	126	60.0

[1] OC was assessed on a 5 point grading scale were 0 indicates no OC and 4 indicates the severest form of OC.

[2] The locations affected with OC were as follows: in the elbow joint (6 affected locations) the medial humeral condyle, the lateral humeral condyle, and the anconeal process; in the hock joint (5 affected locations) the sagittal ridge of the distal tibia, lateral trochlea of talus, and the medial trochlea of talus; in the knee joint (5 affected locations) the lateral femoral condyle, the medial femoral condyle, and the lateral side of a lateral femoral condyle.

[3] AA = ad libitum feeding from weaning until slaughter; AR = ad libitum feeding up to 10 weeks of age, after which feeding was switched to restricted (80% of ad libitum uptake); RA = restricted feeding up to 10 weeks of age, after which feeding was switched to ad libitum; RR = continuously restricted feeding from weaning until slaughter.

[4] Affected is the total number of locations with an OC score higher than 0. Within a row, the prevalence of OC differs significantly if there is no common superscript ($P < 0.05$).

No significant effect was present for the elbow and knee joint. Significant differences were found between the comparisons of the RA treatment with the RR and AR treatments in the hock joint (OR = 3.3, $P = 0.04$ and OR = 8.5, $P = 0.002$, respectively) and at the animal level (OR = 2.5, $P = 0.001$ and OR = 1.9, $P = 0.01$, respectively). The OR results indicate that gilts in the RA treatment had significantly higher odds of being affected with OC than gilts in both the AR and RR treatments in the hock joint and at the animal level. A significant difference was furthermore found in the comparison between the AA and AR treatments in the hock joint (OR = 5.3, $P = 0.01$). The OR result indicates that gilts in the AA treatment had higher odds to be affected with OC than gilts in the AR treatment in the hock joint.

Last, at the animal level, tendencies ($0.05 < P < 0.1$) were found (data not shown) for the comparisons between the AA and RA treatments (OR = 0.7; $P = 0.09$), and the AA and RR treatments (OR = 1.7; $P = 0.06$). The OR results indicate that gilts in the AA treatment had a tendency to have lower odds of being affected with OC than gilts in the RA treatment but higher odds of being affected with OC compared with gilts in the RR treatment.

2.5 DISCUSSION

The aim of this study was to investigate age-dependent effects of dietary restriction, ad libitum versus restricted (80% of ad libitum uptake), on OC prevalence in breeding gilts from weaning until slaughter. Results indicate that there were age-dependent effects of dietary restriction on OC prevalence in breeding gilts after 10 weeks of age. A possible factor involved in the differences seen in OC prevalence may be differences in BW between treatments.

2.5.1 Body weight

A higher feeding level leads to a higher BW, which may impose higher loading pressures on joints carrying the weight, especially at a young age. Joint loading has previously been suggested to play a role in OC development (Nakano and Aherne, 1988; Carlson et al., 1991; Ytrehus et al., 2004bc). As this factor was known to potentially play a vital role in OC development, it was essential that the applied dietary restrictions provided significant contrasts in BW at a relatively young age. This was the case, as the dietary restrictions applied provided

significant contrasts in BW before and after 10 weeks of age in the expected direction. The results of the BW progression hence indicate that the intended effect of the treatments was attained and point at a possible pathway that could be implicated in the differences found in the OC prevalence between treatments in the current study.

2.5.2 Osteochondrosis Prevalence

Overall, almost 60% of the gilts in the current study showed an OC score higher than 0 in at least one of the joints assessed. Differences in OC prevalence between studies exist and can range from 10% to 80%, depending on the joints assessed (Jørgensen et al., 1995; Jørgensen and Andersen, 2000; Ytrehus et al., 2004c; Luther et al., 2007; Busch and Wachmann, 2011; van Grevenhof et al., 2011). Differences in OC prevalence between studies may arise from differences in susceptibility for OC between breeds and sexes (Lundeheim, 1987; van der Wal et al., 1987). In the current study, the overall prevalence of OC was 60%. The OC prevalence could have been higher if the unfavorable treatment (RA treatment in the current study) had been administered to all gilts. For example, the RA treatment showed an OC prevalence of almost 80% (highest prevalence), whereas in the RR treatment group the OC prevalence was 43% (lowest prevalence).

Osteochondrosis lesions have previously been suggested to show bilateral symmetry, as genetic correlations of OC lesions in pigs between bilateral homologues are reported to approach 1 (Jørgensen and Andersen, 2000). In the current study, correlations of OC lesions between bilateral homologues ranged from 0.38 to 0.85, depending on the location assessed. Van Weeren and Barneveld (1999) have indicated that bilateral symmetry in horses is dependent on the joints assessed, which is similar to the results of the current study in gilts. In addition, van Weeren and Barneveld (1999) also indicated that bilateral symmetry of OC lesions at young age is more prevalent than at an older age. This may indicate that the occurrence of bilateral symmetry of OC lesions is influenced by variation in the effect of environmental factors related to aging of the animal.

2.5.3 Treatment Effects

The treatments applied showed significant effects at the pen level on the prevalence of OC. Overall, the RA-treated gilts showed significantly higher odds

of being affected with OC than the AR-and RR-treated gilts in the hock joint and at the animal level. The AA-treated gilts showed significantly higher odds to be affected with OC in the hock joint compared with the AR-treated gilts. Overall, the order of treatments from high to low OC prevalence in the gilts was RA, AA, AR, and RR.

To the authors' knowledge, no study has yet been performed that directly investigated age-dependent effects of dietary restriction on OC prevalence. In a previous experiment (van Grevenhof et al., 2011), dietary restrictions (ad libitum versus restricted) were applied to pigs that were similar to the AA and AR treatments in the current study, resulting in a significantly higher prevalence of OC for the ad libitum-fed pigs. In the current study, differences between the AA and AR treatments were not significant except in the hock joint. Carlson et al. (1988) also showed an increased incidence of OC in pigs fed ad libitum from weaning to slaughter. However, Jørgensen (1995) was not able to show effects of dietary restriction on OC prevalence in pigs. In that study, restrictions were applied from 25 kg BW onward, which is close to 10 weeks of age (the switching point in the current study). That study does not indicate what type of feeding was applied before 25 kg BW. If ad libitum feeding was applied before 25 kg BW, the absence of significant differences between the ad libitum and restricted feeding groups after 25 kg BW would be in line with the results from our study. There are indications that the age interval from 7 to 13 weeks of age is important for the development of OC in pigs (Ytrehus et al., 2004ab). It is clear that if different dietary restrictions are applied at an early age (well before 10 weeks of age), the contrasts in BW will be more pronounced during the time window of OC susceptibility. Possibly, if contrasts in dietary restrictions are applied at an age after the most sensitive period, effects of dietary restriction on BW may not be as pronounced and consequently have less impact on OC development.

2.5.4 Possible Mechanism of Treatment Effects

Joint loading has been implied in the development of OC (Nakano and Aherne, 1988; Carlson et al., 1991; Ytrehus et al., 2004bc). An explanation for the treatment effects in the current study may indeed be the difference in joint loading applied due to differences in BW. The development of growth cartilage within joints is most likely responsive to the demands that the environment (BW in the

current study) places on the joints. An important component of the extracellular matrix of growth cartilage involves proteoglycans, which are produced by chondrocytes and which provide compressive strength to the tissue (reviewed by Greenwald et al., 1978; Kheir and Shaw, 2009). Studies have indicated that (dynamic) loading of articular cartilages increases proteoglycan contents (Buschmann et al., 1995; Little and Ghosh, 1997; Quinn et al., 1999; Spiteri et al., 2010). In addition, there are indications that proliferation of chondrocytes, which has been suggested to be an aspect involved in reparative responses of OC (Ytrehus et al., 2004bd; and reviewed by Ytrehus et al., 2007), can be reduced by mechanical compression of chondrocytes (Alberty et al., 1993; Stokes et al., 2007). Thus, loading of joints may lead to a higher proteoglycan content of the growth cartilage, which can be seen as beneficial. However, when the loading passes a certain threshold or perhaps increases too rapidly, the growth cartilage may not be able to adapt quickly enough, leading to compression of chondrocytes. These loading stresses on the joint can be detrimental to chondrocyte function (Ytrehus et al., 2004d).

In the RA treatment, feeding increased from restricted to ad libitum. After the switch, the BW of those gilts increased rapidly and relatively quickly differed significantly from the continuously restricted fed gilts. This increase in BW may result in a sudden increase of joint loading and surpass the adaptive capacity of the growth cartilage and chondrocytes that were developed under restricted feeding. In the AR treatment, the feeding, in contrast, decreased from ad libitum to restricted. Those joints were likely adapted to cope with higher loads at young age and were subject to relatively lower loads after the switch in feeding regime as compared with ad libitum-fed gilts. Those joints are hence under relatively less strain as to what they had adapted to and may therefore develop less OC. The AA treated gilts have a gradual increase in BW and loading pattern. The growth cartilage develops under ad libitum conditions at young age to cope with the ad libitum conditions after 10 weeks of age; the growth cartilage therefore experiences similarly high loading pressures throughout development. This continuity is likely the crucial difference between the AA and RA treatments, with the RA treatment initiating the development of cartilage before 10 weeks of age that is not able to withstand the sudden increase of loading due to ad libitum feeding after 10 weeks of age. The group of gilts in which the lowest number of affected gilts were found was the RR

treatment. These gilts continuously experience less strain placed on the joints as compared with ad libitum-fed gilts and could therefore be less likely to develop OC.

2.6 CONCLUSION

This study showed the existence of an influence of age on the effects of dietary restriction on OC prevalence. The results show that gilts fed restricted up to 10 weeks of age and then switched to ad libitum feeding (RA treatment) have the highest prevalence of OC affected gilts. The treatments with the lowest OC prevalence were the continuously feed-restricted gilts (RR treatment) and switched to restricted feeding after 10 weeks of age (AR treatment). If the dietary restriction that the animals are subjected to at young age do not match the dietary restriction at later age, the adaptive capacity of the joints may be surpassed. In practice, OC prevalence can be lowered by continuously restricted feeding or by switching to restricted feeding at 10 weeks of age.

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CHAPTER 3

The influence of floor type before and after 10 weeks of age on osteochondrosis in growing gilts[1]

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3.1 ABSTRACT

Osteochondrosis (OC) is a degenerative joint condition developing in a short time frame in young growing gilts that may cause lameness at an older age, affecting welfare and leading to premature culling of breeding sows. Causes of OC are multifactorial including both genetic and environmental factors. Floor type has been suggested to affect OC prevalence and effects might be age-dependent during the rearing period. The aim of this study was to investigate possible age-dependent effects of floor type, conventional concrete partially slatted versus wood shavings as deep bedding, on OC prevalence in gilts (Dutch Large White x Dutch Landrace) at slaughter (24 weeks of age; 106.5 [14.7 SD] kg of BW). At weaning (4 weeks of age; 6.9 [1.3 SD] kg of BW), 212 gilts were subjected to 1 of 4 flooring regimens. Gilts were either subjected to a conventional floor from weaning until slaughter (CC); wood shavings as bedding from weaning until slaughter (WW); a conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding (CW); or wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a conventional floor (WC). After slaughter the elbow, hock, and knee joints were macroscopically examined for OC and scored on a 5-point scale where 0 indicates no OC and 4 indicates the severest form of OC. There was no significant difference ($P > 0.4$) between treatments on the overall OC prevalence for any joint assessed or at the animal level (all joints combined). At the animal level, however, gilts had greater odds to have OC scores 3 and 4 in the CW treatment (OR = 2.3, $P = 0.05$), WC treatment (OR = 2.6, $P = 0.02$), and WW treatment (OR = 3.7, $P < 0.001$) compared with gilts in the CC treatment. The results indicate that there are no age-dependent effects of floor types on overall OC prevalence. However, wood shavings as bedding seems to increase the odds for severe OC and might affect animal welfare in the long term.

Keywords: age, floor type, bedding, gilts, osteochondrosis

3.2 INTRODUCTION

Osteochondrosis **(OC)** involves formation of necrotic growth cartilage in young gilts, which have been associated with leg weakness (Jørgensen and Andersen, 2000; de Koning et al., 2012) and premature culling (Yazdi et al., 2000). Prevalence of OC has been reported to range up to 80% (Busch and Wachmann, 2011; van Grevenhof et al., 2011; de Koning et al., 2013) and culling due to leg weakness can range up to 30% (Stein et al., 1990; Jørgensen and Sørensen, 1998). Breeding gilts last several parities and therefore it is important to reduce OC prevalence to avoid negative effects on welfare and longevity.

Floor types influence joint loading or the risk of traumatic events (such as slipping), which have been suggested to be associated with OC (Nakano and Aherne, 1988; Carlson et al., 1991; Ytrehus et al., 2004bc). A conventional partially slatted floor compared with deep litter has been reported to increase OC prevalence in fattening pigs (van Grevenhof et al., 2011), whereas others could not find an effect of floor type on OC prevalence (Jørgensen, 2003; Scott et al., 2006). Inconsistency in the effect of floor type on OC might be related with age dependency of OC development. Indications exist that a sensitive time period for porcine OC development lies around 10 weeks of age (Ytrehus et al., 2004ab), which hence might imply age-dependent effects of floor types on OC prevalence as found earlier for dietary restriction (de Koning et al., 2013).

The aim of the study was to investigate age-dependent effects of floor type (conventionally partially slatted versus wood shavings) on OC prevalence in breeding gilts at 24 weeks of age, taking into account the abovementioned period of sensitivity for OC development. We hypothesized that a conventional floor increases OC prevalence as it is harder and more slippery. If floor type affects OC prevalence, it could be an environmental factor that might help in reducing OC prevalence in breeding gilts.

3.3 MATERIALS AND METHODS

3.3.1 Ethical Note

Osteochondrosis can cause joint surface irregularities possibly resulting in lameness that might affect welfare of the gilts. Assessments to detect serious impairments of welfare in the gilts were therefore performed daily. Severely lame

or wounded gilts were taken out of the experiment and euthanized. The experiment and all measurements were approved by the Animal Welfare Committee of Wageningen University and Research center in compliance with Dutch Law on Animal Experimentation.

3.3.2 Animals

The experiment was performed using 212 Topigs 20 (Dutch Large White x Dutch Landrace) gilts acquired from a commercial breeding company (TOPIGS, Veldhuizen Wehl, Wehl, The Netherlands). The Topigs 20 line represents a large part of the commercially kept sows in The Netherlands for the production of fattening pigs and previously it was found that OC prevalence within this line of animals ranges up to 60% (de Koning et al., 2013). The gilts were weaned at 27 (2.4 SD) days of age (6.9 [1.3 SD] kg of body weight), after which they entered the experiment. Gilts had continuous access to various enrichment items (such as biting chains, burlap sacks, solid plastic balls, rubber mats), which were changed every 2 to 3 days. Gilts were given ad libitum access to water using drinking bowls and feed using a dry feeder unit.

3.3.3 Treatments

Gilts were assigned 1 of 4 treatments and divided to 32 pens (8.37 m^2 surface area per pen) of 6 to 7 individuals. Assignment to treatments was based on an equal distribution over pens of littermates and body weight that was measured at 19 days of age. Pens were distributed over 4 departments with an equal distribution of treatments per department. Gilts were housed either on a conventional floor consisting of 60% slatted floor (twisted metal bars) and 40% solid floor (epoxy-coated concrete) or on 25 to 50 cm deep bedding of wood shavings (approximately 3 to 5 cm large shavings). Wood shavings were gradually increased to approximately 50 cm bedding during the experiment. Wood shavings were distributed on the same floor type as the conventional floor except that the slatted area was covered with a 0.5 cm thick rubber mat so that wood shavings could not accumulate in the manure pan. The manure areas were removed as much as possible 3 to 4 times a week (approximately 10 to 15 minutes per pen) while gilts remained in their pens. After removal of manure, wood shavings were added to the existing layer of wood shavings to retain a full bedding of wood shavings. Daily

inspections by the animal caretakers ensured that a full bedding was consistently available. Treatments consisted of 4 flooring regimens administered to pens: a conventional floor from weaning until slaughter **(CC)**; wood shavings as bedding from weaning until slaughter **(WW)**; a conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding **(CW)**; wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a conventional floor **(WC)**. The switch in floor type was performed at 10 weeks of age (at an average of 74 days of age) so that the piglets received different floor types during the possible sensitive period for porcine OC development between 7 and 13 weeks of age (Ytrehus et al., 2004ab), during which OC can still be influenced.

Gilts received 4 successive diets according to their age and feed uptake. After weaning gilts received a pelleted weaning diet (11.0 MJ NE/kg; 170 g/kg CP; 12.5 g/kg ileal digestible lysine; 7.2 g/kg calcium; 5.5 g/kg phosphorus). Gilts were switched at 40 days of age to a standard pelleted grower diet (9.86 MJ NE/kg; 165 g/kg CP; 10 g/kg ileal digestible lysine; 5.3 g/kg calcium; 5.3 g/kg phosphorus); at 70 days of age to a standard pelleted rearing diet 1 (9.40 MJ NE/kg; 157 g/kg CP; 8.4 g/kg ileal digestible lysine; 8.5 g/kg calcium; 5.5 g/kg phosphorus); at 104 days of age to a standard pelleted rearing diet 2 (9.24 MJ NE/kg; 140 g/kg CP; 6.9 g/kg ileal digestible lysine; 6.9 g/kg calcium; 5.1 g/kg phosphorus).

3.3.4 Behavioral Assessment

As an indication of behavior performed which could influence results, behavioral activity was assessed at 8 weeks of age, 10 weeks of age (2 and 3 days after the switch in treatments), and at 17 weeks of age. Within each week, each pen was observed 4 times for 10 minutes continuously within 4 days. Observations were carried out from 0800 to 1140 h in the morning and 1400 to 1740 h in the afternoon. The observation sessions were equally distributed for pens and treatments over days. Behavioral sampling was performed, focusing on the behaviors as described in Table 3.1. Within each 10 minute observation session per pen, the frequency of a behavior performed per gilt within the pen were counted. Because of low frequencies of the behaviors observed, behaviors were combined per gilt in the categories play behavior, aggressive behavior, and a category activity behavior. Play behavior consisted of the behaviors scamper, turn, flop, rolling,

Table 3.1. Ethogram (taken with permission from Bolhuis et al., 2006)

Behavior	Description
Aggressive behavior	
Chasing	Actively pursuing another pig
Fighting	Mutual pushing or ramming, or lifting pen mate
Headbutting	Ramming or pushing pen mate with head, without biting
Play behavior	
Scamper	A sequence of at least two forward hops in rapid succession
Turn	Rapid turning around the body axis on the spot
Flop	A rapid drop from an upright position to sternal or lateral recumbence
Rolling	Lying on back and moving from side to side on the floor
Pushing	Pushing the opponent with the head or shoulder
Gamboling	Running across the pen, occasionally accompanied by nudging pen mate gently

pushing, and gamboling. Aggressive behavior consisted of the behaviors chasing, fighting, and headbutting. Activity behavior consisted of the summation of play and aggressive behavior. Frequencies of behaviors were then summed per week as an indication of the number of behaviors performed within a week.

3.3.5 Osteochondrosis Assessment

Osteochondrosis assessment was performed after slaughter of the gilts at a local slaughterhouse. Gilts were slaughtered over a 1-week period. One-half of the population with an equal distribution of treatments and pens was slaughtered within 2 days at an average of 166 days of age with an average body weight of 105.6 (10.3 SD) kg. The other one-half of the population was slaughtered within 2 days at an average of 172 days of age with an average body weight of 110.4 (10.1 SD) kg. Carcasses were stored for 1 day at 4 °C after which the legs from each gilt were collected by dissection through the shoulder and hip joints. The legs were then stored again at 4 °C for a maximum of 2 days until dissection of the elbow, knee, and hock joints. Dissection of joints was performed within 1 day of the same slaughter week and occurred at a random order of gilts. After dissection of the joints, the joint surface was macroscopically assessed for presence and severity of OC using a 5-point grading scale from 0 to 4 (as described by van Weeren and Barneveld, 1999). The OC score 0 indicated no abnormalities, OC score 1 indicated flattening of the cartilage, OC score 2 indicated slight irregularities of the cartilage, OC score 3 indicated severe irregular cartilage, and OC score 4 indicated the severest form of OC with (partially) loose articular cartilage fragments and/or

osteochondral cysts [for the locations assessed see de Koning et al. (2013); for figures depicting OC lesions see van Grevenhof et al., (2011)]. Osteochondrosis was scored by 1 veterinarian specialized in orthopedics, experienced in judging OC, and unaware of the treatments.

3.3.6 Statistics

Behavioral Statistical Model. As the behaviors are frequencies and did not follow a normal distribution, the data was analyzed with a more appropriate distribution using a Poisson distribution with the default log link using PROC GLIMMIX in SAS 9.2 (SAS Inst. Inc., Cary, NC). The statistical model used to assess treatment effects on behavior is as follows:

$$Y_{ijkl} = \mu + Treat_i + Dept_j + Pen_{k(ij)} + \varepsilon_{ijkl},$$

in which Y_{ijk} represents the ijklth observation on behavior with a log link. The mean is represented by μ. $Treat_i$ represents the fixed class effect of treatments administered (i = CC, CW, WC, WW). $Dept_j$ represents the fixed class effect of department in which gilts were housed (j = department 1, 2, 3, 4) and is used to account for environmental variation. $Pen_{k(i)}$ represents the random effect of the experimental unit pen (k = pen 1, 2, …, 32) nested within treatment and department, and is used to assess treatment effects on pen level. The random residual term from a Poisson distribution is represented by ε_{ijkl}. Results are displayed as the estimated least squares (**LS**) means and the SE on the log scale.

Osteochondrosis Affected Locations. The locations within joints on which no OC was found for all gilts were omitted from the analyses as these locations are uninformative. Bilateral symmetry of OC between joints of the bilateral homologues is indicated using Spearman rank correlations on the original OC scores using PROC CORR in SAS 9.2.

Grouping of Osteochondrosis Scores. Osteochondrosis was scored on an ordinal scale of 0 to 4. For each joint separately (joint level), the sufficient required number of observations of each combination of treatment and OC score could not be attained for application of ordinal logistic regression (Stokes et al., 2000) but was attained at the animal level (all joints combined). For the joint level, OC scores were grouped as a 0 and 1 variable to accommodate binary logistic regression,

where 0 indicates no abnormalities (OC score 0) and 1 indicates an OC score greater than 0. This procedure was also performed at the animal level. Additionally at the animal level, ordinal logistic regression was possible when grouping the OC scores to attain a sufficient number of observations between OC scores and treatments. For ordinal logistic regression, OC scores at the animal level were grouped into a variable with 3 categories: no OC (OC score 0), mild OC (OC scores 1 and 2), and severe OC (OC scores 3 and 4). To accommodate a repeated measurement analysis, the ordinal logistic regression method was approximated using a binary distribution and modeling the cumulative logits of ordinal logistic regression [for further details about this procedure and SAS code see Stokes et al. (2000) p. 538].

Binary Logistic Regression. The OC scores were analyzed on pen level, which is the experimental unit. Osteochondrosis in joints was scored on the bilateral homologues and therefore observations within a gilt within a pen are not independent. A repeated measurement analysis was performed using PROC GLIMMIX in SAS 9.2. The statistical model used to assess treatment effects on OC prevalence is as follows:

$$\text{Logit } P(y_{ijklm} = 1) = \alpha + \text{Treat}_i + \text{Dept}_j + Pen_{k(ij)} + Gilt(Dam)_l + \varepsilon_{ijklm},$$

in which the model estimates the probability (P) that the ijklmth observation of OC (y) for a joint or at the animal level is 1. Logit is the link function used to model the mixed linear regression analysis. The α component represents the estimate of the log odds of being affected with OC disregarding the independent variables [baseline odds (Kleinbaum and Klein, 2010)]. Treat_i represents the fixed class effect of treatments administered (i = CC, CW, WC, WW). Dept_j represents the fixed class effect of department in which gilts were housed (j = department 1, 2, 3, 4) and is used to account for environmental variation. $Pen_{k(ij)}$ represents the random effect of the experimental unit pen (k = pen 1, 2, …, 32) nested within treatment and department, and is used to assess treatment effects on pen level. $Gilt(Dam)_l$ represents the random term of gilts nested within dams from which the gilts originated. A repeated measurement analysis was performed on the binary OC scores from the different locations assessed for OC with gilts as the subject. The random residual term from a binary distribution is represented by ε_{ijklm}. Addition of

other variables such as slaughter weight, slaughter age, or birth weight did not significantly ($P > 0.1$) influence treatment effects on OC prevalence.

Ordinal Logistic Regression. The OC scores were analyzed on pen level, which is the experimental unit. As with the binary logistic regression model, a repeated measurement analysis was performed using PROC GLIMMIX in SAS 9.2. The statistical model used to assess treatment effects on OC prevalence is as follows:

$$\text{Logit } P(y_{ijklmn} = 1) = \alpha + \text{Treat}_i + \text{Dept}_j + \text{Logtype}_k + (\text{Treat*Logtype})_{ik} + Pen_{l(ij)} + Gilt(Dam)_m + \varepsilon_{ijklmn}.$$

The terms in the ordinal logistic regression model remain similar as to the binary logistic regression model except for the addition of the cumulative logits modelled with ordinal logistic regression ($\text{Logtype}_k = 1, 2$). In addition, an interaction is present between the cumulative logits and treatments [$(\text{Treat*Logtype})_{ik}$]. This interaction allows the assessment of a difference between the prevalence of OC categories for the different treatments. Addition of other random variables such as slaughter weight, slaughter age, or birth weight did not significantly ($P > 0.1$) influence treatment effects on OC prevalence.

The prevalence of OC is presented on the ordinal scale and as the total number of OC affected joint locations and gilts. When significant treatment effects are present, odds ratios (**OR**) are presented to indicate effect size.

3.4 RESULTS

Eleven out of the 212 gilts were removed before the end of the experiment due to health or welfare problems (3 gilts from the CC group, 3 gilts form the CW group, 3 gilts from the WC group, 2 gilt from the WW group). The joints were assessed for OC status where possible after these animals were removed. Removals could not be attributed to treatment effects and were relatively equally distributed among treatments.

3.4.1 Behavior

The estimated LS means from the statistical model concerning behaviors performed at 8, 10, and 17 weeks of age are displayed in Table 3.2. No significant

effects were present for aggressive behavior and activity behavior at all ages ($P > 0.1$). A significant difference was found for play behavior at 10 weeks of age ($P = 0.03$) and a tendency for a difference at 17 weeks of age ($P = 0.08$). At 10 weeks of age, the gilts on wood shavings (CW and WW treatments) showed numerically more play behavior compared with gilts on conventional flooring (CC and WC treatments). However, the significant differences were only found for the CW treatment compared with the CC and WC treatments, and for the WC treatment compared with the CW and WW treatments. At 17 weeks of age, the gilts on wood shavings again displayed numerically more play behavior compared with gilts on conventional flooring.

Table 3.2. Estimated LS means (SE)[1] for aggressive behavior, play behavior, and activity behavior[2] at 8, 10, and 17 weeks (wk) of age per treatment[3].

	Treatments			
	CC	CW	WC	WW
Aggressive behavior				
8 wk	0.96 (0.16)	0.55 (0.18)	1.07 (0.15)	0.68 (0.17)
10 wk	0.66 (0.20)	0.19 (0.23)	0.67 (0.20)	0.55 (0.20)
17 wk	0.77 (0.19)	0.52 (0.21)	0.66 (0.20)	0.65 (0.20)
Play behavior				
8 wk	1.80 (0.34)	1.18 (0.36)	2.12 (0.33)	1.64 (0.34)
10 wk	0.61 (0.35)^{ac}	1.60 (0.31)^{b}	0.04 (0.40)^{c}	1.09 (0.33)^{ab}
17 wk	-1.26 (0.47)^{ac}	-0.35 (0.38)^{bc}	-1.47 (0.51)^{a}	-0.05 (0.37)^{b}
Activity behavior				
8 wk	2.24 (0.24)	1.70 (0.26)	2.44 (0.24)	2.01 (0.25)
10 wk	1.40 (0.23)	1.87 (0.21)	1.18 (0.24)	1.69 (0.21)
17 wk	0.90 (0.17)	0.89 (0.17)	0.82 (0.17)	1.23 (0.14)

[a-c] Within a row, the frequency of behavior performed differs significantly between treatments if there is no common superscript at 10 weeks of age ($P < 0.05$) and tended to differ between treatments at 17 weeks of age ($P \leq 0.08$).

[1] Estimated least squares means (SE) from the Poisson model on a natural log scale.

[2] Activity behavior is the summation of aggressive behavior and play behavior.

[3] CC = conventional floor from weaning until slaughter (51 gilts); CW = conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding (50 gilts); WC = wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a conventional floor (51 gilts); WW = wood shavings as bedding from weaning until slaughter (52 gilts).

3.4.2 Osteochondrosis Affected Locations

Locations affected by OC in the elbow joint (total of 3 locations) were the medial humeral condyle (bilateral homologues) and the lateral humeral condyle (only in the left elbow joint); in the hock joint (total of 4 locations) were the lateral trochlea of the talus (bilateral homologues) and the medial trochlea of the talus (bilateral homologues); in the knee joint (total of 2 locations) was the medial femoral condyle (bilateral homologues).

In the elbow joint the prevalence of OC scores greater than 0 was low (Table 3.3) and lesions were equally divided between the medial and lateral humeral condyles (data not shown). In the hock joint, the majority of OC scores greater than 0 were found on the medial trochlea of the talus (93% of the total prevalence of OC scores greater than 0) of which 33% were scored as a 3 or a 4 (data not shown). Significant correlations ($P < 0.001$ for all correlations) between bilateral homologues were found for the medial femoral condyle (correlation of 0.39), the lateral trochlea of the talus (correlation of 0.59), and the medial trochlea of the talus (correlation of 0.52).

3.4.3 Osteochondrosis Prevalence

Descriptive results of the prevalence of the locations affected with OC within a joint and at the animal level are presented in Table 3.3. The hock joint had the greatest prevalence of OC affected locations and the greatest prevalence of OC scores 3 and 4. The elbow joint had the least prevalence of OC affected locations. The percentage of OC scores 3 and 4 (more severe OC) of the overall prevalence of OC scores greater than 0 was for the elbow joint 82%, for the hock joint 31%, for the knee joint 57%, and at the animal level 40%.

The number of locations affected with OC does not necessarily represent the number of gilts affected with OC, as a gilt may have bilateral symmetry of OC lesions at the joint level or have several joints affected with OC at the animal level. Descriptive results of the prevalence of gilts affected with their greatest OC score is displayed in Table 3.4. For example, if a gilt has OC scores on 2 joint locations with an OC score 1 and an OC score 4, then the greatest OC score is 4 and that animal is counted within the OC score 4 category of Table 3.4. Again, the hock joint showed the greatest number of gilts affected with OC and the elbow joint showed the least number of gilts affected with OC. The percentage of gilts with OC

Table 3.3. Prevalence (n and %) of osteochondrosis (OC)[1] on the locations within the elbow joint, hock joint, knee joint, and at the animal level (all joints combined) assessed per treatment[2] and overall prevalence.

	CC		CW		WC		WW		Overall	
	n	%	n	%	n	%	n	%	n	%
Elbow										
0	151	98.7	147	98.0	149	97.4	154	98.7	601	98.2
1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2	0	0.0	0	0.0	2	1.3	0	0.0	2	0.3
3	1	0.7	2	1.3	1	0.7	0	0.0	4	0.7
4	1	0.7	1	0.7	1	0.7	2	1.3	5	0.8
Affected[3]	2	1.3	3	2.0	4	2.6	2	1.3	11	1.8
Hock										
0	165	80.9	169	84.5	164	80.4	180	86.5	678	83.1
1	2	1.0	2	1.0	5	2.5	4	1.9	13	1.6
2	34	16.7	18	9.0	21	10.3	9	4.3	82	10.1
3	3	1.5	8	4.0	7	3.4	11	5.3	29	3.6
4	0	0.0	3	1.5	7	3.4	4	1.9	14	1.7
Affected	39	19.1	31	15.5	40	19.6	28	13.5	138	16.9
Knee										
0	93	91.2	91	91.0	91	89.2	87	83.7	362	88.7
1	6	5.9	5	5.0	6	5.9	3	2.9	20	4.9
2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
3	2	2.0	2	2.0	3	2.9	8	7.7	15	3.7
4	1	1.0	2	2.0	2	2.0	6	5.8	11	2.7
Affected	9	8.8	9	9.0	11	10.8	14	13.9	46	11.3
Animal										
0	409	89.1	407	90.4	404	88.0	421	90.0	1641	89.4
1	8	1.7	7	1.6	11	2.4	7	1.5	33	1.8
2	34	7.4	18	4.0	23	5.0	9	1.9	84	4.6
3	6	1.3	12	2.7	11	2.4	19	4.1	48	2.6
4	2	0.4	6	1.3	10	2.2	12	2.6	30	1.6
Affected	50	10.9	43	9.6	55	12.0	47	10.0	195	10.6

[1] Osteochondrosis was assessed on a 5-point scale where 0 indicates no OC and 4 indicates the severest form of OC for all the joint locations (n and % of total n) assessed.

[2] CC = conventional floor from weaning until slaughter (51 gilts); CW = conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding (50 gilts); WC = wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a conventional floor (51 gilts); WW = wood shavings as bedding from weaning until slaughter (52 gilts).

[3] Affected is the total number of locations with an OC score greater than 0.

Table 3.4. Prevalence (n and %) of gilts of the total number of gilts within each treatment with a greatest osteochondrosis (OC) score[1] in the elbow joint, hock joint, knee joint, and at the animal level (all joints combined) assessed per treatment[2] and overall prevalence.

	CC		CW		WC		WW		Overall	
	n	%	n	%	n	%	n	%	n	%
Elbow										
0	49	96.1	47	94.0	47	92.2	50	96.2	193	94.6
1	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
2	0	0.0	0	0.0	2	3.9	0	0.0	2	1.0
3	1	2.0	2	4.0	1	2.0	0	0.0	4	2.0
4	1	2.0	1	2.0	1	2.0	2	3.8	5	2.5
Affected[3]	2	3.9	3	6.0	4	7.8	2	3.8	11	5.4
Hock										
0	28	54.9	27	54.0	24	47.1	35	67.3	114	55.9
1	0	0.0	1	2.0	3	5.9	3	5.8	7	3.4
2	20	39.2	13	26.0	14	27.5	4	7.7	51	25.0
3	3	5.9	7	14.0	4	7.8	6	11.5	20	9.8
4	0	0.0	2	4.0	6	11.8	4	7.7	12	5.9
Affected	23	45.1	23	46.0	27	52.9	17	32.7	90	44.1
Knee										
0	43	84.3	43	86.0	41	80.4	41	78.8	168	82.4
1	5	9.8	3	6.0	6	11.8	3	5.8	17	8.3
2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
3	2	3.9	2	4.0	3	5.9	4	7.7	11	5.4
4	1	2.0	2	4.0	1	2.0	4	7.7	8	3.9
Affected	8	15.7	7	14.0	10	19.6	11	21.2	36	17.6
Animal										
0	22	43.1	20	40.0	18	35.3	25	48.1	85	41.7
1	3	5.9	4	8.0	6	11.8	5	9.6	18	8.8
2	18	35.3	11	22.0	13	25.5	2	3.8	44	21.6
3	6	11.8	10	20.0	8	15.7	10	19.2	34	16.7
4	2	3.9	5	10.0	6	11.8	10	19.2	23	11.3
Affected	29	56.9	30	60.0	33	64.7	27	51.9	119	58.3

[a,b] Significant differences ($P < 0.05$) between treatments were present for the severe OC lesions (OC score 3 and 4) at the animal level. Within a row, the prevalence of OC differs significantly if there is no common superscript ($P < 0.05$).

[1] OC was assessed on a 5 point scale where 0 indicates no OC and 4 indicates the severest form of OC.

[2] CC = conventional floor from weaning until slaughter (n = 51); CW = conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding (n = 50); WC = wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a conventional floor (n = 51); WW = wood shavings as bedding from weaning until slaughter (n = 52).

[3] Affected is the total number of gilts with an OC score greater than 0.

scores 3 and 4 of the overall prevalence of gilts with OC scores greater than 0 was for the elbow joint 82%, for the hock joint 36%, for the knee joint 53%, and at the animal level 48%.

3.4.4 Treatment Effects

Descriptive results show that the prevalence of OC was greatest in the WC treatment except for the knee joint (Table 3.4). In the knee joint, OC prevalence was numerically greatest for the gilts in the WW treatment. The treatment or treatments with numerically the least number of gilts affected with OC in the elbow joint were the CC and WW treatments; in the hock joint the WW treatment; in the knee joint the CW treatment; and at the animal level the WW treatment. The WW treatment, therefore, seems to numerically be the treatment with the least number of gilts affected with OC. However, the WW treatment contained the greatest number of gilts affected with an OC score 3 and 4 at the animal level.

When OC was analyzed as a binary variable with binary logistic regression, no significant differences ($P > 0.4$) were present between treatments for the different joints or at the animal level. For the ordinal logistic regression model performed for the animal level, a significant effect was found for the interaction of logtype with treatment ($P < 0.001$). The interaction effect indicated that there was a greater odds to be affected within the category severe OC (OC scores 3 and 4) for gilts in the CW treatment (OR = 2.3, $P = 0.05$), WC treatment (OR = 2.6, $P = 0.02$), and WW treatment (OR = 3.7, $P < 0.001$) compared with gilts in the CC treatment.

3.5 DISCUSSION

The aim of this study was to investigate age-dependent effects of floor type, conventional partially slatted versus wood shavings (25 to 50 cm of bedding), during the rearing period in breeding gilts on the prevalence of OC at 24 weeks of age. Floor type might affect OC prevalence and increase animal welfare. This would be in compliance with the EU directive 2008/120/EC stating that pig husbandry requires environmental enrichment of which bedding on floors would be a good candidate as supported by studies (Spoolder et al., 2000; Bolhuis et al., 2006; Averos et al., 2010). Results indicate that there does not seem to be a clear age-dependent effect of floor type on OC prevalence. However, the prevalence of

severe OC lesions seems to be affected by floor type.

3.5.1 Osteochondrosis Prevalence

The overall prevalence of gilts affected with OC in the elbow and knee joint was relatively low (approximately 5% and 18%, respectively). In a previous study which assessed the age-dependent effects of dietary restriction on OC prevalence in the same joint locations in the same genetic crossbred line of gilts (de Koning et al., 2013), overall OC prevalence in the elbow and knee joints were greater (approximately 18% and 41%, respectively). The overall prevalence of gilts affected with OC in the hock joint, in contrast, was greater in the current study (approximately 44%) compared with our previous study (approximately 20%). Similarly, the overall number of severe OC lesions (OC score 3 and 4) in the hock joint in the current study (16%) was greater than in our previous study (3%). However, the number of severe OC lesions in the hock joint is roughly equal between the CC treatment (approximately 6%) of the current study and the comparable continuously fed ad libitum treatment from our previous study (approximately 8%). The difference in OC prevalence is likely influenced by the difference in treatments imposed in this study and the previous study. However, the prevalence of OC is known to vary between studies and seems to have a large range depending on the joint locations assessed. The prevalence of any form of OC (joint deformities indicative of OC) in any of the joints assessed might range up to 80% (Lundeheim, 1987; Jørgensen, 1995, 2000; Jørgensen et al., 1995; Stern et al., 1995; Jørgensen and Andersen, 2000; Kadarmideen et al., 2004; Ytrehus et al., 2004c; Jørgensen and Nielsen, 2005; Luther et al., 2007; Busch and Wachmann, 2011; van Grevenhof et al., 2011). Reasons for this large range in OC prevalence between studies are likely various and might include factors such as breed and sex effects (Lundeheim, 1987; van der Wal et al., 1987), genetic predisposition towards OC development (Ytrehus et al., 2004c), dietary restriction or type of housing applied (van Grevenhof et al., 2011; de Koning et al., 2013).

Phenotypic bilateral symmetry of OC lesions as indicated by Spearman rank correlations was significant but low to moderate, ranging between 0.3 and 0.6. This is in concordance with a previous study performed by our group showing correlation coefficients in a similar range (de Koning et al., 2013). These results indicate that some phenotypic symmetry of OC lesions is to be expected but is not

rigorously present and the joints from both sides are needed for proper OC assessment of an animal.

3.5.2 Treatment Effects

The different floor types used did not result in a significant difference in overall OC prevalence, nor were there indications of significant age-dependent differences in OC prevalence. Concerning overall OC prevalence, the WC and WW treatments had numerically the greatest and least numbers, respectively, of OC affected gilts in the elbow joint, hock joint, and at the animal level. Concerning only severe lesions (OC score 3 and 4), gilts in the CC treatment had numerically consistently the least number of severe OC lesions, whereas gilts in WW treatment had the greatest number of gilts affected with severe OC lesions in the hock joint, knee joint, and at the animal level. Significant effects were only present at the animal level, where the CC treatment contained significantly the least number of gilts affected with severe OC compared with the other treatments. There was no clear pattern of possible age-dependent effects of floor types. However, it seems that if gilts experience wood shavings as bedding either before or after 10 weeks of age or both, the number of severe OC lesions increase compared with gilts kept continuously on a conventional concrete partially slatted floor. Previously, age-dependent effects of dietary restriction on OC prevalence were determined and a significant effect of the conditions that gilts experienced before and after 10 weeks of age on OC prevalence was found (de Koning et al., 2013). This is apparently not the case with floor types. However, in the current experiment, all gilts were fed ad libitum, which may have reduced contrasts between treatments.

To the knowledge of the authors, no study has been performed to determine whether the effect of floor type on OC prevalence is age-dependent. In contrast to our results, van Grevenhof et al. (2011) showed that pigs housed on a conventional floor were more affected with OC than pigs housed on wood shavings as bedding. In agreement with our study, they also found that pigs housed on wood shavings as bedding had a greater prevalence of severe OC lesions. In addition, the difference of floor type found by van Grevenhof et al. (2011) was less present in pigs with restricted access to feed. This may indicate that effects of floor type can be influenced by the dietary restriction applied to pigs and may indicate that dietary restriction is a more important factor in reducing OC prevalence than floor type.

Other studies were unable to report significant effects of floor types on OC prevalence (Jørgensen, 2003; Scott et al., 2006), which is in agreement to our results for overall OC prevalence.

3.5.3 Possible Mechanism of Treatment Effects

The results of this study did not support our hypothesis that a conventional floor would result in a greater prevalence of OC. Loading of joints and trauma to joints has been suggested to be associated with OC (Nakano and Aherne, 1988; Carlson et al., 1991; Ytrehus et al., 2004bc). Impact loading on joints in normal motion would presumably be larger on concrete (hard) floors compared with housing systems with wood shavings as bedding. Wood shavings as bedding would likely absorb more of the impact loading and thereby reduces the risk to develop OC. In addition, the conventional floor would be more slippery compared with wood shavings as bedding. Conventional floors would, therefore, likely lead to more trauma to joints due to slipping of gilts and thereby increase the odds to develop OC. However, this study showed that gilts experiencing wood shavings as bedding before or after 10 weeks of age or both have greater odds to be affected with severe OC than gilts housed continuously on a conventional floor. A possible speculative explanation to this might lie in the behavioral activity of the animals. In the current study, gilts on wood shavings showed more play behavior after 10 weeks of age compared with gilts on conventional flooring. However, this was not reflected by the variable activity behavior, which was a summation of aggressive behavior and play behavior. Likely, the aggressive behavior affected the total frequency of activity behavior masking the effects of the frequency of play behavior. Other studies indicate that pigs housed on straw bedding or deep litter systems (somewhat comparable to wood shavings as bedding) are more active (Morgan et al., 1998; Lay Jr. et al., 2000; Bolhuis et al., 2005, 2006; Scott et al., 2006; van Grevenhof et al., 2011) and show more play behaviors (Lay Jr. et al., 2000; Bolhuis et al., 2005, 2006) compared with pigs in conventional pens. A greater level of activity or play behavior might increase joint loading or increase the risk for trauma to the joints. Any mild OC lesions that would be present could then be aggravated by the joint loading and trauma, resulting in the progression to severe lesions. Conversely, gilts on a conventional floor might be less active and therefore less at risk to aggravate existing lesions. This might be in line with

findings in horses where it has been shown that there may be an optimum between activity level and (intensity of) mechanical loading. In foals it has been shown that restriction of exercise and exercise in paddocks of a very large size or consisting of rough terrain or both increases the risk of development of OC lesions (Lepeule et al., 2013; Praud et al., 2013). This explanation of more activity leading to significantly more severe OC lesions, however, is not reflected by the observed behavior at 8 weeks of age before the treatment switch where there is not a clear difference in play behaviors performed. It is therefore likely that other, as of this point unknown, factors also have had an influence in the current results. In more practical terms it is important to notice that OC lesions within the classes 3 and 4 are more likely to lead to clinical effects or discomfort. Therefore, an increase in severe lesions is likely of more practical importance in terms of threats to welfare and longevity of sows that have to last several parities. In this manner, floor bedding could endanger animal welfare on the long term with regards to OC prevalence.

3.6 CONCLUSION

This study could not indicate age-dependent effects of the type of floor during the rearing period of gilts on the overall prevalence of OC. However, gilts kept on wood shavings as bedding either before or after 10 weeks of age or both had a greater odds to be affected with severe OC lesions compared with gilts that were kept on a conventional floor from weaning until slaughter at 24 weeks of age. The exact biological mechanism to wood shavings as bedding increasing the odds of severe OC lesions remains elusive but may be related to a disturbance of the equilibrium between activity and mechanical loading of the joints. In practical terms, it seems that wood shavings as bedding at a relatively young age is to be avoided to reduce possible problems arising from OC at a later age (i.e., lameness).

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CHAPTER 4

Associations of conformation and locomotive characteristics in growing gilts with osteochondrosis at slaughter.[1]

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4.1 ABSTRACT

Osteochondrosis (OC) and abnormalities in conformation and locomotive characteristics (CLC) have been associated with premature culling in sows. Several CLC have been suggested to be associated with OC and might help as an in vivo indicator for and increased risk of having OC. The aim of this study was to investigate associations of OC with CLC assessed at several ages in growing gilts from 2 separate experiments over the effects of dietary restriction (Exp. 1) and floor type (Exp. 2) on OC prevalence. In Exp. 1, gilts (n=211) were subjectively assessed for CLC at, on average, 4, 9, 11, 16, and 24 weeks of age. In Exp. 2, gilts (n=212) were subjectively assessed for CLC at, on average, 4, 9, 11, 16, and 22 weeks of age. Assessment was done on 10 conformation and 2 locomotive characteristics using a 9-point grading scale by 2 observers. At, on average, 26 weeks of age in Exp. 1 and 24 weeks of age in Exp. 2, gilts were slaughtered and the knee, elbow, and hock joints were macroscopically assessed for OC. The CLC most frequently associated with OC were O shape or X shape of the hind legs, straight or bowed hind legs, and straight or sickled hock. X-shaped hind legs were associated with OC at slaughter in the knee joint at 4, 9, and 24 weeks of age; and at the animal level (all joints taken together) at 4, 9, and 16 weeks of age. Straight or bowed hind legs were associated with OC at slaughter in the knee joint at 4 and 11 weeks of age; in the hock joint at 11 weeks of age; and at the animal level at 4, 9, 11, and 22 weeks of age. Straight or sickled hock was associated with OC at slaughter in the knee joint at 4 weeks of age; in the hock joint at 9 and 22 weeks of age; and at the animal level at 9 and 22 weeks of age. Results show that several CLC assessed at several ages were associated with OC, but consistent associations of a type of CLC in every assessment could not be found. The associations of CLC with OC are, therefore, difficult to be used as an in vivo indicator of increased risk for OC.

Keywords: conformation and locomotive characteristics, growing gilts, osteochondrosis

4.2 INTRODUCTION

Osteochondrosis **(OC)** in the epiphyseal growth cartilage of a joint involves a disturbance in the process of ossification in young growing pigs, which has been associated with abnormalities in conformation and locomotive characteristics **(CLC)** (Jørgensen and Andersen, 2000; de Koning et al., 2012). Conformation and locomotive characteristics may be associated with OC as either a cause or a consequence of OC. Studies report a reduced longevity with increased severity of leg weakness (which includes CLCs; Jørgensen and Sørensen, 1998; Jensen et al., 2010) and OC (Yazdi et al., 2000).

The CLC appear to have an association with OC to varying degrees (Jørgensen, 1995, 2000; Jørgensen et al., 1995; Stern et al., 1995; Jørgensen and Andersen, 2000; Luther et al., 2007; Kirk et al., 2008; de Koning et al., 2012). However, the type of CLC associated with OC and the measure and direction of associations differ between studies. Partly, these differences may be caused by differences in development of OC and CLC abnormalities over time (van Steenbergen et al., 1990; Ytrehus et al., 2004ab). This complicates how and to what extent CLC are associated with OC and makes CLC as an in vivo indicator of OC status uncertain.

Previously, the age-dependent effects of dietary restriction or floor type on OC prevalence were described in 2 separate experiments (de Koning et al., 2013, 2014). The aim of this study was to investigate whether CLC in gilts from these experiments, assessed at different ages, have a consistent direction of association with OC status at slaughter at 5 to 6 months of age when corrected for the age-dependent effects of dietary restriction or floor type. If CLC during early life, in which OC development takes place, have an association with OC in gilts, this could help elucidate the association CLC have with OC and will provide further insight in the use of CLC as an in vivo indicator of OC in gilts.

4.3 MATERIALS AND METHODS

Two experiments were performed in which either the age-dependent effects of dietary restriction on OC prevalence was assessed (**Exp. 1**) or the age-dependent effects of floor type on OC prevalence was assessed (**Exp. 2**). During both experiments, CLC were assessed at several ages.

4.3.1 Ethical Note

Osteochondrosis can cause articular surface irregularities possibly resulting in lameness that might affect welfare of the gilts. Assessments to detect serious impairments of welfare in the gilts were therefore performed daily. Severely lame or wounded gilts were taken out of the experiment and euthanized. The experiments and all measurements were approved by the Animal Welfare Committee of Wageningen University and Research center in compliance with Dutch Law on Animal Experimentation.

4.3.2 Animals

Both experiments were performed using Topigs 20 (Dutch Large White x Dutch Landrace) gilts acquired from a commercial breeding company (in the first experiment, 211 gilts were acquired from Van Beek SPF Varkens BV, Lelystad, The Netherlands; in the second experiment, 212 gilts were acquired from TOPIGS, Veldhuizen Wehl, Wehl, The Netherlands). This crossbred line represents a large part of the commercially kept sows in The Netherlands that will produce finishing pigs. The gilts entered the experiment after weaning at, on average, 4 weeks of age. All gilts were housed in 8.37 m^2 pens. In Exp. 1, all gilts were housed on a 60% slatted floor and 40% solid floor. In Exp. 2, gilts were housed either on a 60% slatted floor and 40% solid floor or on a 25- to 50-cm-deep bedding of wood shavings (see treatments), of which the wood shavings were gradually increased to 50 cm during the experiment. Gilts had access to various enrichment items (such as biting chains, burlap sacks, solid plastic balls, and / or rubber mats) at all times, which were switched every 3 to 4 days. Gilts had ad libitum access to water at all times through a drinking nipple.

4.3.3 Treatments

In both experiments, gilts were mixed and housed immediately after weaning in 32 pens of 6 to 7 individuals and divided in 4 treatment groups with respect to dietary restriction (Exp. 1) or floor type (Exp. 2), based on an equal distribution of littermates and body weight measured 1 week before the start of the experiment. The 32 pens were divided over 4 rooms within 1 stable (8 pens per room) with an equal distribution of the treatments per room.

In Exp. 1, age-dependent effects of dietary restriction were assessed and gilts were per pen exposed to 1 of 4 treatments of feeding regimes as described in de Koning et al. (2013). Briefly, treatments consisted of: ad libitum feeding continuously from weaning until slaughter **(AA)**; restricted feeding (80% of ad libitum) continuously from weaning until slaughter **(RR)**; ad libitum feeding from weaning until 10 weeks of age, after which feeding levels were switched to restricted feeding **(AR)**; or restricted feeding from weaning until 10 weeks of age, after which feeding levels were switched to ad libitum feeding **(RA)**. For further details of the treatments imposed, the reader is referred to de Koning et al. (2013).

In Exp. 2, age dependent effects of floor type were assessed and gilts were per pen exposed to 1 of 4 treatments of floor type regimens as described in de Koning et al. (2014). Briefly, treatments consisted of a conventional floor from weaning until slaughter **(CC)**; wood shavings as bedding from weaning until slaughter **(WW)**; a conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding **(CW)**; or wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a conventional floor **(WC)**. For further details of the treatments imposed, the reader is referred to de Koning et al. (2014).

4.3.4 Conformation and Locomotive Characteristics Assessment

The CLC of all gilts were assessed 5 times during the experiments. In Exp. 1, gilts were assessed at, on average, 4, 9, 11, 16, and 24 weeks of age. In Exp. 2, gilts were assessed at, on average, 4, 9, 11, 16, and 22 weeks of age. The CLC were assessed over a 2-d period (half of the population on each day with an equal distribution of treatments). Methodology was based on methods described by van Steenbergen (1989) and Jørgensen and Vestergaard (1990). Two locomotive and 10 conformation characteristics were scored on a scale of 1 to 9 (Table 4.1; see the aforementioned studies for figures describing the conformation characteristics). The scoring scale for the locomotive characteristics include a normal state at score 1, with scores greater than 1 indicating deviations in the locomotive characteristic (for example, stiff gait or swaying hindquarters). The scoring scale of all conformation characteristics contains a normal conformation at score 5 and the severity in deviations above or below 5 indicates a certain type of characteristic (for example, X-shaped legs or O-shaped legs). Scoring of CLC for each gilt was

Table 4.1. Conformation and locomotive characteristics subjectively assessed on a 9-point grading scale in gilts [modified from van Steenbergen (1989) and Jørgensen and Vestergaard (1990)]

Trait	Scoring		
Locomotive characteristics	Score 1		Score 9
Swaying hindquarters	No swaying		Severe swaying
Gait movement and pattern	Quick or smooth		Slow or difficult
Conformation characteristics	Score 1	Normal (5)	Score 9
Width of hams	Narrow		Broad
Front legs			
O shape or X shape	O shape		X shape
Claw size[3]	Inner claw smaller		Outer claw smaller
Sickled or buckled	Sickled		Buckled
Steep or weak pasterns	Steep		Weak
Hind legs			
O shape or X shape	O shape		X shape
Claw size	Inner claw smaller		Outer claw smaller
Straight or sickled hock	Straight		Sickled
Straight or bow leg	Straight		Bow
Steep or weak pasterns	Steep		Weak

performed independently by 2 observers at the same time while a maximum of 4 pigs were walking freely in a concrete flooring hallway. Due to practical circumstances, half of the gilts of the CLC assessment at 4 weeks of age in Exp. 2 was performed by 1 observer and the other half by 2 observers. Observer agreement (see below) is therefore based on half the population of the CLC assessment at 4 weeks of age in Exp. 2.

4.3.5 Osteochondrosis Assessment

At the end of the experiment, gilts were slaughtered at a local slaughterhouse and joints were harvested for macroscopical evaluation of the irregularities of the articular surfaces indicative of OC as described in de Koning et al. (2013, 2014). Briefly, gilts were slaughtered over a 1-week period. Half of the population (equal distribution of treatments and pens) was slaughtered within 2

days at, on average, 185 days of age in Exp. 1 and 166 days of age in Exp. 2. The other half of the population was slaughtered within 2 days at, on average, 192 days of age in Exp. 1 and 172 days of age in Exp. 2. After slaughter, the elbow, knee, and hock joints were dissected to be macroscopically assessed for prevalence and severity of OC on a total of 22 locations (see de Koning et al., 2013). Joints were scored on a 5-point grading scale from 0 to 4 (as described by van Weeren and Barneveld, 1999), with score 0 indicating no abnormalities and score 4 indicating severe abnormalities. Osteochondrosis was scored by 1 veterinarian who specialized in orthopedics and was unaware of the treatments and experienced in judging OC.

4.3.6 Statistics

Conformation and Locomotive Characteristics Data. The subjective CLC scores of both judges were averaged and used as fixed covariates in statistical analyses. As an indication of observer agreement, overall observer agreement was calculated by the number of times the observers agreed on CLC scores divided by the total number of observations. Spearman rank correlations were calculated, as an indication of changes in CLC between 2 consecutive ages. Spearman rank correlations were only calculated for those CLC assessments in which less than half of the gilts were contained in 1 CLC score. When more than half of the gilts are contained within 1 CLC score, the correlation coefficient approaches 0 and does not reflect the correlation between assessments appropriately.

For the conformation characteristics, deviations above or below score 5 relate to a certain characteristic, which both could result in differences of OC status. If a CLC is taken as a linear covariate in statistical models, then it cannot account for scores below and above 5 both being associated with greater odds to be affected with OC. Therefore, quadratic associations of the conformation characteristics were assessed in statistical analyses to account for this type of scoring scale used. The scores for the conformation characteristics need to be subtracted by the 'normal' score (score 5) for appropriate quadratic analyses (de Koning et al., 2012). The reason for this is when the derivative of a quadratic relationship [$y = (x-5)^2 + (x-5) + intercept$, in which x is a conformation score] is equated to 0 when the linear term is nonexistent, it will result in the vertex of the parabola of the function at score 5 (x = 5). If no such transformation occurred, then

the vertex of the parabola would result in score 0 (x = 0) and would therefore be incorrect considering the scale used. In this way, the statistical analyses will provide better insight of whether a linear term existed. The locomotive characteristics are all on a linear scale with the normal value at score 1. Because no optimum value is present in the middle of the scoring scale, no quadratic relationships were assessed for the locomotive characteristics.

Osteochondrosis Data. The data of the OC scores are on an ordinal scale. However, due to an insufficient number of observations of each combination of treatment and OC score along with the addition of CLC as covariates, ordinal logistic regression provided convergence problems. Therefore, the OC scores were transformed to a 0 (no OC) and 1 (OC score higher than 0) variable. This transformation allows for appropriate binary logistic regression to be applied.

Osteochondrosis Statistical Model. The effects of dietary restriction or floor type on the prevalence and severity of OC have been discussed previously (de Koning et al., 2013, 2014) and will not be presented here. Associations between CLC and OC were analyzed separately for each experiment. The CLC were added to the statistical model as fixed covariates. Analyses were performed using PROC GLIMMIX in the statistical software package SAS 9.2 (SAS Inst., Inc., Cary, NC). The statistical model used to assess whether CLC are associated with OC prevalence over the age-dependent effects of dietary restriction or floor type is as follows;

$$\text{Logit } P(y_{ijklmn} = 1) = \alpha + \text{Treat}_i + \text{Room}_j + Pen_{k(ij)} + Gilt(Dam)_k + (\text{CLCmeas}+\text{CLCmeas}^2)_m + \varepsilon_{ijklmn},$$

In which the model estimates the probability (P) that the ijklmnth observation of OC (y) for a joint or at the animal level (all joints combined) is 1 (OC score larger than 0). Logit is the link function used to model the mixed linear regression analysis. The α component represents the estimate of the log odds of being affected with OC disregarding the independent variables [baseline odds (Kleinbaum and Klein, 2010)]. Treat_i represents the fixed class effect of treatments administered (i = AA, AR, RA, RR in Exp. 1; i = CC, CW, WC, WW in Exp. 2). Room_j represents the fixed class effect of room in which gilts were housed (j = room 1, 2, 3, 4) and is used to account for environmental variation. $Pen_{k(ij)}$ represent the random effect of

the experimental unit pen (k = pen 1, 2, …, 32) nested within treatment and room, and is used to assess OC scores on pen level for the treatment effects. A random term was added consisting of gilts nested within dams from which the gilts originated to account for dam effects [$Gilt(Dam)_l$; l = dam 1, 2, …., 46 in Exp. 1; l = dam 1, 2, …., 40 in Exp. 2]. All CLC from one single age at which CLC were assessed from 1 experiment were added to the model. Backward elimination was then performed on the CLC (as described in de Koning et al., 2012) until only significant effects ($P < 0.05$) of CLC on OC remained in the model. All other effects, as described in the model above, remained in the model at all times to account for 'environmental' variation. This process was repeated for the 5 ages at which CLC were assessed per experiment. Quadratic relationships were assessed for the conformation characteristics using both the linear term and the accompanying quadratic term [(CLCmeas + $CLCmeas^2$)], in which CLCmeas is the CLC assessed within a measurement from one of the 5 ages at which CLC were assessed. Nonexistent quadratic relationships were removed from the model and only the linear term remained in the process of backward elimination. No quadratic relationships were assessed for the locomotive characteristics. A repeated measurement analysis was performed with gilts as the subject, indicating that the random residual term from a binary distribution (ε_{ijklm}) shows dependency of the joint locations assessed for OC within each gilt. The above-described statistical procedure was not performed for the elbow joint in Exp. 2 as prevalence of OC in the elbow joint (5.4%) was deemed too low for appropriate analysis.

Significant effects of CLC on OC are presented as the regression coefficients β and the SE (β [SE]) from the statistical model. When few observations are present at the boundaries of a measured scale (in this case the CLC scoring scale), they may have large influence on the outcome of an analysis (Ott and Longnecker, 2001). After backward elimination, analysis was performed again without these observations to determine whether the association remains present for those CLC scores that contained less than or equal to 5 observations. When associations became insignificant ($P > 0.05$) as a consequence, these issues are presented.

4.4 RESULTS

The detailed prevalence of OC has been described elsewhere. In Exp. 1, the percentage of gilts affected with OC in the elbow joint was 18%; in the hock joint, 20.4%; in the knee joint, 40.5%; and at the animal level, 60% (de Koning et al., 2013). In Exp. 2, the percentage of gilts affected with OC in the elbow joint was 5.4% (as mentioned not used for analysis in this study); in the hock joint, 44.1%; in the knee joint, 17.6%; and at the animal level, 58.3% (de Koning et al., 2014).

4.4.1 Prevalence and agreement of Conformation and Locomotive Characteristics

Prevalence of CLC assessed for all 5 observations for Exp. 1 are shown in Figure 4.1A and Exp. 2 are shown in Figure 4.1B. For most CLC, the majority of animals were scored as or close to normal conformation. For all conformation characteristics throughout the 5 observations, at least 89% (or more) of the scores range from 4 to 6, except in the second experiment for steep or weak pasterns of the front legs and hind legs, and claw size of the hind legs. Although the scoring scale for all CLC range from 1 through 9, scores 1 , 2, 8, and 9 (severe deviations from normal) for conformation characteristics were not present in Exp. 1, and only limitedly present for some conformation characteristics in Exp. 2. This indicates that there were no severe deviations from normal conformation. Additionally, for the locomotive characteristics, no severe deviations from normal were rigorously present.

Overall observer agreement was, in general, higher than 0.5 (Table 4.2 and 4.3). The highest overall observer agreement was found for the first 2 observations in which CLC were assessed. Relatively high observer agreements (> 0.75) throughout all observations were found in Exp. 1 (Table 4.2) for O shape or X shape of the front legs, claw size of the front legs, O shape or X shape of the hind legs, and swaying hindquarters, and in Exp. 2 (Table 4.3) for claw size of the front legs. For all other CLC, observer agreement ranged between 0.32 and 0.75.

Spearman rank correlation between consecutive ages at which the CLC were assessed were generally low to moderate. In the first experiment (Table 4.2), correlations of approximately 0.6 for 2 or more assessments were found for width of the hams after the second assessment. In Exp. 2 (Table 4.3), correlations higher

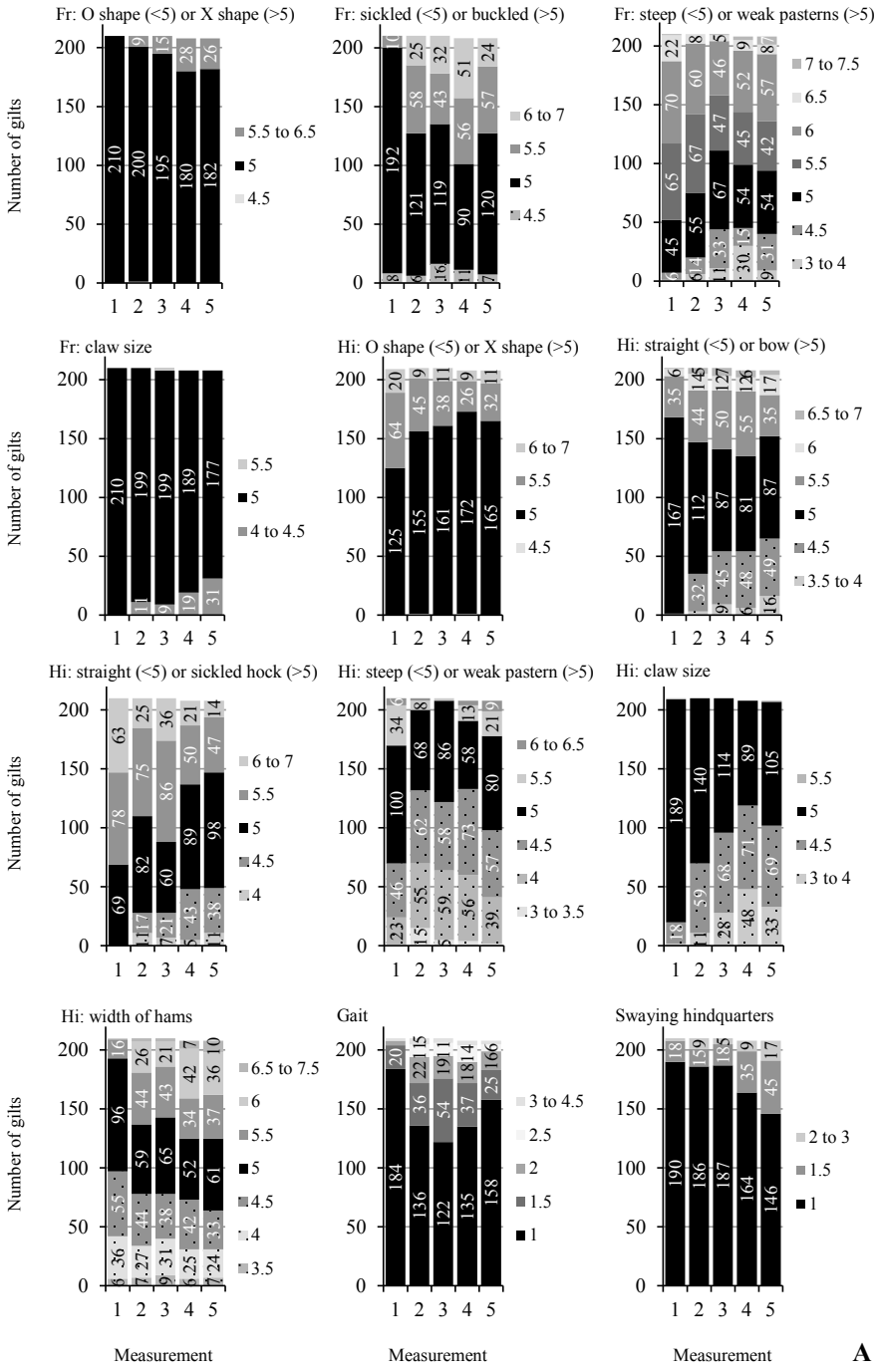

Figure 4.1AB. Prevalence of conformation and locomotive characteristics (CLC) assessed at 5 ages during (A) Exp. 1 and (B) Exp. 2. Assessments of CLC were performed at, on average, 4, 9, 11, 16, and 22 (Exp. 2) or 24 weeks of age (Exp. 1). The y-axis in each figure represents the number of gilts. *(Continued on the next page)*

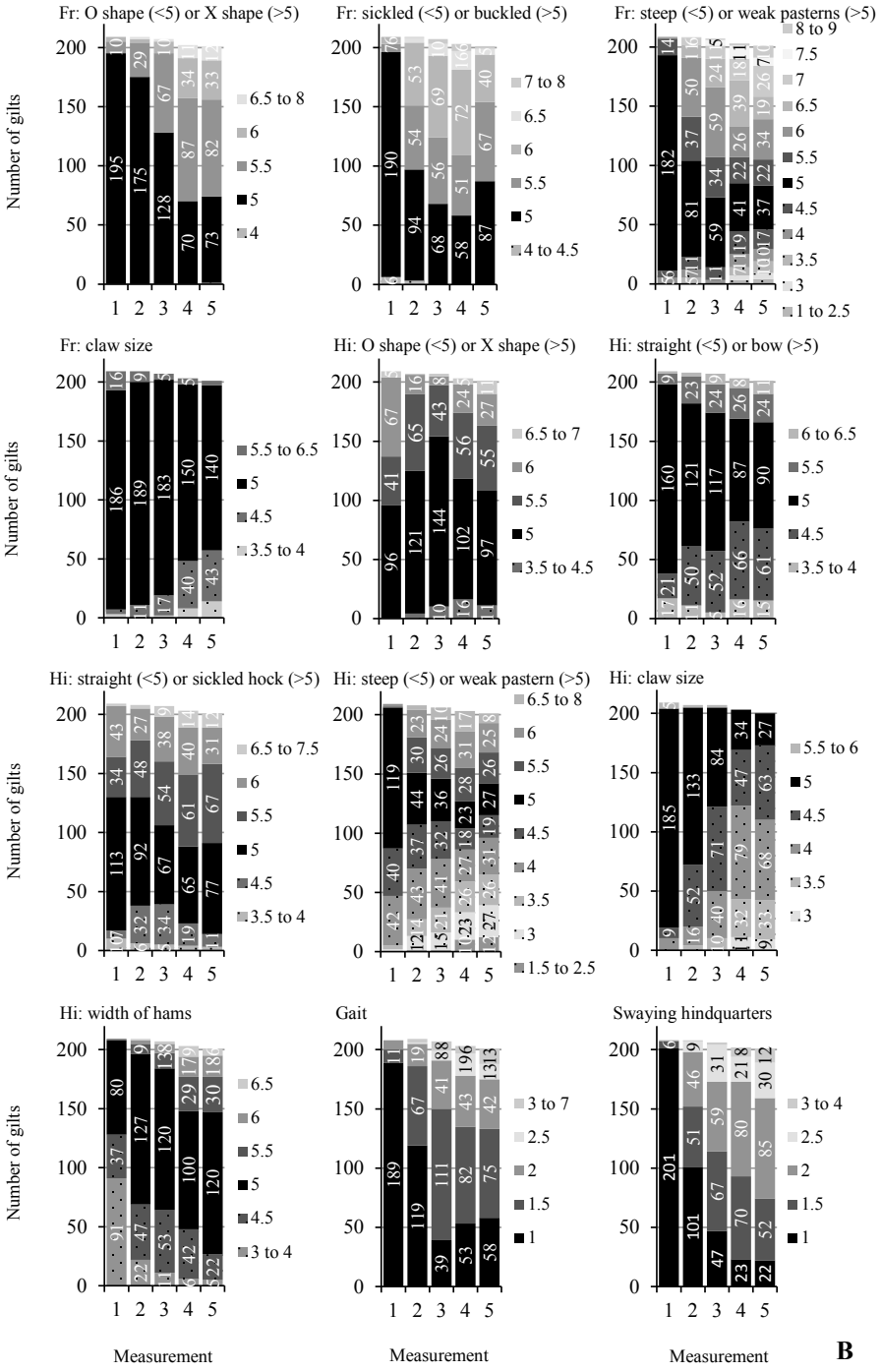

Figure 4.1AB *(continued).* The x-axis represents the assessments. Each conformation characteristic is preceded with either an "Fr" for front legs or an "Hi" for hind legs. For the conformation characteristics, a CLC score of 5 indicates normal conformation. *(Continued on the next page)*

than 0.6 for 2 or more assessments were found for width of the hams, and steep or weak pasterns of the front legs and hind legs after the third assessment. These low to moderate correlations indicate that changes in the CLC are not consistent over time. Note, however, that for many CLC the scores remain relatively close to the normal score and remains relatively consistent over time.

4.4.2 Associations in Exp. 1

Several CLC were significantly associated with OC ($P < 0.05$) after backward elimination was performed in the statistical models. Associations of CLC with OC are depicted in Figure 4.2 (black lines). Significant effects of CLC on OC are presented as the regression coefficients β and the SE (β [SE]) from the statistical model.

Knee Joint. At 4 weeks of age, the CLC associated with less OC in the knee joint were X-shaped hind legs (-0.92 [0.42]), bowed hind legs (-1.37 [0.59]), steep pasterns on the front legs (0.55 [0.26]), and sickled hocks (-0.77 [0.32]); at 9 weeks of age, steep and weak pasterns on the front legs (linear term 1.16 [0.43]; quadratic term -1.08 [0.42]), and X shaped hind legs (-1.37 [0.52]); at 11 weeks of age, sickled or buckled front legs (linear 0.59 [0.59]; quadratic -1.32 [0.63]), and bowed hind legs (-0.62 [0.25]); at 16 weeks of age, swaying hindquarters (-1.50 [0.53]); and at 24 weeks of age, steep pasterns of the front legs (0.55 [0.2]), X-shaped hind legs (-0.93 [0.47]), and a smooth and quick gait (0.69 [0.31]). No significant effects remained ($P > 0.05$) after removal of the low number of animals (≤ 5) at the boundaries of the scoring scale from the analysis for the CLC steep pasterns at 4 weeks of age, sickled or buckled front legs at 11 weeks of age, and X-shaped hind legs at 24 weeks of age.

Hock Joint. No associations were found between OC and the CLC assessed at 9 and 16 weeks of age. At 4 weeks of age, the CLC associated with less OC in the hock joint were broad hams (-1.0 [0.44]); at 11 weeks of age, bowed hind legs (-0.83 [0.42]) and a smooth and quick gait (0.86 [0.38]); and at 24 weeks

Figure 4.1AB *(continued).* For the claw size characteristic, a CLC score of 5 indicates equally sized claws, <5 indicates smaller inner claws, and >5 indicates bigger inner claws. For the width of the hams characteristic, a CLC score of <5 indicates narrow hams and >5 indicates broad hams. For the locomotive characteristics gait and swaying hindquarters, a CLC score of 1 indicates normal locomotion and scores above 1 indicates a worse form of locomotion. For display purposes, CLC scores for which there were less than 5 observations were combined.

Table 4.2. Overall[1] observer agreement in Exp. 1 of the conformation and locomotive characteristics subjectively assessed at several ages (in weeks [wk] of age) and phenotypic Spearman correlations between 2 consecutive assessments[2].

	4 wk		9 wk		11 wk		16 wk		24 wk
Characteristic	Agree	Corr.[3]	Agree	Corr.	Agree	Corr.	Agree	Corr.	Agree
Swaying hindquarters	0.91	-	0.92	-	0.91	-	0.83	-	0.78
Gait	0.90	-	0.74	-	0.67	-	0.72	-	0.84
Width of the hams	0.62	0.32	0.5	0.59	0.52	0.58	0.51	0.71	0.57
Front legs									
O shape or X shape	1.00	-	0.95	-	0.94	-	0.87	-	0.88
Claw size	1.00	-	0.95	-	0.95	-	0.91	-	0.86
Sickled or buckled	0.91	-	0.68	-	0.67	-	0.63	-	0.62
Steep or weak pasterns	0.53	0.29	0.55	0.47	0.53	0.59	0.53	0.43	0.53
Hind legs									
O shape or X shape	0.68	-	0.78	-	0.8	-	0.86	-	0.83
Claw size	0.91	-	0.72	-	0.66	-	0.66	-	0.64
Straight or sickled hock	0.62	0.12	0.48	0.26	0.41	0.31	0.49	0.35	0.58
Straight or bow leg	0.82	-	0.57	-	0.46	0.40	0.39	0.30	0.52
Steep or weak pasterns	0.58	0.29	0.60	0.41	0.69	0.26	0.54	0.21	0.59

[1] Overall agreement was calculated by dividing the number of times observers agreed by the total number of observations.

[2] In Exp. 1, correlations were calculated between 4 and 9 weeks of age, 9 and 11 weeks of age, 11 and 16 weeks of age, and 16 and 24 weeks of age. Correlation coefficients are placed between the corresponding assessments.

[3] Corr. = Spearman rank correlation coefficient. Correlations were only performed for those assessments in which less than half of the animals ($n < 105$) were in 1 CLC score (when this is not the case, the coefficient cannot be calculated appropriately). For those characteristics where a coefficient cannot be calculated, there was relatively no change in CLC scores and relatively no variation seen.

Table 4.3. Overall[1] observer agreement in Exp. 2 of the conformation and locomotive characteristics subjectively assessed at several ages (in weeks [wk] of age) and phenotypic Spearman correlations between 2 consecutive assessments[2].

	4 wk		9 wk		11 wk		16 wk		22 wk
Characteristic	Agree	Corr.[3]	Agree	Corr.	Agree	Corr.	Agree	Corr.	Agree
Swaying hindquarters	0.94	-	0.70	0.50	0.47	0.35	0.45	0.45	0.53
Gait	0.89	-	0.66	-	0.32	0.23	0.43	0.22	0.48
Width of the hams	0.61	-	0.70	-	0.63	0.60	0.58	0.70	0.69
Front legs									
O shape or X shape	0.90	-	0.85	-	0.66	-	0.47	0.49	0.52
Claw size	0.84	-	0.91	-	0.89	-	0.76	-	0.75
Sickled or buckled	0.89	-	0.70	0.53	0.68	0.46	0.66	0.51	0.64
Steep or weak pasterns	0.8	-	0.68	0.46	0.64	0.74	0.47	0.88	0.59
Hind legs									
O shape or X shape	0.58	-	0.66	-	0.73	-	0.60	0.56	0.61
Claw size	0.88	-	0.71	-	0.58	0.32	0.56	0.53	0.51
Straight or sickled hock	0.56	-	0.59	0.40	0.50	0.37	0.43	0.52	0.54
Straight or bow leg	0.68	-	0.61	-	0.60	-	0.48	0.22	0.53
Steep or weak pasterns	0.55	-	0.58	0.34	0.53	0.75	0.47	0.77	0.53

[1] Overall agreement was calculated by dividing the number of times observers agreed by the total number of observations.

[2] In Exp. 2, correlations were calculated between 4 and 9 weeks of age, 9 and 11 weeks of age, 11 and 16 weeks of age, and 16 and 22 weeks of age. Correlation coefficients are placed between the corresponding assessments.

[3] Corr. = Spearman rank correlation coefficient. Correlations were only performed for those assessments in which less than half of the animals (n < 105) were in 1 CLC score (when this is not the case, the coefficient cannot be calculated appropriately). For those characteristics where a coefficient cannot be calculated, there was relatively no change in CLC scores and relatively no variation seen.

of age, buckled front legs (-1.41 [0.65]), narrow and broad hams (linear 0.004 [0.36]; quadratic 0.60 [0.28]), and a straight and sickled hock (linear -0.70 [0.63]; quadratic -2.27 [1.13]). No significant effects remained ($P > 0.05$) after removal of the low number of animals (≤ 5) at the boundaries of the scoring scale from the analysis for the CLC gait at 11 weeks of age and broad hams at 24 weeks of age.

Elbow Joint. No associations were found between OC and the CLC assessed at 4, 11 and 24 weeks of age. At 9 weeks of age, the CLC associated with less OC in the elbow joint were sickled front legs (1.25 [0.57]); at 16 weeks of age, equally sized claws or larger deviations from equally sized claws in the direction of smaller inner claws of the hind legs (linear -5.16 [1.95]; quadratic -5.08 [1.97]), steep pasterns of the hind legs (1.12 [0.47]), and a smooth and quick gait (1.23 [0.45]).

Animal Level. At 4 weeks of age, the CLC associated with less OC at the animal level were X-shaped hind legs (-0.70 [0.26]); at 9 weeks of age, X-shaped hind legs (-0.88 [0.30]), and bowed hind legs (-0.53 [0.17]); at 11 weeks of age, bowed hind legs (-0.49 [0.16]); at 16 weeks of age, equally sized claws or larger deviations from equally sized claws in the direction of smaller inner claws of the hind legs (linear -1.92 [0.58]; quadratic -1.55 [0.56]), X-shaped hind legs (-0.67 [0.32]), and swaying hindquarters (-0.83 [0.33]); at 24 weeks of age, steep pasterns of the front legs (0.26 [0.12]). No effects remained after removal of the low number of animals (≤ 5) at the boundaries of the scoring scale from the analysis for the CLC X-shaped hind legs at 16 weeks of age and steep pasterns of the front legs at 24 weeks of age.

4.4.3 Associations in Exp. 2

Several CLC were significantly associated with OC ($P < 0.05$) after backward elimination was performed in the statistical models. Associations of CLC with OC are depicted in Figure 4.2 (blue lines).

Knee Joint. No associations were found between OC and the CLC assessed at 4, 9, and 22 weeks of age. At 11 weeks of age, the CLC associated with more OC were smaller inner claws on the front legs (-3.04 [1.38]); and at 16 weeks of age, X shaped front legs (1.40 [0.6]).

Hock Joint. No associations were found between OC and the CLC assessed at 11 and 16 weeks of age. At 4 weeks of age, the CLC associated with

less OC in the hock joint were steep pastern of the front legs (0.94 [0.45]); at 9 weeks of age, sickled hocks (-0.59 [0.24]); at 22 weeks of age, sickled hocks (-0.85 [0.23]), and less sickled and buckled front legs (linear -1.27 [0.62]; quadratic 0.91 [0.43]). No significant effects ($P > 0.05$) remained after removal of the low number of animals (≤ 5) at the boundaries of the scoring scale from the analysis for the CLC sickled or buckled front legs at 22 weeks of age.

Animal Level. No associations were found between OC and the CLC assessed at 11 and 16 weeks of age. At 4 weeks of age, the CLC associated with less OC at the animal level were less straight and bowed hind legs (linear 0.45 [0.30]; quadratic 0.66 [0.34]); at 9 weeks of age, sickled hocks (-0.52 [0.16]); at 22 weeks of age, swaying hindquarters (-0.37 [0.16]), sickled hocks (-0.45 [0.16]), and less straight and bowed hind legs (linear 0.11 [0.16]; quadratic 0.39 [0.20]). No significant effects ($P > 0.05$) remained after removal of the low number of animals (≤ 5) at the boundaries of the scoring scale from the analysis for the CLC straight and bowed hind legs at 4 weeks of age.

4.5 DISCUSSION

The aim of this study was to investigate the associations of CLC with OC over the previously modelled effects of dietary restriction or floor type on OC prevalence. The CLC were subjectively assessed at several ages and various associations with OC were found.

4.5.1 Prevalence and Observer Agreement of Conformation and Locomotive Characteristics

The gilts that were used in both experiments did not seem to suffer from severe deviations of the assumed normal conformation. The gilts were scored near normal for all the CLC assessed and scores 1, 2, 8, and 9 (severe deviations from normal) were not rigorously present. Any differences in CLC between animals are therefore assumed to be marginal and correspond to a previous study (de Koning et al., 2012). Other studies also report very little variation in CLC in pigs and sheep (van Steenbergen, 1989; Jørgensen and Vestergaard, 1990; Janssens and Vandepitte, 2004; Janssens et al., 2004; Tarrés et al., 2006). Possibly, commercial husbandry has selected for animals against deviations from the optimal state (Tarrés et al., 2006).

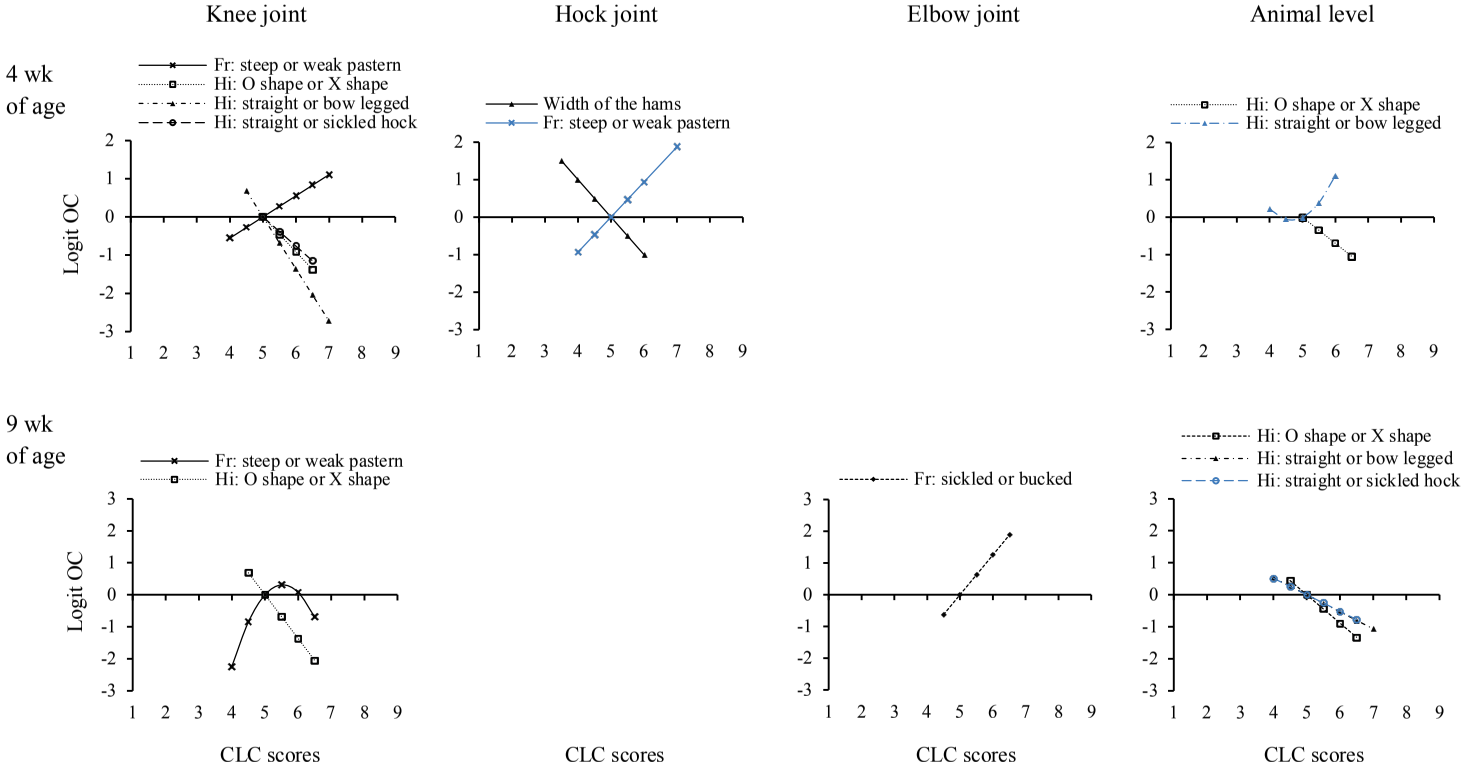

Figure 4.2. Estimated phenotypic associations of conformation and locomotive characteristics (CLC) with osteochondrosis (OC) at slaughter from the statistical models on a logit scale from Exp. 1 (black lines) and Exp. 2 (blue lines). In Exp. 1, CLC were assessed at, on average, 4, 9, 11, 16, and 24 weeks (wk) of age. In Exp. 2, CLC were assessed at, on average, 4, 9, 11, 16, and 22 weeks of age. Each of the 5 assessments are ordered in rows and indicated with the age of the assessment. The CLC were associated with OC in the knee joint, hock joint, elbow joint, and at the animal level (all joints combined). The y-axis shows the estimated OC association on a logit scale based on the regression coefficients from statistical analyses. The x-axis shows the 9-point grading scale used to assess CLC. *(Continued on the next page)*

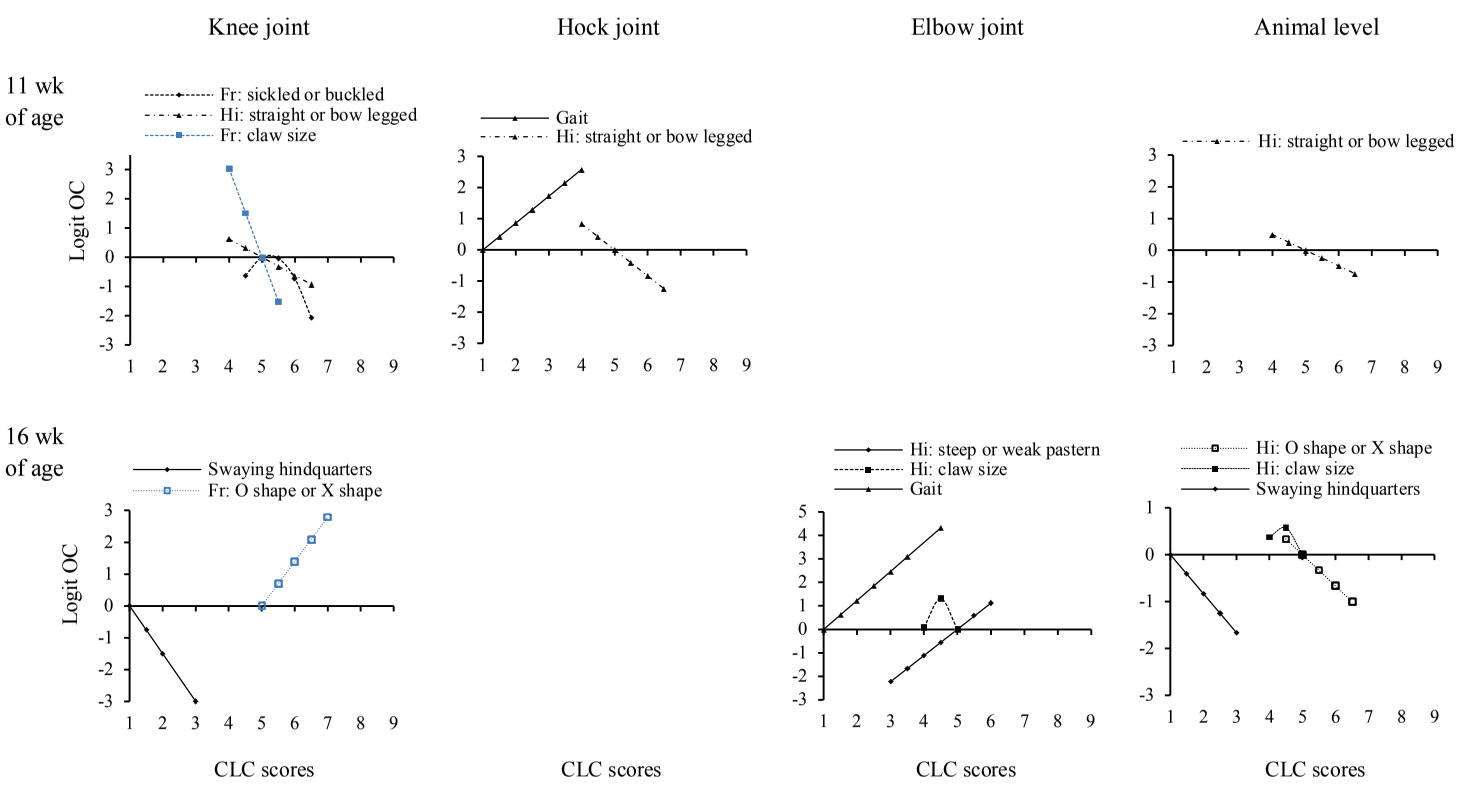

Figure 4.2 ***(continued).*** Only the estimated associations are shown for which CLC scores were present. Only the significant ($P < 0.05$) associations of OC with CLC are depicted. Each conformation characteristic is preceded with either an "Fr" for front legs or an "Hi" for hind legs. For the conformation characteristics, a CLC score of 5 indicates normal conformation and deviations below or above 5 correspond to the first and last conformation characteristic in the definition, respectively. For the claw size characteristic, a CLC score of 5 indicates equally sized claws, <5 indicates smaller inner claws, and >5 indicates bigger inner claws. *(Continued on the next page)*

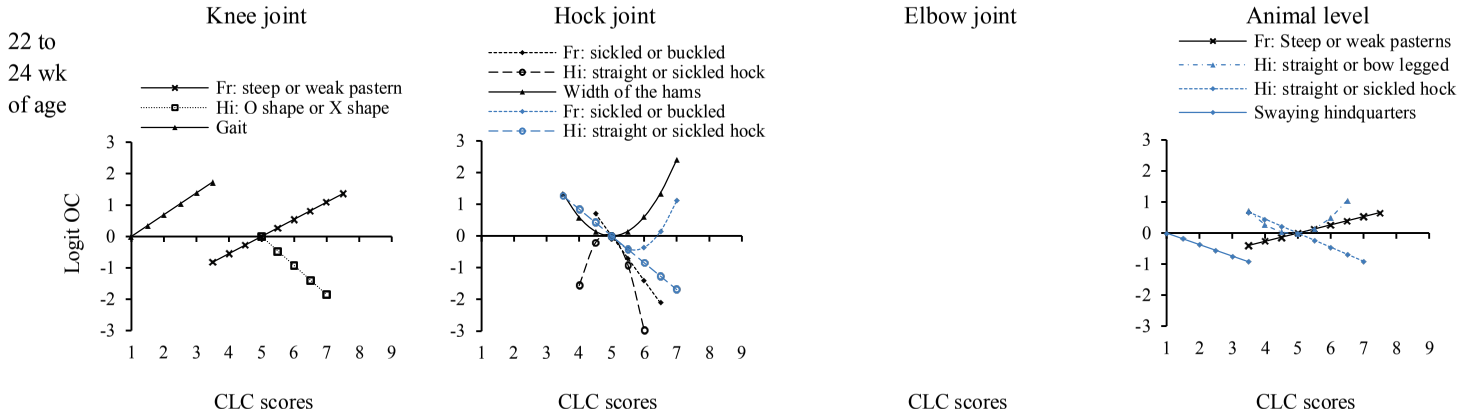

Figure 4.2 ***(continued).*** For the width of the hams characteristic, a CLC score of <5 indicates narrow hams and >5 indicates broad hams. For the locomotive characteristics gait and swaying hindquarters, a CLC score of 1 indicates normal locomotion and scores above 1 indicates a worse form of locomotion.

An issue with subjective assessment of CLC is observer agreement. Different people assessing CLC will likely draw from their own views and experiences as to what is abnormal and to what extent. In the current studies, observer agreement showed a large range. The range in observer agreement indicates that not all CLC are equally easy to assess. Observer agreement was relatively high (0.80 and higher) for some CLC: in Exp. 1 for O shape or X shape of the front legs, claw size of the front legs, O shape or X shape of the hind legs, and swaying hindquarters; in Exp. 2 for claw size of the front legs. Observer agreement for both experiments was relatively low (generally between 0.3 and 0.65) for straight or bow hind legs and straight or sickled hocks, indicating difficulty of the observers to agree on the extent of deviation from normal. Observer disagreement for all CLC was likely more an issue in animals that deviated only slightly from normal, which might pose difficulty in correctly assessing the animal as deviating. Other studies also indicate that observer agreement can be low but can be improved with training and experience (van Steenbergen, 1989; Mawdsley et al., 1996; Veerkamp et al., 2002; Janssens and Vandepitte, 2004; Janssens et al., 2004).

4.5.2 Associations Between Conformation and Locomotive Characteristics and Osteochondrosis

Various CLC at the 5 CLC assessments were found to have an association with OC in different joints or at the animal level. However, associations were relatively low and a clear pattern is not recognizable. In Exp. 1, the elbow and hock joints showed no CLC that were repeatedly found to be associated with OC. However, in the knee joint and at the animal level, several CLC were found to be associated with OC over several ages. In the knee joint, these CLC were X-shaped hind legs (4, 9, and 24 weeks of age), steep or weak pasterns of the front legs (4, 9, and 24 weeks of age), and straight or bowed hind legs (4 and 11 weeks of age). At the animal level, these were X-shaped hind legs (4, 9, and 16 weeks of age) and bowed hind legs (9 and 11 weeks of age). In Exp. 2, several CLC were found to be associated over several ages in the hock joint and the animal level. At the hock joint, the CLC was straight or sickled hock (9 and 22 weeks of age). At the animal level, these CLC were straight or bowed hind legs (4 and 22 weeks of age) and straight or sickled hocks (9 and 22 weeks of age). Between experiments, the hock

joint and the animal level showed similar CLC that were associated with OC, although they were found at different assessments or found only once over all assessments within each experiment. These CLC were for the hock joint sickled or buckled front legs and straight or sickled hocks. For the animal level, these CLC were straight or bowed hind legs, straight or sickled hocks, and swaying hindquarters.

Other studies assessing associations between OC and CLC report various associations depending on the joints assessed and include swaying hindquarters, stiff gait, buckled front legs, X-shaped front or hind legs, steep or weak pasterns of the front legs, and sickled hock (Jørgensen, 1995, 2000; Jørgensen et al., 1995; Stern et al., 1995; Jørgensen and Andersen, 2000; Luther et al., 2007; Kirk et al., 2008; de Koning et al., 2012), of which most CLC are also found to have an association with OC in the current study. However, the strength of the association with OC or even the direction of the association can differ between studies or within studies, as seems to be the case in the current study. For example Jørgensen and Andersen (2000) indicated that associations between CLC and OC can be opposite in different breeds for swaying hindquarters, gait characteristics, weak pasterns, and X-shaped front legs. Similar results were attained when comparing a previous study (de Koning et al., 2012) using Tempo x Topigs 40 pigs with the current study using Dutch Large White x Dutch Landrace gilts for swaying hindquarters and X-shaped hind legs. Such opposing results indicate that associations between OC and CLC from 1 breed cannot be translated directly to another breed. Finally, whether CLC of the front or hind legs are associated with OC in the front or hind legs or both may differ (Jørgensen et al., 1995; Kirk et al., 2008), as is the case in the current study.

Previously, results were presented on the associations of CLC assessed at, on average, 22 weeks of age with OC assessed at 24 weeks of age (de Koning et al., 2012). The key difference in the current study is that CLC were assessed at different ages to examine whether associations between CLC and OC are consistent over time and could have an predictive value for OC at an early age, which does not seem to be the case in the current study. Those CLC that were found repeatedly within and between experiments in the current study may indicate the more important CLC to monitor, though they might be associated with OC at different ages and / or in different directions, providing difficulties for correct

assessment of associations with OC. Literature also reports difficulties in consistent associations between OC and CLC. Aasmundstad et al. (2014) have indicated that phenotypic correlations of OC and several exterior traits are generally low, which seems to be in concordance with the current study and provides further indications that use of subjectively assessed CLC for association with OC may be limited. Therefore, in our view, CLC are likely not a good candidate to be used as an in vivo indicator for increased risk to have OC.

4.5.3 Variability of Conformation and Locomotive Characteristics Over Time

A major factor that might complicate interpretation of which CLC are associated with OC is time dependency of CLC occurrences. In the current study, most correlations between consecutive CLC assessments were quite low, which indicate that for the majority changes in the CLC do not seem to remain consistent for a large part of the gilts assessed as time progresses. However, for some CLC, correlations could not be validly calculated as the majority (more than half) of the gilts were continuously scored within the same CLC score (often close to normal as discussed above). In that sense, those CLC remained relatively constant throughout the experiment but because the majority is scored within 1 CLC score, it becomes more difficult to find differences in associations with OC (due to less variation). Additionally, associations between CLC and OC within one assessment did not necessarily occur again in the next assessment, indicating that associations of CLC with OC can be variable. The data of the current study were also analyzed based on the moment a deviation from normal conformation or locomotion occurred and persisted towards the end of the experiment (data not shown). In this way, it would be possible to assess whether there is an association for the period of time an animal has a deviating CLC with OC at slaughter. This method of analysis, however, generally was inadequate for the current data because of a low number of observations (frequently less than 10 animals in the different categories) in which animals consistently were deviating in the same direction from a certain age towards the end of the experiment, again indicating that changes in the CLC are not consistent throughout the experiments or that the CLC do not show variation in CLC scores. The data were also analyzed by assessing associations between OC at slaughter and whether gilts deviated in any CLC in the experiment irrespective of when these deviations occurred or for how long (data not shown). This method of

analysis also did not provide any association with OC and is likely because of CLC inconsistency over time in the experiment or because of low variation in OC scores. Time dependency of CLC has also been indicated by van Steenbergen et al. (1990). It therefore seems that any association found between CLC and OC might depend on the age at which CLC are assessed and cannot directly be translated from one study to another. Other studies have assessed CLC once relatively close to slaughter at roughly 90 to 120 kg of body weight (Jørgensen, 1995; Jørgensen et al., 1995; Stern et al., 1995; Jørgensen and Andersen, 2000; de Koning et al., 2012; Aasmundstad et al., 2014). This practice might not give full insight into the association of CLC at young age during the important time frame for OC development if any exists.

4.5.4 Possible Pathways of Associations Between Conformation and Locomotive Characteristics and Osteochondrosis

The possible pathway of how CLC and OC are related to each other are difficult to determine. It brings up the discussion of whether CLC cause OC or whether OC causes CLC, as previously discussed (de Koning et al., 2012). The CLC might be causative of OC as a deviating CLC from normal might impose unbalanced load bearing within a joint, which in turn can cause a local overload within a joint (Grøndalen and Grøndalen, 1974), possibly leading to factors that can affect OC development such as risk of vascular disruption within the growth cartilage (Ytrehus et al., 2004b; Olstad et al., 2008, 2011; and reviewed by Ytrehus et al., 2007; Laverty and Girard, 2013; McCoy et al., 2013) or altered functioning of chondrocytes (Quinn et al., 1999; Davisson et al., 2002). On the other hand, OC might be causative for CLC when OC becomes severe enough to such an extent that it impairs normal joint movement, resulting in CLC abnormalities, as is suggested to be a mechanism by others (Jørgensen, 1995; Jørgensen et al., 1995). This could then result in abnormal movement such as lameness or stiffness. A note to this study is that OC was analyzed as a 0 and 1 trait, indicating that all forms of OC were categorized into 1 variable which might miss associations with severe OC and CLC. However, there was relatively little severe OC found in the current experiments (de Koning et al., 2013, 2014), which might indicate that OC was not the cause for deviating CLCs. Other types of analyses considered that take severity of OC into account (as ordinal logistic regression) provided convergence problems

and could not be performed.

As discussed above, CLC seem to be variable over time. Osteochondrosis, however, seems to be age dependent as well. As suggested by results of Ytrehus et al. (2004ab), a main time frame for OC development likely takes place around 10 weeks of age. As CLC are variable over time and OC seem to be age dependent, it complicates their association. Taking this into account, there are several possibilities of how CLC are associated with OC at the 5 CLC assessments performed in the current study (Figure 4.3). The first possibility is that animals at a young age (4 weeks of age in the current study) have a certain conformation that remains until the sensitive time period of OC development (around 10 weeks of age), leading to a causative factor for OC development. This seems unlikely considering the low correlations found in the current study between the CLC assessments at 4 and 9 weeks of age, aside from the CLC in which the majority of the animals were consistently scored as normal, as well as the associations within an experiment not always recurring in both assessments. The second possibility is that CLC assessed at a young age (4 weeks of age in the current studies) are still liable to change throughout the sensitive time period for OC development. This might indicate that CLC assessed at this time frame (9 and 11 weeks of age in the current studies) have a higher impact on OC development as a causative factor, as abnormalities in CLC might cause local overloading of joints in the sensitive time period of OC development. Any changes in the CLC occurring after the sensitive time period of OC development might be of less importance as a causing factor. A

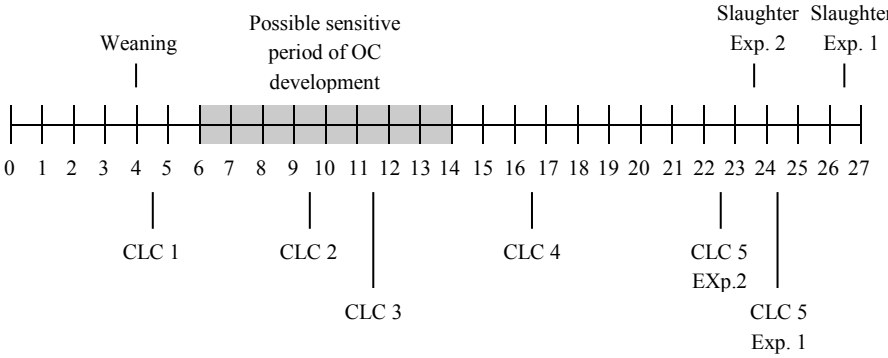

Figure 4.3. Timeline (in weeks of age) of conformation and locomotive characteristics (CLC) assessed with the possible sensitive time period for osteochondrosis (OC) development. The CLC 1, 2, 3, 4, and 5 represent the 5 assessments of CLC performed for all gilts in Exp. 1 and Exp. 2.

third possibility is that the period of OC development is highly dynamic where OC lesions develop but can also be repaired or regress (Woodard et al., 1987; Henson et al., 1997; Dik et al., 1999; van Weeren and Barneveld, 1999; Ytrehus et al., 2004bc; Olstad et al., 2007). Any CLC in that period might then be unreliable as OC lesions might still be repaired and are thus not a causative factor for OC development. Only the CLC assessed after OC development (22 to 24 weeks of age in the current study) might then more reliably be associated with OC in such a way that the presence of OC lesions lead to CLC (as the CLC were not affecting OC development in the sensitive time frame). Although in the current studies several CLC are associated more than once with OC during the rearing period, inconsistency still remains to conclude which of the aforementioned possibilities is likely true. The CLC are relatively easy to assess throughout the life of an animal, whereas OC is usually assessed after slaughter of the animals. It, therefore, remains problematic to conclude whether OC or CLC are causative of each other. However, due to inconsistencies in the current study in the direction of association with OC and repeated associations of similar CLC over all assessments, it seems that using subjectively assessed CLC at young age as an indicator of OC status in growing gilts is unreliable. Certainly, studies assessing the association of CLC and OC need to take into account that both conditions are age dependent to unravel their association with each other and may include in vivo measurements of OC such as X-ray imaging or computed tomography scanning methods of the joints. Additionally, new studies indicate that there might be possibilities in objectively assessing gait and stance parameters using, for example, motion capture or pressure mat analysis (Meijer et al., 2014; Stavrakakis et al., 2014). This will, however, require more material and time for an on-farm assessment as compared to subjective assessment of CLC but may result in more reliable findings.

4.6 CONCLUSION

The associations of CLC in growing gilts assessed at several ages on OC prevalence at slaughter were studied. Several CLC were found to be associated with OC at different ages, although no clear consistent associations could be found. The CLC that were more frequently associated with OC were X-shaped hind legs, straight or bow hind legs, straight and sickled hocks, swaying hind quarters, and steep or weak pasterns of the front legs. These might indicate the more important

CLC to monitor. However, the aim of this study was to find consistent associations of CLC with OC in the same direction throughout the early life of breeding gilts that could possibly be used as a tool for assessing OC in vivo. Because of relatively inconsistent associations over several ages or between experiments, the use of CLC as an in vivo measurement to assess OC status in growing gilts is uncertain or at the least very complicated. The practical usefulness of recording CLC is, therefore, at this point limited.

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CHAPTER 5

Association of natural (auto-) antibodies in young gilts with osteochondrosis at slaughter [1]

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5.1 ABSTRACT

Osteochondrosis (OC) develops at a young age and has been associated with lameness and reduced longevity of sows. Early detection of OC is therefore beneficial for selection against OC. Possibly, immunological components within the blood may serve as an indicator for OC development and could, therefore, be used as a biomarker. Levels of naturally occurring (auto-) antibodies (N[A]Ab) have been associated with homeostatic imbalance and various forms of inflammation, and may have an association with OC. The aim of this study was to investigate possible associations between the presence and levels of N(A)Ab of the IgM and IgG isotypes at an early age with OC in growing gilts at slaughter (24 weeks of age). Plasma samples were obtained from 212 Topigs 20 (Dutch Large White x Dutch Landrace) gilts at 6, 10, and 24 weeks of age and analyzed for N(A)Ab titers against 11 (auto-) antigens using ELISA. After slaughter, the elbow, hock, and knee joints were macroscopically examined for OC status. Due to low prevalence of OC in the elbow joint (5.4%), the elbow joint was not taken into account in analyses. Significant ($P \leq 0.05$) associations with OC in both the hock joint and at the animal level (all joints combined) were found for IgM titers against chondroitin sulfate A at 6 weeks of age (OR 1.4 and 1.5), actin at 6 weeks of age (OR 1.4 and 1.3), thyroglobulin at 24 weeks of age (OR 1.5 and 1.3), and IgG titers against insulin at 6 weeks of age (OR 1.7 and 1.4). Additionally, significant ($P \leq 0.05$) associations with OC were found at the knee joint for IgM titers against albumin at 6 weeks of age (OR 2.3), at the hock joint for IgM titers against keyhole limpet hemocyanin at 6 weeks of age (OR 1.4), and at the animal level for IgM titers against actin at 24 weeks of age (OR 1.3). This study indicated for the first time associations between the presence and levels of N(A)Ab at a young age and OC at 24 weeks of age in breeding gilts.

Keywords: gilts, naturally occurring antibodies, osteochondrosis

5.2 INTRODUCTION

Osteochondrosis (**OC**) has been associated with leg weakness, lameness, and reduced longevity of pigs (Dewey et al., 1993; Jørgensen and Sørensen, 1998; Yazdi et al., 2000). Detecting OC at early age would benefit selection against animals with increased risk for OC, especially breeding and multiplier sows that have to last several parities. However, early detection methods of OC at young age seems limited.

Osteochondrosis involves the formation of necrotic cartilage due to vascular disruption in epiphyseal growth cartilage, which is suggested to occur around 10 weeks of age with a time frame of several weeks (Ytrehus et al., 2004abc; Olstad et al., 2014) and has been associated with the presence of cartilage breakdown products, hormones, and minerals within the blood (Sloet van Oldruitenborgh-Oosterbaan et al., 1999; Billinghurst et al., 2004; Gangl et al., 2007; de Grauw et al., 2011). Natural (auto-) antibodies (**N[A]Ab**) are defined as being present within the body without prior specific immune activation or antigenic challenge (reviewed by Avrameas, 1991; Avrameas et al., 2007) and have been implicated in maintaining homeostasis of the body by clearing debris from damaged cells (reviewed by Lutz et al., 2009; Binder, 2012; Elkon and Silverman, 2012), thereby preventing or reflecting inflammation. Presence and (changing) levels of N(A)Ab might thus be a possible new serological marker for increased risk of OC in pigs. This could provide evidence for a role of the immune system in the occurrence of OC as suggested for horses and pigs (Osborne et al., 1995; Rangkasenee et al., 2013).

The aim of this study was to assess whether levels of the IgM and IgG isotypes of N(A)Ab are present in young gilts and are associated with OC at slaughter over the previously described and modeled effects of floor type (de Koning et al., 2014). Levels of N(A)Ab might then add to predict risk of OC affliction in breeding gilts at an early age.

5.3 MATERIALS AND METHODS

5.3.1 Animals and Treatments

This association study is part of a study that assessed the age dependent effects of floor type on OC prevalence (de Koning et al., 2014). The experiment

consisted of 212 Topigs 20 (Dutch Large White x Dutch Landrace) gilts acquired from a commercial breeding company (TOPIGS, Veldhuizen Wehl, Wehl, the Netherlands). Gilts were assigned to 1 of 4 treatments and 1 of 32 pens of 6 to 7 individuals after weaning at, on average, 4 weeks of age, based on an equal distribution of littermates and body weight measured 1 week before the start of the experiment. Gilts from 1 dam were equally divided over treatments as much as possible to prevent that 1 litter received only 1 treatment. Pens consisted of 8.37 m^2 of surface area and were equally divided over 4 departments. Gilts were housed either on a 60% slatted floor and 40% solid floor (conventional) or on 25 to 50 cm deep bedding of wood shavings. Briefly, treatments consisted of a conventional floor from weaning until slaughter (**CC**); wood shavings as bedding from weaning until slaughter (**WW**); a conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding (**CW**); wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a conventional floor (**WC**). Manure areas were removed as much as possible up to 4 times a week, after which wood shavings were replenished. Gilts were given ad libitum access to water and feed. For further details see de Koning et al. (2014).

5.3.2. Antibody Titer Assessment

Blood Sampling. Blood samples were collected in EDTA tubes before the treatment switch at 6 and 10 weeks of age from the jugular vein and at slaughter at 24 weeks of age. Blood samples were centrifuged for 10 minutes at 3000 *g* at 4°C and plasma was stored at -20°C until use.

Enzyme Linked Immunosorbent Assay. The IgM and IgG antibody titers were assessed by indirect two-step enzyme linked immunosorbent assay (**ELISA**) against 11 exo- and auto-antigens: keyhole limpet hemocyanin (**KLH**), bovine actin (**ACT**), ovalbumin (**OVA**), and porcine albumin (**ALB**), -hematin (**HEMA**), -hemoglobin (**HEMO**), -thyroglobulin (**THYRO**), -chondroitin sulfate A (**CS-A**), -insulin (**INS**), and –myosin (**MYO**), all from Sigma-Aldrich Co. LLC. (St. Louis, Missouri), and egg white lysozyme (**LYSO**) (Merck Millipore, Billerica, Massachusetts), as shown in Table 5.1.

Ninety-six-wells ELISA microtiter medium binding plates (Greiner Bio-One GmbH, Frickenhausen, Germany) were coated with antigens diluted in carbonate/bicarbonate buffer (0.05 M Na_2CO_3, 0.05 M $NaHCO_3$ pH 9.6) as

specified in Table 5.1. Plates were incubated overnight at 4°C and subsequently washed with tap water containing 0.05% Tween 20. For assessment of antibody titers against HEMA, HEMO, MYO, and THYRO, an additional blocking step was done with 2% horse serum during 1 hour prior to administration of plasma samples. Optimal dilutions for samples and standard controls were determined beforehand. Plasma samples were administered to the plates in 4-step serial dilutions at a 1:3 ratio with a starting dilution of 1:30 in phosphate buffered saline (PBS) pH 7.2 containing 0.5% horse serum and 0.05% Tween 20. In the final assessment of the IgG antibody titers against CS-A, MYO, and INS, the plasma samples were diluted in PBS buffer with bovine serum instead of horse serum and administered to the plates with a 1:4 starting dilution and 4-step serially diluted at a 1:2 ratio. Plasma earlier collected from a sow served as a standard control in two columns on all plates (standard controls). Starting dilutions of the standard controls, which were 8-step serially diluted at a 1:2 ratio, are displayed in Table 5.1. After administration of plasma samples and standard controls, plates were incubated at room temperature for 1.5 hours and subsequently washed with tap water containing 0.05% Tween 20. Isotype specific peroxidase conjugated anti-porcine antibodies were administered to the plates 1:40,000 diluted for IgG (horse radish peroxidase [PO] conjugated goat anti porcine IgG-Fc, Bethyl Lab. Inc., Montgomery, Texas), and 1:20,000 diluted for IgM (IgM/PO, Bethyl Lab. Inc., Montgomery, Texas) in PBS containing 0.5% horse serum and 0.05% Tween 20, and incubated for 1.5 hours. After washing, sodium acetate buffer pH 5.5 with tetramethylbenzidine and urea hydrogen peroxide (comparable with 0.05% H_2O_2) were added and incubated for 10 min at room temperature to initiate the enzymatic color reaction. The reaction was stopped with 50 µL of 1.25 M H_2SO_4. Optical density (**OD**) of each sample was measured with a Multiscan GO (Thermo Fisher Scientific, Vantaa, Finland) at a wavelength of 450 nm.

Titer Calculation. Titers were calculated as described earlier (Frankena, 1987) and as used repeatedly (Schrama et al., 1997; Bolhuis et al., 2003; Lammers et al., 2004; Parmentier et al., 2004; Star et al., 2007; van Knegsel et al., 2007; 2012; Reimert et al., 2014). Briefly, the ODs of the standard controls were averaged and logit values of the ODs were calculated by taking the natural logarithm of the OD of a well divided by the maximum averaged OD of the standard controls (**maxOD**) minus the respective OD:

Table 5.1. Antigen coating and starting dilutions of the standard controls (std. con.) used for IgG and IgM at the 3 assessments at 6, 10, and 24 weeks (wk) of age.[1]

Antigens	Coating (µg/ml)	IgG std. con.			IgM std. con.		
		6 wk	10 wk	24 wk	6 wk	10 wk	24 wk
Exo-antigens							
KLH	4	1:40	1:40	1:40	1:80	1:80	1:40
Lysozym	4	1:40	1:10	1:10	1:160	1:80	1:80
Ovalbumin	4	1:40	1:10	1:10	1:80	1:40	1:40
Actin	4	1:40	1:40	1:40	1:40	1:40	1:40
Auto-antigens							
Albumin	4	1:5	1:5	1:5	1:20	1:40	1:10
Chondroitin sulfate A[2]	8	1:5	1:5	1:5	1:20	1:20	1:20
Hematin	8	1:20	1:20	1:20	1:80	1:40	1:40
Hemoglobin	8	1:40	1:10	1:10	1:80	1:80	1:80
Insulin[2]	5	1:5	1:5	1:5	1:20	1:20	1:20
Myosin[2]	4	1:20	1:20	1:5	1:80	1:80	1:80
Thyroglobulin	4	1:20	1:80	1:20	1:80	1:80	1:80

[1] Appropriate starting dilutions were assessed beforehand.

[2] At the third assessment at 24 weeks of age, coating of the microtiter plates was 1µg / ml to acquire appropriate signals.

$$Logit\ OD\ = \ln \frac{OD}{(\max OD - OD)}$$

A linear regression line of the logit ODs against the respective $\log_2$ dilution values of the standard controls was determined. The last positive well was placed standard at the sixth row of the standard controls. The estimated logit OD at the last positive well (**OD lpw**) was calculated with the estimated linear regression function using the $\log_2$ dilution value of the last positive well. For each 4-step serially diluted plasma sample of the gilts, the logit OD was calculated of the OD closest to 50% of the maxOD according to the previous logit OD formula (**Logit OD_{sample}**). Titers of the plasma samples were then calculated by taking the Logit OD_{sample} and the $\log_2$ dilution value at which that OD occurs [**$Log_2(Dilution_{sample})$**], the OD lpw, and the regression coefficient β from the estimated linear regression function of the standard controls, using the following formula:

$$Titer = \frac{\text{OD lpw} - [\text{Logit OD}_{sample} \ - \ \beta \ * \ \text{Log}_2(Dilution_{sample}\ )]}{\beta}$$

5.3.3 Osteochondrosis Assessment

Slaughtering procedure and OC assessment was performed as previously described (de Koning et al., 2014). Briefly, animals were slaughtered at on average 24 weeks of age and carcasses were stored for 1 d at 4 °C, after which all 4 legs from each gilt were collected. After collection, the legs were stored at 4 °C for a maximum of 2 d until dissection of the elbow, knee, and hock joints of all legs. After dissection of the joints, the joint surface was macroscopically assessed for presence and severity of OC using a 5-point grading scale from 0 to 4 (as described by van Weeren and Barneveld, 1999) on a total of 22 locations (de Koning et al., 2013). No abnormalities were indicated with score 0, flattening of the cartilage was indicated with score 1, slight irregularities were indicated with score 2, severe irregular cartilage was indicated with score 3, and OC score 4 indicated the severest form of OC with (partially) loose articular cartilage fragments and /or osteochondral cysts [for figures depicting OC lesions see van Grevenhof et al., (2011)]. Osteochondrosis was scored by one veterinarian specialized in orthopedics, unaware of the treatments, and experienced in judging OC.

5.3.4 Statistics

Antibody Titers Data. The antibody titers were used as covariates in statistical modeling. Missing values were generated when OD signals were non-optimal. Adequate OD signals of plasma samples and standard controls for valid IgG titer calculation could not be attained for the antigens MYO and CS-A at all moments, ALB at 6 weeks of age, and THYRO at 24 weeks of age, and were therefore not assessed in statistical analyses. For IgG, OD signals of plasma binding several antigens were attained but often low (OD lower than 0.1) or did not follow a continuous decline throughout the 4-step dilutions. This was true for IgG titers binding ALB at 10 and 24 weeks of age, HEMA at 6 weeks of age, THYRO at 6 and 10 weeks of age, and INS at all 3 moments. However, for some gilts the OD signal was strong indicating that IgG titers against ALB, HEMA, THYRO, and INS were present. This suggested that some animals do not have IgG antibodies binding those antigens (weak OD signals) but others do (strong OD signals). As weak OD signals present problems with correct titer calculation, the titer could not be validly calculated for ALB, HEMA, THYRO, and INS at the mentioned ages. In these cases, titers were converted to a 0 and 1 variable. A 0 indicates that there was

not a good OD signal present, indicating that there were no titers present. An 1 indicates that there was an OD signal present, which indicates that there were titers present. Those titers that were converted to a binary variable were consequently entered in the models as class effects.

Osteochondrosis Data. The locations for which no OC was found for all animals were omitted from the analysis as they are not informative. Ordinal logistic regression analysis as described in de Koning et al. (2014) to analyze association of N(A)Ab titers with OC, was deemed not appropriate as fit statistics indicated an inappropriate model fit under the expected distribution due to insufficient observations between treatment groups, OC scores for each joint separately, and continuous variables or class effects. Each OC score obtained from the macroscopic assessment was converted to a binary variable in which 0 indicates no OC (OC score 0) and 1 indicates an OC score higher than 0 (any form of OC). This transformation allows for more appropriate binary logistic regression to be applied (de Koning et al., 2014) as described below in more detail.

Statistical procedure. A principal component analysis (**PCA**) was performed separately on the N(A)Ab for each isotype and the age at which N(A)Ab titers were assessed to determine whether strong associations exist between the N(A)Ab within an assessment and whether the N(A)Ab could be summarized in 1 component to be used for further statistical analysis. Only the antibody titers that were not converted to binary variables were analyzed. The PCA was performed using PROC PRINCOMP in the statistical software package SAS 9.2 (SAS Institute Inc. 2002 - 2008, Cary, NC, USA).

The effects of floor type on OC prevalence were described elsewhere (de Koning et al., 2014). To assess associations of N(A)Ab titers with OC prevalence, the titers from one age were added separately as a linear covariate to the previously developed statistical logistic regression model (see de Koning et al., 2014), except for the antibody titers that were converted to a binary variable, which were added to the model as class effects. Statistical analyses were not performed for the elbow joint as OC prevalence (5.4%) was too low. The previously described statistical logistic regression model includes the fixed class effects of treatments imposed (CC, CW, WC, WW), fixed class effects of departments at which the gilts were kept (department 1, 2, 3, 4), the random effect of the experimental unit pen nested within treatment and department (pen 1, 2, …, 32), and the random effect of gilts

nested within dams from which the gilts descended (dam 1, 2, ….., 40). Fixed interaction effects between treatments imposed and the N(A)Ab titers were assessed only at the hock joint and at the animal level (OC prevalence for the knee joint was too low to assess interaction effects) and presented in the text when significant. The analysis for each joint separately and at the animal level was performed as a repeated measurement analysis on the binary OC scores from the locations assessed within each joint of the bilateral homologues for OC with gilts as the subject. Analyses were performed using PROC GLIMMIX in the statistical software package SAS 9.2. For the titers that were significantly ($P \leq 0.05$) associated with OC, the average titer for OC affected animals and OC unaffected animals, the regression coefficient (β) from the statistical model and accompanying SE, and the odds ratio (**OR**) corresponding to the β are displayed.

5.4 RESULTS

The percentage of gilts affected with any form of OC was 44.1% in the hock joint, 17.6% in the knee joint, and 58.3% at the animal level. As mentioned before, OC prevalence in the elbow joint was too low (5.4%) to be taken into account for statistical analysis (de Koning et al., 2014).

5.4.1 Principal Component Analysis

The results of the first components created by PCA for both isotypes and age assessment are shown in Table 5.2. For both isotypes at all moments, the first component only explained less than 43% of the total variance, except for IgM at 24 weeks of age which explained approximately 52% of the total variance. The created first components all had an eigenvector loading pattern that pointed in the same direction and were roughly equally loaded between the N(A)Ab titers, but loading patterns were relatively low: approximately 0.3 to 0.4 for IgM, and 0.3 to 0.5 for IgG. The proportion of explained variance along with the strength of the loading pattern suggested that the N(A)Ab titers were only slightly associated with each other. For IgM, all other created components explained less than 11% (data not shown). For IgG, all other created components explained less than 22% (data not shown). Although the components for IgG after the first component seem to explain more variance, a clear distinctive pattern was not recognized. For both isotypes at each moments, more than half of the components created (total created

Table 5.2. Results from principal component analysis (PCA) performed on the IgG and IgM titers against the antigens measured for each assessment at 6, 10, and 24 weeks (wk) of age. [1]

Item	IgM			IgG[2]		
	6 wk	10 wk	24 wk	6 wk	10 wk	24 wk
Variance accounted (%)	42.3% (n=158)	40.0% (n=202)	52.3% (n=195)	34.2% (n=173)	32.8% (n=162)	26.2% (n=163)
Eigenvectors						
KLH	0.31	0.30	0.30	0.56	0.48	0.44
Actin	0.38	0.37	0.27	0.51	0.40	0.35
Lysozyme	0.29	0.22	0.28	0.26	0.30	0.43
Ovalbumin	0.34	0.31	0.32	0.34	0.50	0.39
Hemoglobin	0.27	0.33	0.28	0.49	0.44	0.51
Hematin	0.28	0.29	0.31		0.28	0.30
Thyroglobulin	0.30	0.29	0.31			
ChondroA	0.29	0.27	0.32			
Insulin	0.25	0.28	0.29			
Myosin	0.28	0.38	0.31			
Albumin	0.30	0.25	0.32			

[1] The variance accounted for and the eigenvector loading pattern are displayed for the first components calculated from the PCA, which by default is the component that explains the most variance of the original variables. Missing values were present for each individual variable and created a total of missing values that resulted in different total observation used for the analysis for the different assessments.

[2] Only the antigens were assessed for IgG for which titers could be calculated (see materials and methods).

components is equal to the number of variables entered) was necessary to attain a proportion of explained variance of at least 80%. The PCA was therefore not successful as a variable reducing method for which the components could be used for further statistical analysis.

5.4.2 Associations Between Natural (Auto-) Antibodies and Osteochondrosis

Significant associations ($P \leq 0.05$) between N(A)Ab and OC are shown in Table 5.3. Significant associations were predominantly found for IgM titers at 6 weeks of age. At the knee joint, each unit increase in IgM titers at 6 weeks of age against ALB was associated with a 2.3 greater odds to be affected with OC. At the hock joint, each unit increase in IgM titers against KLH at 6 weeks of age was associated with a 1.4 greater odds to be affected with OC. At the animal level, each unit increase in IgM titers at 24 weeks of age against ACT was associated with a

Table 5.3. Average natural (auto-) antibody (N[A]Ab) titers and the regression coefficient and odds ratio (OR) from statistical models for osteochondrosis (OC) in the knee joint, hock joint and at the animal level (all joints combined).[1]

Item	Isotype	Age (wk)[2]	Average (SE) titer[3]		β (SE)[4]	OR[5]	*P*-value[6]
			No OC	OC affected			
Knee							
Albumin (n=176)	IgM	6	3.41 (0.07) (n=145)	3.95 (0.16) (n=36)	0.83 (0.33)	2.3	0.01
Hock							
KLH (n=198)	IgM	6	3.50 (0.07) (n=110)	3.73 (0.08) (n=88)	0.33 (0.17)	1.4	0.05
Actin (n=200)	IgM	6	4.78 (0.07) (n=112)	5.02 (0.08) (n=88)	0.35 (0.17)	1.4	0.04
Thyroglobulin (n=201)	IgM	24	7.06 (0.06) (n=113)	7.24 (0.07) (n=88)	0.43 (0.18)	1.5	0.02
Chondroitin Sulf. A (n=184)	IgM	6	0.73 (0.05) (n=114)	0.94 (0.06) (n=82)	0.58 (0.23)	1.5	0.01
Insulin (n=200)	IgG	6	-	-	0.54 (0.26)	1.7	0.04
Animal level							
Actin (n=200)	IgM	6	4.70 (0.08) (n=84)	5.02 (0.07) (n=116)	0.27 (0.12)	1.3	0.02
(n=201)	IgM	24	6.83 (0.07) (n=84)	7.10 (0.07) (n=117)	0.26 (0.11)	1.3	0.02
Thyroglobulin (n=201)	IgM	24	7.04 (0.07) (n=84)	7.21 (0.06) (n=117)	0.27 (0.13)	1.3	0.03
Chondroitin Sulf. A (n=184)	IgM	6	0.71 (0.06) (n=74)	0.90 (0.05) (n=110)	0.35 (0.16)	1.4	0.02
Insulin (n=200)	IgG	6	-	-	0.37 (0.18)	1.4	0.04

[1] Only the titers against the antigens that were significantly associated with OC are displayed. The titers against the antigens were assessed in statistical models as linear covariates, except for insulin which was assessed as either no titer present or titer present (see materials and methods). Therefore, no averages could be calculated for titers against insulin. The number of observations used for each antigen assessed differs as signals from some observations from the ELISA assessment were deemed bad and were treated as missing values. *(continued on the next page)*

1.3 greater odds to be affected with OC. At the animal level an interaction effect (not shown in Table 5.3) was found for IgM titers against MYO assessed at 10 weeks of age and treatments imposed, which specified that each unit increase in IgM titers against MYO was associated with 1.1 greater odds to be affected with OC for gilts in the CC treatment; 0.4 lower odds to be affected with OC for gilts in the CW treatment; 1.5 greater odds to be affected with OC for gilts in the WC treatment; and associated with no change in OC status for gilts in the WW treatment (OR = 1.0). For both the hock joint and at the animal level, each unit increase in IgM titers against ACT at 6 weeks of age, THYRO at 24 weeks of age, and CS-A at 6 weeks of age was associated with 1.3 to 1.5 greater odds to be affected with OC. The only significant IgG titer association with OC was found for (the class effect) INS at 6 weeks of age at the hock joint and at the animal level, which indicated that gilts with titers against INS had 1.7 to 1.4 greater odds, respectively, to be affected with OC. The difference between the average titers of gilts affected with OC and gilts not affected with OC was in general a difference of less than half a titer point.

5.5 DISCUSSION

In this study, we found evidence that presence, levels, and isotypes of natural (auto-) antibodies (N[A]Ab) in gilts at an early age are associated with an increased odds for osteochondrosis (OC) at slaughter at 24 weeks of age.

5.5.1 Analysis of Natural (Auto-) Antibodies

Levels and isotypes of N(A)Ab were associated with maintenance of homeostasis and prevention of disease by clearance of damaged cells, intracellular components, cell waste products or neo-epitopes (reviewed by Ochsenbein and

Table 5.3 continued

[2] The age is depicted in weeks (wk).

[3] Average titers and SE were calculated for OC affected animals and animals that were not affected with OC as an indication of the extent of the differences found.

[4] The regression coefficients (β) and the SE are presented from the statistical models where the titers of the N(A)Ab were assessed as linear covariates on OC.

[5] Odds ratios (OR) corresponding to the regression coefficients indicate the odds of a gilt to have OC with a 1 unit titer increase.

[6] The *P*- values are given corresponding to the regression coefficients of the titers from the statistical models.

Zinkernagel, 2000; Zelenay et al., 2007; Lutz et al., 2009; Ehrenstein and Notley, 2010; Binder, 2012; Bouhlal and Kaveri, 2012; Elkon and Silverman, 2012; Grönwall et al., 2012; Santos-Argumedo, 2012; Avrameas et al., 2007). It was proposed that clearance of these components restricts damaging inflammatory reactions in various tissues including bones, joints, cardiovascular tissues, and renal tissues. In the present study, several antigens were analyzed for N(A)Ab titers. Most of the antigens used can be considered as auto-antigens as they were generated and harvested from pigs. Other antigens were not auto-antigens (KLH, LYSO, ACT and OVA). The choice of antigens assessed was arbitrary as this was (to our knowledge) the first attempt to relate N(A)Ab with OC in pigs. The antigen KLH has been used frequently as a model antigen for general assessment of NAb titers within various species including pigs (Reimert et al., 2014), cattle (van Knegsel et al., 2007; van Knegsel et al., 2012), and chicken (Lammers et al., 2004; Star et al., 2007). Chondroitin sulfate A is a component of proteoglycans which is a major component of growth cartilage (Byers et al., 1992; Cortes et al., 2009; Mourao, 1988; and reviewed by Greenwald et al., 1978; Roughley, 2006; Kheir and Shaw, 2009). Actin and myosin are components of the cellular cytoskeleton including chondrocytes (Hale et al., 1983; Durrant et al., 1999; Knight et al., 2006; Sanz-Ramos et al., 2012; Sauter et al., 2012). All other antigens were assessed more arbitrarily to assess whether other N(A)Ab titers could be found within pigs and whether they were associated with OC.

For the majority of tested antigens the ELISA provided clear and adequate signals for the IgM isotype. For the IgG isotype, less clear and adequate signals were found for the antigens MYO and CS-A at all 3 moments, ALB at 6 weeks of age, and THYRO at 24 weeks of age. These results suggest that either IgG antibodies against these antigens were not present in the plasma samples, or that IgM levels were that high that binding of IgG antibodies was inhibited by competition. For ALB, HEMA, THYRO, and INS a relatively large part of the gilts showed clear IgG signals, which indicates that some gilts had IgG titers against these antigens. It was previously suggested that N(A)Ab are mainly of the IgM isotype (reviewed by Ochsenbein and Zinkernagel, 2000; Schwartz-Albiez et al., 2009) which may explain the stronger signals obtained for IgM. However, others show that IgG N(A)Ab are present as well (Cukrowska et al., 1996; Nagele et al., 2013; and reviewed by Avrameas, 1991; Avrameas et al., 2007). It was proposed

that a shift from IgM to IgG binding auto-antigens may reflect disease status of an individual, but in the current study we could not find strong evidence for IgG binding antigens in association with OC.

The PCA results suggest that the N(A)Ab titers were associated with each other to a small degree and indicated that an increase in titers for one of the antigens assessed is associated to a small degree with an increase of titers against other antigens. Thus, PCA suggested that different N(A)Ab titers are likely independent entities as was partially suggested earlier for poultry (Berghof et al., 2010). This urges that more antigens should be assessed to attain information about relations between N(A)Ab and disease/condition.

5.5.2 Associations of Natural (Auto-) antibodies and Osteochondrosis

To our best knowledge, this is the first study that suggest associations between OC and both N(A)Ab isotypes and idiotypes in pigs. Others associated OC with increased levels of immune complexes and antibodies binding collagen breakdown products in horses (Osborne et al., 1995) or a variety of hormones, plasma components (growth- and, parathyroid hormones, calcium, etc.), and collagen breakdown biomarkers in horses and pigs (Sloet van Oldruitenborgh-Oosterbaan et al., 1999; Billinghurst et al., 2004; Gangl et al., 2007; Frantz et al., 2010; de Grauw et al., 2011). Various associations of immune reactivity were found with osteoarthritis (reviewed by Haseeb and Haqqi, 2013), which is an advanced degenerative joint disorder and therefore not comparable with OC development at young age. Recently it was indicated in pigs that there are genes associated with the immunological system in relation with OC in pigs (Rangkasenee et al., 2013), indicating an unknown role for the immune system in OC as either a primary contributor of OC development or a secondary response of OC development. The mechanisms that explain the current associations of N(A)Ab with OC remain unknown and speculative. Osteochondrosis lesions are reported to develop around 10 weeks of age, with a time window spanning several weeks before and after 10 weeks of age (Ytrehus et al., 2004abc; Olstad et al., 2014) and remaining relatively stable thereafter. Therefore, associations of N(A)Ab at young age (6 weeks of age in the current study) with OC may reflect the initial clearance of debris resulting from the initial phases of OC onset and development before 10 weeks of age. Alternatively, N(A)Ab levels may indicate immune competence of

gilts at an early age that determine the reactions to developing OC lesions. Since several N(A)Ab at very young age were associated with OC it is unlikely that these N(A)Ab are causative of OC as noted partially by Osborne et al. (1995) for immune complexes associated with various joint disorders. Hardly any associations were found of N(A)Ab at 24 weeks of age and OC, which suggests that development of OC may have ceased.

Various antigens tested are related with chondrocyte physiology or cartilage. The ACT (at 6 and 24 weeks of age), THYRO (at 24 weeks of age), CS-A (at 6 weeks of age), MYO (at 10 weeks of age), and INS (at 6 weeks of age) antigens were associated with OC at both the hock joint and the animal level. Chondroitin sulfate A is a component of the cartilage matrix, and ACT and MYO are components of the cytoskeleton of chondrocytes as discussed above. These components may be released during chondrocyte necrosis during OC development and cleared by N(A)Ab, as indicated for CS-A in rheumatoid arthritis (György et al., 2008). Insulin has been implicated with proliferation and survival of chondrocytes (Böhme et al., 1992; Alini et al., 1996; Henson et al., 1997) and thyroglobulin is necessary for the production of thyroid hormones necessary for chondrocyte differentiation (Böhme et al., 1992; Ballock and Reddi, 1994; Alini et al., 1996; Jiang et al., 2008; Wang et al., 2010), but explanations of the associations of N(A)Ab with OC remain difficult and speculative. Finally, associations of OC with N(A)Ab titers against KLH and ALB were found, which do not have a clear direct relation with OC. It is tempting to speculate that association of OC and N(A)Ab to KLH and ALB reflect general non-specific enhancement of (auto-) antibody levels in response to increased debris levels. In this sense it is necessary for future studies to put more focus on antigens or neo-forms thereof related to OC (such as cartilage matrix components) to indicate to what degree the association of different N(A)Ab with OC are specific for OC development.

The difference in average titers for OC affected and OC of unaffected animals were on the whole less than half a titer point, which suggests that N(A)Ab are less appropriate as a predictive characteristic, although N(A)Ab titers were assessed systemically and associated with a very localized affliction. It is unknown whether a shorter time interval between estimation of N(A)Ab levels and exact moment of OC development and assessment in an individual animal would result in larger differences in titer points between affected and unaffected pigs. In

correspondence with our study, other studies assessing systemic N(A)Ab antibody titers with a certain condition also often found less than 1 or 0.5 titer point differences in pigs (Schrama et al., 1997; Bolhuis et al., 2003; Reimert et al., 2014) and dairy cattle (van Knegsel et al., 2007; van Knegsel et al., 2012). These studies indicate that systemic differences in N(A)Ab levels, although small, can be associated to different aspects of the body or different disease status (Nagele et al., 2013). A complicating factor is that the analysis required the combining of OC into a binary variable, which may miss the effect of severe lesions (severe damage) to the joint and the reaction of N(A)Ab thereof. However, due to the small differences found, the current study cannot indicate that N(A)Abs are an accurate in vivo tool for the risk of OC development in practice.

5.6 CONCLUSIONS

Possible associations were found in pigs between plasma N(A)Ab levels and isotypes in early life with OC at slaughter (24 weeks of age). However, associations found were relatively small. Predominantly, associations of OC were found with IgM antibodies measured at 6 weeks of age binding actin, chondroitin sulfate A, albumin, and IgG antibodies measured at 6 weeks of age binding insulin. The exact mechanism of associations between N(A)Ab and OC remain elusive and, therefore, their use as an in vivo indicator at young age for OC at slaughter remains speculative and awaits further studies. Future studies need to elaborate on the repertoire of antigens that are possibly recognized to assess whether ‘antigen specific’ N(A)Ab titers have a stronger association with OC before N(A)Ab can be used in vivo as an indicator of OC status.

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CHAPTER 6

Effects of a high carbohydrate diet and arginine supplementation during the rearing period of gilts on osteochondrosis prevalence[1]

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6.1 ABSTRACT

Osteochondrosis (OC) is a consequence of areas of necrotic growth cartilage early in life and is suggested to be associated with lameness and premature culling of sows. Insulin, glucose, and insulin-like growth factor-1 (IGF-1) are associated with OC in horses and are affected by dietary composition. If dietary composition can affect OC through metabolic parameters in sows, it could be a tool in practice to reduce OC prevalence. This study examined if OC prevalence in rearing gilts can be influenced by dietary carbohydrates and / or arginine by affecting IGF-1, insulin, glucose, and nitric oxide (NO) levels. Gilts (n=212; Dutch Large White x Dutch Landrace) were acquired after weaning (4 weeks of age). At 6 weeks of age, gilts were subjected to a 2x2 factorial treatment design of dietary carbohydrate and arginine level. Carbohydrate level consisted of 12.5% cornstarch and 12.5% dextrose added to a basal diet (C+) versus an isocaloric diet in which cornstarch and dextrose were replaced with 8.9% soya bean oil (C-). Arginine supplementation consisted of 0.8% arginine supplemented to a basal diet (A+) versus 1.64% alanine as the isonitrogenous control (A-). At 24 weeks of age, regular blood samples of 34 gilts around feeding were taken and assessed for insulin, glucose, IGF-1, and NO levels. After slaughter at 25 weeks of age, OC was scored on the elbow, knee, and hock joints. Gilts in the C- treatment had higher glucose and insulin levels 90 minutes after feeding onwards and higher IGF-1 levels than gilts in the C+ treatment ($P < 0.05$). Arginine supplementation did not significantly affect metabolic parameters. Arginine supplementation tended to decrease OC prevalence ($P = 0.07$) at the animal level (all joints combined) and in the knee joint. Carbohydrate treatment affected prevalence of OC only in the knee joint in which gilts in the C- treatment had higher odds to have OC (OR = 2.05, CI: 1.18-3.58) than gilts in the C+ treatment. Additionally, body weight at slaughter was significant when added to the statistical model ($P < 0.01$) in the knee joint and the animal level (per 10 kg increase OR=1.33, CI=1.11-1.6 and OR=1.17, CI=1.05-1.31, respectively), while the treatment effects became non-significant. This study found effects of carbohydrates on OC prevalence in gilts at slaughter. The dietary treatment effects found in the current study likely have been mediated through effects on body weight.

Keywords: arginine, carbohydrate, fat, gilts, osteochondrosis

6.2 INTRODUCTION

Formation of necrotic cartilage due to vascular disruption at young age in the epiphyseal growth cartilage is the first step in osteochondrosis (**OC**) development and suggested as a cause of lameness and premature culling in sows (Yazdi et al., 2000; Olstad et al., 2014; de Koning et al., 2015; and reviewed by Ytrehus et al., 2007). Reparative attempts by chondrocytes and vasculature have been suggested to occur (Ytrehus et al., 2004bd; 2007; 2008ac; and reviewed by Ytrehus et al., 2007). Feeding practices may affect OC prevalence in sows by affecting chondrocyte functioning or growth.

Diets could influence OC through metabolic parameters such as glucose, insulin, and insulin-like growth factor-1 (**IGF-1**) as shown in horses (Ralston, 1996; Sloet van Oldruitenborgh-Oosterbaan et al., 1999; Pagan, 2001). Insulin and IGF-1 are suggested to affect survival and proliferation of chondrocytes (Böhme et al., 1992; Hunziker et al., 1994; Alini et al., 1996; Henson et al., 1997a) and are involved in growth (Balage et al., 2001; Laron, 2001). Arginine, a nitric oxide precursor, has been indicated as an angiogenesis signal in hypoxic tissues (Schwarzacher et al., 1997; Murohara et al., 1998; Duan et al., 2000b; Dulak et al., 2000; Hazeleger et al., 2007). Hypothetically, insulin, IGF-1, and arginine may aid in reparative responses of chondrocytes and vasculature to OC. However, insulin and IGF-1 can increase growth and weight gain that can affect OC prevalence (de Koning et al., 2013). Carbohydrate rich diets affect insulin and IGF-1 levels in pigs (Wientjes et al., 2012, 2013). It may be possible that both a carbohydrate rich diet and supplementation with arginine affect the prevalence of OC.

The aim of the study was to assess if dietary carbohydrate and arginine level in the rearing period of breeding gilts affect OC prevalence at 6 months of age. If OC prevalence is affected, this could be used as a dietary strategy to reduce OC prevalence in practice.

6.3 MATERIALS AND METHODS

6.3.1 Ethical Note

Osteochondrosis can cause lameness affecting welfare of the gilts. Gilts were daily inspected for impairments of welfare. Severely lame or wounded gilts were taken out of the experiment and euthanized. The experiment and all

measurements were approved by the Animal Welfare Committee of Wageningen University and Research center in compliance with Dutch law on animal experimentation.

6.3.2 Animals

The experiment was performed using 212 Topigs 20 (Dutch Large White x Dutch Landrace) gilts acquired after weaning at 27 (2.6 SD) days of age and 7.0 (1.5 SD) kg of body weight (**BW**) from a commercial breeding company (TOPIGS, Veldhuizen Wehl, Wehl, The Netherlands). Previous research showed an OC prevalence within this line of animals of up to 60% at approximately 6 months of age (de Koning et al., 2013, 2014). Gilts were housed in a 8.37 m^2 pen with a conventional floor consisting of 60% slatted floor (twisted metal bars) and 40% solid floor (epoxy-coated concrete). Enrichment items were provided at all times (such as biting chains, burlap sacks, solid plastic balls, rubber mats) and different items were made available within pens every 2 to 3 days. Gilts were weighed every 2 weeks after weaning until slaughter at 176 (4.4 SD) days of age. Gilts had continuous access to water through a drinking nipple. For approximately the first 2 weeks after weaning, all gilts received a similar weaning diet administered ad libitum to adapt to housing conditions after weaning. The BW of the gilts was measured once every 2 weeks after weaning throughout the experiment.

6.3.3 Treatments

Gilts were assigned to 1 of 4 treatment groups and 1 of 32 pens of 6 to 7 individuals after weaning, based on an equal distribution of BW measured 1 week before the start of the experiment. Gilts from 1 dam were equally divided over treatments as much as possible to prevent that 1 litter received only 1 treatment. Pens were divided over 4 departments (8 pens per department) with an equal distribution of treatments within each department. Treatments were administered from approximately 40 (2.6 SD) days of age onwards. Treatments consisted of a 2x2 factorial design of dietary carbohydrate and arginine supplementation on a commercial basal diet (diets produced by Research Feed Plant, ForFarmers BV, Heijen, The Netherlands), which remained similar for all animals (see Table 6.1 for the added dietary component composition). The carbohydrate level consisted of a diet with 12.5% cornstarch and 12.5% dextrose added to the commercial basal diet

Table 6.1. Contents as fed of the high and low dietary carbohydrate treatments for the 3 successive diets[1].

Diet	GE (MJ)	DM (g)	CP (g)	CF (g)	Carb + Sug (g)
Grower diet					
C+A+	15.98	882.2	169.1	29.4	594.0
C-A+	15.70	752.6	165.3	116.5	343.2
C+A-	15.99	885.7	169.7	28.7	594.0
C-A-	15.63	752.2	166.6	111.8	343.2
Rearing diet 1					
C+A+	15.89	894.1	167.1	19.5	574.0
C-A+	15.46	755.6	167.6	107.1	323.0
C+A-	15.76	891.8	167.0	18.0	574.0
C-A-	15.35	754.3	167.3	105.7	323.0
Rearing diet 2					
C+A+	15.67	889.7	139.8	15.0	617.0
C-A+	15.40	758.5	135.2	109.3	366.6
C+A-	15.64	892.8	139.5	14.9	617.0
C-A-	15.20	754.1	135.0	101.2	366.6

[1] Gilts received 3 successive diets based on their age and weight development. Dietary treatments per diet consisted of a 2x2 factorial design of carbohydrates and arginine. C+ = high carbohydrate, low fat diet; C- low carbohydrate, high fat diet; A+ diets supplemented with 0.8% arginine; A- diets supplemented without arginine but with 1.64% alanine as the isonitrogenous control.

[2] The content of the components are as fed: for the C+ diets per 1 kg of C+ diet, for the C- diets per 839 g of C- diet (the isocaloric amount of feed). Carbohydrate and sugar content according to the Centraal Veevoederbureau (CVB 2007). GE = gross energy (ISO-9831, 1998); DM = dry matter (ISO-6496, 1999); CP = crude protein (ISO-5983-2, 2005); CF = crude fat (ISO-6492, 1999).

(high carbohydrate diet; **C+**) versus a diet in which the cornstarch and dextrose was replaced with an isocaloric 8.9% soya bean oil (low carbohydrate diet; **C-**). This contrast of carbohydrate level was determined from previous experiments as the largest possible contrast conceivable in terms of food uptake and digestion (Wientjes et al., 2013). Arginine (L-arginine, Daesang Corp., Seoul, South Korea) supplementation consisted of supplementation with 0.8% arginine supplemented to the commercial basal diet (**A+**) versus 1.64% alanine (L-alanine, Omya Hamburg GmbH, Hamburg, Germany) supplemented to the basal diet as the isonitrogenous control (**A-**) as described by others (Li et al., 2010; He et al., 2011; Shan et al., 2012; Zheng et al., 2013). The 2x2 factorial treatment design resulted in the following treatment combinations termed treatment groups: **C+A+, C+A-, C-A+, C-A-**. The dietary treatments were fed isocaloric and were initially administered at approximately 95% of ad libitum energy uptake determined in a previous experiment using the same line of gilts (de Koning et al., 2013). During the current

experiment, it was observed that the amount of feed presented to the high carbohydrate diet resulted in regular feed residuals (see Figure 6.1), which could cause differences in energy uptake with the isocalorically fed low carbohydrate diets. Therefore, the amount of feed administered to all groups was lowered to approximately 85 to 90% of the estimated ad libitum intake. All gilts received feed in 2 portions per day (8:00 h and 16:00 h) in 2 troughs with ample feeding space and with metal bars separating individual feeding places. Feed residuals were collected and weighed after each day in the morning before the first feeding bout.

After weaning, all gilts received first the pelleted weaning diet (10.3 MJ NE/kg, 171 g/kg CP, 12.7 g/kg ileal digestible lysine). Subsequently, gilts changed to 3 successive diets in time each containing 1 of the 4 dietary treatments, adapted to their feed uptake and age (Table 6.1). Gilts were switched at 40 days of age to a pelleted grower diet (based on 1 kg of the C+ diet: 10.1 MJ NE/kg, 12.1 g/kg ileal digestible lysine; 6.3 g/kg calcium; 5.5 g/kg phosphorus); at 77 days of age to a pelleted rearing diet 1 (based on 1 kg of the C+ diet: 9.7 MJ NE/kg, 10.0 g/kg ileal digestible lysine; 8.0 g/kg calcium; 5.5 g/kg phosphorus); at 116 days of age to a pelleted rearing diet 2 (based on 1 kg of the C+ diet: 9.5 MJ NE/kg, 6.5 g/kg ileal digestible lysine; 8.0 g/kg calcium; 5.5 g/kg phosphorus).

6.3.4 Blood Sampling

To determine if dietary treatments affected glucose and insulin profiles, regular blood samples were taken at the end of the experiment at 167 (2.5 SD) days of age of 10 randomly selected gilts per treatment group. Blood sampling occurred through cannulation of the ear vein. In 6 gilts cannulation was not successful as the veins were too small (number of gilts sampled: C+A+ n=8; C+A- n = 10; C-A+ n=8; C-A- n=8). The insulin and glucose profiles could not be obtained at younger age, as the veins in the ear are still too small for our cannulation procedure. Cannulation of the ear vein was performed as described in Wientjes et al. (2012) for adult sows. Gilts were individually housed in a 2.6 m^2 pen 2 days before blood sampling, serving as a habituation period to individual housing. Gilts were cannulated one day before blood sampling and were fixated in a nose sling during the cannulation procedure. One ear was scrubbed with disinfectant and a medical PVC catheter with an inner diameter of 0.8 mm and outer diameter of 1.6 mm (Rubber BV, Hilversum, The Netherlands) was inserted 40 cm into a suitable

(broad and straight) ear vein. The catheter at the site of cannulation was taped to the ear of the gilt and the ear was fixated against the head of the gilt. The other end of the catheter was secured with a one-way luer-lock stopcock® (Vygon, Veenendaal, The Netherlands). The catheter was externally placed and secured in a fabric pouch fixated with medical tape on the back of the gilt. After the procedure, the gilts received 0.4 mg·kg^{-1} Novem®20 (containing meloxicam). Regular blood samples were taken 1 day after cannulation around the morning (08:00 h) feeding time at -24, -12, 0 (feeding time), 12, 24, 36, 48, 60, 84, 120, 156, 228, 300, 372, and 444 minutes relative to feeding. Gilts were allowed to eat an isocaloric feed amount (11.4 MJ NE) for a maximum of 30 minutes to ensure profiles of glucose and insulin were measured as close to a short feeding bout as possible. Only 1 gilt from the C+A+ treatment had a substantial amount of feed left over after 30 minutes of approximately 40%. Statistical analyses were performed with and without this gilt and indicated as such in the results section. All sampling time points were used to determine glucose and insulin profiles. Plasma IGF-1 levels of the gilts were determined 228 minutes after feeding. Plasma nitric oxide (**NO**) levels were determined in 30 randomly selected gilts per treatment group at approximately 70 (2.5 SD) days of age by puncture of the jugular vein and at the cannulation procedure. Plasma NO was determined as NO is a product of arginine metabolism. Blood samples were collected in EDTA tubes and were centrifuged for 10 minutes at 3000 *g* at 4°C to obtain plasma and was stored at -20°C until use.

6.3.5 Plasma Analysis

Plasma glucose, insulin, and IGF-1 analyses were performed as described in Wientjes et al. (2012) using commercial kits following manufacturers' instructions. Briefly, plasma glucose levels (in mg·dL^{-1}) were assessed in duplicate for all sampling time points during cannulation using a glucose oxidase-peroxidase kit (Roche Diagnostics Nederland BV, Almere, The Netherlands) after protein precipitation of 50 µl plasma using 500 µl 0.3M trichloroacetic acid. Plasma insulin levels (in µU·mL^{-1}) were assessed in duplicate for all sampling time points during cannulation using a RIA kit (PI-12K Porcine Insulin RIA-kit®, Millipore, St. Charles, MO, USA). Plasma IGF-1 levels (in ng·mL^{-1}) were assessed in duplicate using an IRMA kit (IRMA IGF-1 A15729®, Immunotech, Marseille, France) after ethanol/HCL extraction. Plasma total NO levels (in µmol·L^{-1}) were determined by

indirect assessment of nitrite concentration (where the nitrate present was converted to nitrite) and were assessed in duplicate for the blood samples taken at 10 and 24 weeks of age using a total NO kit (Parameter™ Total nitric oxide KGE001, R&D Systems Inc., Minneapolis, MN, USA) after ultrafiltration of plasma samples using a 10K MW filter and centrifugation for 40 minutes at 16000 *g* (Pierce® Concentrator PES 88513, Thermo Scientific, Rockford, IL, USA).

6.3.6 Osteochondrosis Assessment

Osteochondrosis assessment was performed after slaughter as described in de Koning et al. (2013, 2014). One half of the gilt population was slaughtered within 2 days at 173 (2.7 SD) days of age, the other half of the gilt population was slaughtered within 2 days at 180 (2.6 SD) days of age with an equal distribution of treatments on each day of slaughter. After slaughter, carcasses were stored for 1 day at 4 °C, after which the legs were collected by dissection through the elbow and hip joints and stored at -20 °C for a maximum of 5 days. After thawing for 2 days, the hock, knee, and elbow joints were dissected in 1 day for each half of the population in random order of gilts. After dissection, the joint surfaces were macroscopically scored for any irregularities indicative of OC on a total of 22 locations (see de Koning et al., 2013). Scoring of OC was performed using a 5-point grading scale from 0 to 4 with score 0 indicating no OC and score 4 indicating the severest form of OC as described previously (van Weeren and Barneveld, 1999). Osteochondrosis was scored by one veterinarian specialized in orthopedics, experienced in judging OC, and unaware of the treatments.

6.3.7 Statistical Analysis

Statistical analyses were performed for BW progression and growth rate, feed residuals, feed efficiency, blood sampling parameters, and OC scores. Several (successive) measurements were taken for several of these parameters and, therefore, observations cannot be considered independent. Repeated measurement analyses were performed and suitable variance-covariance structures were evaluated using Akaike's corrected information criterion. In each of the statistical models, non-significant higher order interaction effects were removed from the model using backward elimination until either the (higher order) interaction effects were significant ($P < 0.05$) or only main effects remained in the model.

Body Weight and Growth Rate. The average daily growth rate was calculated for each interval between BW measurements by taking the increase in weight within the interval divided by the number of days of the interval. The analyses of growth rates and BW were performed using PROC MIXED in SAS 9.2 (SAS Inst. Inc., Cary, NC). The carbohydrate treatment (C+, C-), arginine treatment (A+, A-), the time points at which BW or growth rates were measured (12 measurements), and their interaction effects were assessed as fixed class effects in the statistical model. A random effect of the experimental unit pen (32 pens) nested within treatments was added to assess the treatment effects on BW and growth rates on pen level. A repeated measurement analysis was performed with gilts as the subject, applying a heterogeneous first order auto-regressive variance-covariance structure for the BW model and an ante-dependent variance-covariance structure for the growth rate model. The model was not convergent when fixed effects of department (4 departments), in which the gilts were kept, were added to the model and assessed in four way interaction effects of fixed class effects. Three way interaction effects of the treatments with measurements and department were assessed, but did not improve the model and were therefore not included in the model. Addition of dam effect (38 dams) did not result in further improvement of the model. Significant results are displayed as the least squares means and the corresponding SE.

Feed Residuals. Feed residuals were calculated as the average daily feed residuals per week and per pen and expressed as the proportion of daily feed given during the corresponding week. Feed residuals were analyzed from the start of administering dietary treatments until 88.8 (2.6 SD) days of age. After 88 days of age, the number of pens having feed residuals were too few for statistical analysis (on average 4 pens per week until the end of the experiment) and were restricted mainly to the C+A+ treatment. As feed residuals were calculated as a proportion, the data was analyzed using binomial logistic regression with a logit link using PROC GLIMMIX in SAS 9.2. The carbohydrate treatment (C+, C-), arginine treatment (A+, A-), the time points at which feed residuals were measured (8 measurements), and their interaction effects were assessed as fixed class effects in the statistical model. A repeated measurement analysis was performed with pens (32 pens) as the subject, applying a heterogeneous first order auto-regressive variance-covariance structure. As with the analysis of BW progression, the same

issues and outcomes were encountered for the feed residuals when department (4 departments) was added to the model. Therefore, department was not included in the model. Significant results are displayed as the inverted logit least squares means with corresponding CI.

Feed Efficiency. Total energy consumed (in mega joule per net energy, **MJ NE**) over the entire experiment was determined by calculating the sum of the average feed eaten per gilt in a pen per day multiplied by the estimated energy within each feed. Total weight gain over the experimental period was determined. The energy required for 1 kg of BW gain was calculated by dividing the total consumed energy by the total weight gain. This indicates whether group-housed fed gilts are equally efficient in growing with the same amount of energy consumed. Feed efficiency was analyzed with PROC MIXED in SAS 9.2. The carbohydrate treatment (C+, C-), arginine treatment (A+, A-), and their interaction effects were assessed as fixed class effects in the statistical model. Dams (38 dams) were added as a random effect and the departments (4 departments) in which the gilts were housed were added as fixed class effects to the model. A random effect of the experimental unit pen (32 pens) nested within treatments and department was added to assess the treatment effects on feed efficiency on pen level. Significant results are displayed as the least squares means and the corresponding SE.

Glucose, Insulin, and Insulin-Like Growth Factor-1. Levels of glucose, insulin, and IGF-1 were statistically analyzed using PROC MIXED in SAS 9.2. As the gilts were individually housed for blood sampling, the gilts were the experimental unit and department effects were not assessed. To approximate normality, the natural logarithms of glucose and IGF-1 values were calculated, and the base 10 logarithms of the insulin values were calculated. The carbohydrate treatment (C+, C-), arginine treatment (A+, A-), the time points at which blood samples were taken (only for glucose and insulin analyses; 15 measurements), and their interaction effects were assessed as fixed class effects in the statistical model. For glucose and insulin, a repeated measurement analysis was performed with gilts as the subject, applying a first order ante-dependent variance-covariance structure. Significant results are displayed as the least squares means and the corresponding SE on their respective logarithm scales.

Nitric Oxide. Levels of NO were statistically analyzed using PROC MIXED in SAS 9.2. To approximate normality, the natural logarithms of NO values were calculated. The carbohydrate treatment (C+, C-), arginine treatment (A+, A-), and their interaction effects were assessed as fixed class effects in the statistical models at 10 weeks of age and 24 weeks of age. For the statistical model at 10 weeks of age, dam (36 dams) was added as a random effect and the departments (4 departments) in which the animals were housed were added as fixed class effects. Additionally, for the statistical model at 10 weeks of age, a random effect of the experimental unit pen (32 pens) nested within treatment and department was added to assess the treatment effects on NO on pen level. Significant results are displayed as the least squares means and the corresponding SE on a natural logarithm scale.

Osteochondrosis Scores. Osteochondrosis was scored on several locations within a joint on an ordinal scale of 0 to 4 for each joint separately. However, ordinal logistic regression was deemed not appropriate and could not be performed validly because of a low number of observations of each combination of treatment and OC score (Stokes et al., 2000). As a consequence, OC scores were grouped as a 0 and 1 variable to accommodate binary logistic regression as described previously (de Koning et al., 2013, 2014), where 0 indicates no abnormalities (OC score 0) and 1 indicates an OC score greater than 0. The OC scores were analyzed using PROC GLIMMIX in SAS 9.2. Statistical analyses were not performed for the elbow joint as the prevalence of OC was low (< 8%). The carbohydrate treatment (C+, C-), arginine treatment (A+, A-), and their interaction were assessed as fixed class effects in the statistical model. Dams (38 dams) were added as a random effect and the departments (4 departments) in which the gilts were housed were added as fixed class effects. A random effect of the experimental unit pen (32 pens) nested within treatment and department was added to assess the treatment effects on OC on pen level. A repeated measurement analysis was performed on the binary OC scores from the different locations assessed for OC with gilts as the subject, applying a variance component structure. After backward elimination was performed, the BW of the gilts at slaughter was added as a fixed covariate to the statistical models to assess whether BW at slaughter had an effect on OC prevalence over the treatment effects. Results are displayed as odds ratios (**OR**) and their confidence interval (**CI**) to indicate effect size.

6.4 RESULTS

A total of 11 gilts were euthanized before the end of the experiment due to health and welfare problems that were deemed unrelated to treatment effects such as diarrhea after weaning, fighting, pneumonia, inflamed wounds on claws, severe tail biting, and an atrial septal defect. The number of gilts euthanized before the end of the experiment were 5 gilts from the C+A+ group (of which 2 were euthanized before dietary treatments were given), 4 gilts from the C-A- group (of which 1 was euthanized before dietary treatments were given), 1 gilt from C-A+ group, and 1 gilt from the C+A- group. The joints of these animals were assessed and taken into account in analyses where appropriate.

6.4.1 Bodyweight and Feed Residuals

The progression of BW, average daily growth rate, and feed residuals per treatment are shown in Figure 6.1A-C. No significant differences between treatments were present in BW until 68 days of age. A significant interaction effect between carbohydrate and arginine treatments was present after approximately 68 days of age until the end of the experiment. After 68 days of age, gilts in the C+A+ treatment had a lower BW ($P < 0.01$) when compared with gilts in the other treatment groups (Figure 6.1A), which likely follows from higher feed residuals being present for the C+A+ treatment throughout the experiment (Figure 6.1C). Additionally, at approximately 138 days of age until the end of the experiment, the gilts in the C+A- treatment had a lower BW than gilts in the C-A- treatment ($P < 0.05$).

A significant interaction effect between carbohydrate and arginine treatments was present for the average daily growth rates (Figure 6.1B). Overall, gilts in the C+A+ treatment had lower growth rates ($P < 0.05$) than gilts in the other treatment groups. These were significantly lower in the 2-week periods from 40 to 82 days of age and from 110 to 169 days of age. Additionally, gilts in the C-A- treatment had the highest growth rates, which were significantly higher ($P < 0.03$) than for gilts in the treatments C+A- at 54 to 68 days of age, C-A+ at 82 to 96 days of age, and C+A- from 110 to 151 days of age (Figure 1B). The BW of the gilts at the last measurement at the end of the experiment was for the C+A+ treatment 85.2 (1.5 SE) kg, for the C+A- treatments 95.7 (1.5 SE) kg, for the C-A+ treatments 99.2 (1.5 SE) kg, and for the C-A- treatments 101.3 (1.5 SE) kg.

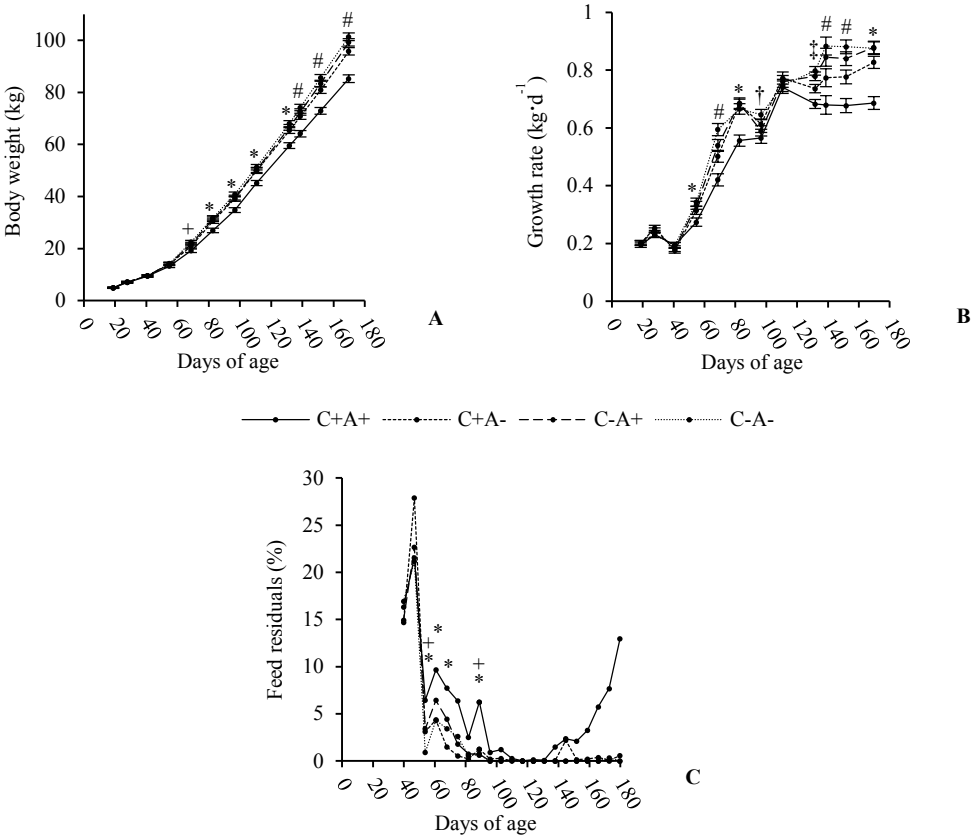

Figure 6.1A-C. Bodyweight progression (A), average daily growth rates (B), and feed residuals (C) for each treatment group. Treatments consisted of a 2x2 factorial design of carbohydrates and arginine. C+ = high carbohydrate diet; C- = low carbohydrate diet; A+ = diets supplemented with arginine; A- = diets supplemented without arginine. (B) Growth rates were calculated as the average daily growth rates between 2 BW measurements. Thus, each point indicates the average daily growth rate from the previous BW measurement. (C) Feed residuals were calculated as the average proportion of feed left over compared with the total amount of feed given per gilt and per day within a week. Symbols indicate the following significant differences ($P \leq 0.05$) where no common superscript is found for A: + indicates C+A+^{a}, C+A-^{ab}, C-A+^{b}, C-A-^{b}; * indicates C+A+^{a}, C+A-^{b}, C-A+^{b}, C-A-^{b}; # indicates C+A+^{a}, C+A-^{b}, C-A+^{bc}, C-A-^{c}. For B, symbols indicate the following significant differences ($P \leq 0.05$) where no common superscript is found: * indicates C+A+^{a}, C+A-^{b}, C-A+^{b}, C-A-^{b}; # indicates C+A+^{a}, C+A-^{b}, C-A+^{bc}, C-A-^{c}; † indicates C+A+^{a}, C+A-^{ab}, C-A+^{a}, C-A-^{b}; ‡ indicates C+A+^{a}, C+A-^{b}, C-A+^{c}, C-A-^{c}. For C, only significant differences were found between the interaction effect of time points at which feed residuals were measured and the main effects of treatments: + indicates significant difference between C+ and C- diets; * indicates significant difference between A+ and A- diets. Statistical analyses for the feed residuals were performed until approximately 88 days of age, afterwards feed residuals were few and mainly restricted to the C+A+ treatment. For display purposes, CI bars for the feed residuals are not shown, but can be found in Table 6.2.

Table 6.2. Least squares mean (LSmean) feed residuals in % and confidence intervals (CI) per treatment group[1].

days of age[2]	C+A+ LSmean % (CI)	C+A- LSmean % (CI)	C-A+ LSmean % (CI)	C-A- LSmean % (CI)
39	16.3 (10.3-24.9)	14.7 (9.0-23.1)	14.9 (9.2-23.3)	16.9 (10.8-25.6)
46	21.6 (15.5-29.1)	27.9 (21.1-35.8)	22.7 (16.5-30.3)	21.2 (15.2-28.7)
53	6.4 (3.5-11.5)	3.1 (1.3-7.3)	3.4 (1.5-7.7)	0.9 (0.2-4.5)
60	9.7 (6.6-13.9)	4.4 (2.5-7.6)	6.4 (4.0-10.1)	4.3 (2.4-7.5)
67	7.7 (4.7-12.4)	1.5 (0.5-4.6)	4.4 (2.3-8.4)	3.4 (1.6-7.1)
74	6.4 (3.3-12.1)	0.5 (0.1-5.3)	1.8 (0.5-6.2)	2.6 (0.9-7.2)
81	2.5 (1.2-5.2)	0.2 (0.0-2.8)	0.7 (0.2-2.9)	0.6 (0.1-2.7)
88	6.2 (4.0-9.6)	1.3 (0.5-3.4)	0.7 (0.2-2.7)	0.6 (0.2-2.6)

[1] Treatments consisted of a 2x2 factorial design of carbohydrates and arginine. C+ = high carbohydrate diet; C- low carbohydrate diet; A+ diets supplemented with arginine; A- diets supplemented without arginine.

[2] Only the data are shown for which statistical analyses were performed. Analyses were performed from start of dietary treatments until 88 days of age. After 88 days of age, a low number of pens presented with feed residuals and concerned mainly the C+A+ treatment.

An increase in feed residuals was present one week after the start of the dietary treatments (at approximately 46 days of age), but subsequently decreased rapidly in all treatment groups (Figure 6.1C; the CI are presented in Table 6.2 for display purposes instead of in the figure). No significant interaction effects between treatments were present for feed residuals ($P > 0.05$). Significant effects of carbohydrate ($P = 0.04$) and arginine ($P = 0.01$) treatments were present (Figure 6.1C). The effect of carbohydrate treatment indicated that gilts in the C+ treatment presented with higher feed residuals ($P \leq 0.05$) when compared with gilts in the C- treatment at 53 and 88 days of age. The effect of arginine treatment indicated that gilts in the A+ treatment presented with higher feed residuals ($P \leq 0.05$) when compared with gilts in the A- treatment at 53, 60, 67, and 88 days of age. After 82 days of age, mainly gilts in the C+A+ treatment showed increasingly more feed residuals towards the end of the experiment (from 2% to 13% feed residuals), while gilts in the other treatments showed feed residuals close to 0% (Figure 6.1C).

6.4.2 Feed efficiency

Significant ($P = 0.02$) interaction effects were found between carbohydrate and arginine treatments for the feed efficiency over the entire experimental period. Gilts in the C+A+ treatment (27.3 [0.55 SE] MJ NE) had a significantly ($P < 0.001$) lower feed efficiency compared with gilts in the C-A+ (23.2 [0.53 SE] MJ NE), C-A- (22.9 [0.53 SE] MJ NE), and C+A- (24.6 [0.53 SE] MJ NE) treatments. Additionally, gilts in the C+A- treatment had a lower feed efficiency compared with gilts in the C-A+ ($P = 0.05$) and C-A- ($P = 0.01$) treatment. The results suggest that gilts receiving the low carbohydrate diet were more efficient in gaining BW.

6.4.3 Glucose, Insulin, Insulin-Like Growth Factor-1, and Nitric Oxide

Glucose. Glucose levels peaked at 36 minutes after feeding, declined thereafter until 84 minutes after feeding and then increased again (Figure 6.2A-C). No significant interaction effects between carbohydrate, arginine, and sampling points were present ($P > 0.05$; Figure 6.2A). A significant ($P = 0.03$) overall interaction effect between carbohydrate and arginine treatments indicated that, overall, gilts in the C-A- treatment had significantly higher glucose levels than gilts in the C+A- treatment ($P < 0.01$), while the other treatment groups did not differ significantly from each other (Figure 6.2A). This significant interaction effect is reflected in the significant ($P = 0.03$) main effect of carbohydrate treatment, which indicated that gilts in the C- treatment had overall higher levels of glucose than gilts in the C+ treatment and is particularly evident in the period between 90 minutes and 444 minutes after feeding (Figure 6.2B). As stated before, one gilt from the C+A+ treatment had a relatively large amount of feed left 30 minutes after feeding. When this gilt was excluded from the statistical model, only the main effect of carbohydrate treatment remained with similar results. Arginine treatment did not have a significant effect ($P > 0.05$) on glucose levels (Figure 6.2C).

Insulin. Insulin levels peaked at 48 minutes after feeding and steadily declined afterwards (Figure 6.2D-F). No significant interaction effect between carbohydrate and arginine treatments was present ($P > 0.05$; Figure 6.2D). A significant ($P = 0.02$) effect of carbohydrate treatment was present for insulin levels (Figure 6.2E). This effect indicated that before feeding, gilts in the C- treatment showed higher levels of insulin ($P \leq 0.02$) when compared with gilts in

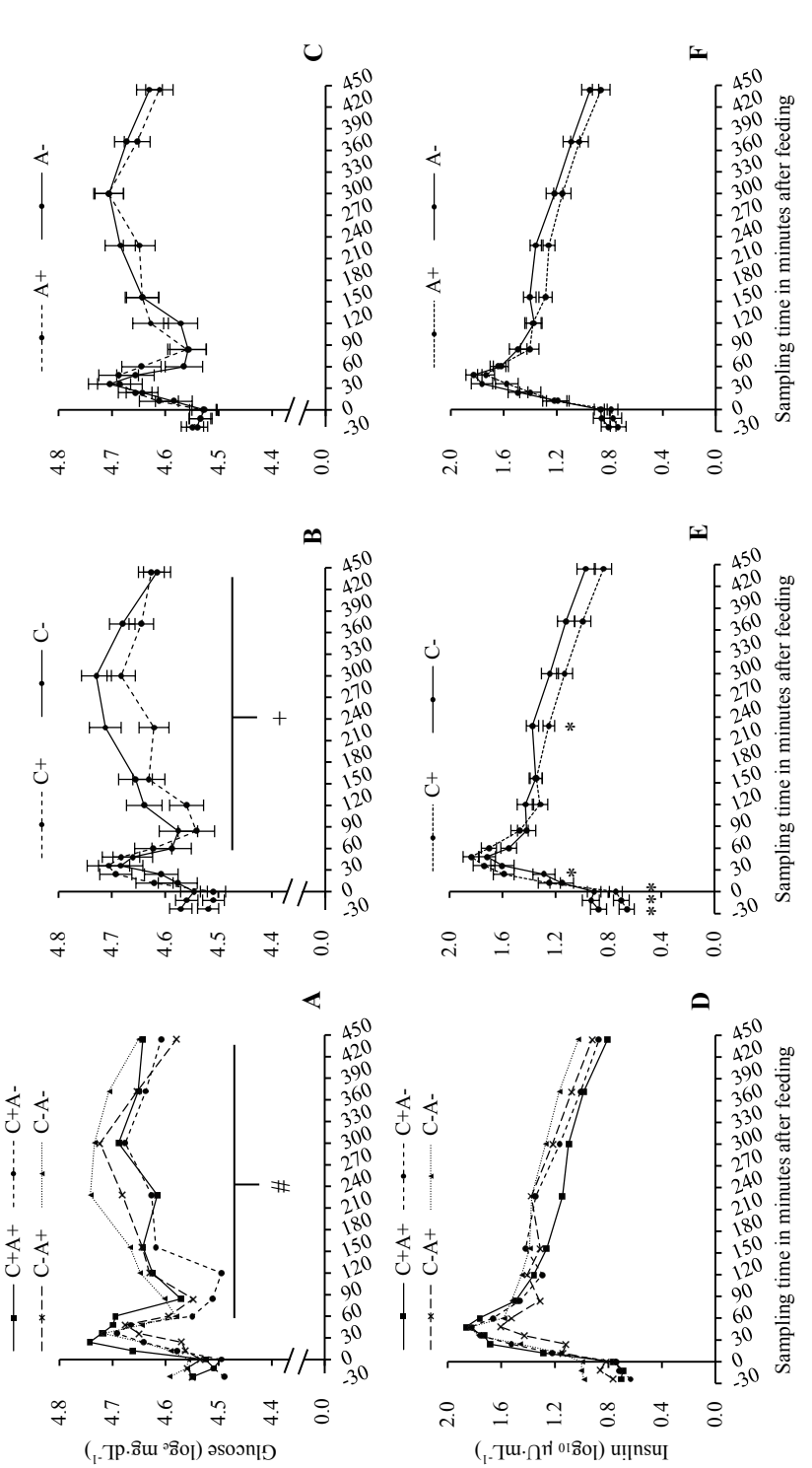

Figure 6.2A-I. Profiles of glucose (A-C), insulin (D-F), and level of insulin-like growth factor-1 (IGF-1; G-I) in gilts at 24 weeks of age (n=34). Blood sampling was performed at regular intervals before feeding (sampling time -24, -12, 0 minutes before feeding) and after feeding (sampling time 12, 24, 36, 48, 60, 84, 120, 156, 228, 300, 372, and 444 minutes after feeding). Treatments consisted of a 2x2 factorial design of carbohydrates and arginine. C+ = high carbohydrate diet; C- = low carbohydrate diet; A+ = diets supplemented with arginine; A- = diets supplemented without arginine. *(Continued on the next page)*

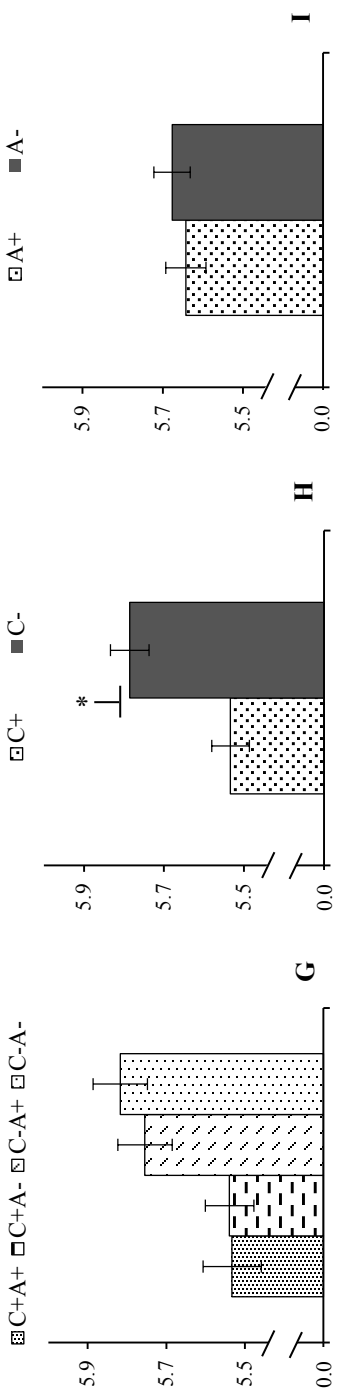

Figure 6.2A-I. ***(continued)*** The profiles and levels of glucose, insulin, and IGF-1 are shown for each treatment group (A, D, and G, respectively), for each main effect of carbohydrate treatment (B, E, and H, respectively), and for each main effect of arginine treatment (C, F, and I, respectively). Significant differences are indicated with symbols and specify the following significant differences were no common superscript is found: * indicates $C+^{a}$, $C-^{b}$; # indicates overall interaction effect $C+A+^{ab}$, $C+A-^{a}$, $C-A+^{ab}$, $C-A-^{bc}$ where C-A- has higher levels overall then C+A-; + indicates overall main effect $C+^{a}$, $C-^{b}$ where C- has higher levels overall then C+. For display purposes, SE bars are not shown for the treatment groups for insulin (A) and glucose (D). Pooled SE of glucose for C+A+ is 0.04 SE, C+A- is 0.04, C-A+ is 0.04 SE, and C-A- is 0.04 SE. Pooled SE of insulin for C+A+ is 0.09 SE, for C+A- is 0.08, C-A+ is 0.09 SE, and C-A- is 0.09 SE.

the C+ treatment at -24 minutes, -12 minutes, and 0 minutes. After feeding, gilts in the C+ treatment showed a higher insulin response than the gilts in the C- treatment and this was significantly different at 24 minutes after feeding ($P = 0.01$). Insulin steadily declined after the insulin peak. However, gilts in the C- treatment showed higher insulin levels from 120 minutes onwards after feeding and this was significantly different at 228 minutes after feeding ($P = 0.05$). Removal of the single gilt with large feed residuals 30 minutes after feeding (see above) did not influence statistical outcome. Arginine treatment did not have a significant effect ($P > 0.1$) on insulin levels (Figure 6.2F).

Insulin-like growth factor-1. No significant interaction effect ($P > 0.05$) between carbohydrate and arginine treatments was present for IGF-1 levels (Figure 6.2G). A significant main effect of carbohydrate treatment ($P < 0.01$) was present for IGF-1 levels (Figure 6.2H), which indicated that gilts in the C- treatment had significantly higher levels of IGF-1 than gilts in the C+ treatment (Figure 6.2H). Removal of the single gilt with large feed residuals 30 minutes after feeding (see above) did not influence statistical outcome. No significant effect ($P > 0.05$) of arginine on IGF-1 level was present (Figure 6.2I).

Nitric Oxide. Levels of plasma NO are shown in Figure 6.3A-B. No significant ($P > 0.05$) interaction effect between carbohydrate and arginine treatments was found at either 10 weeks of age or 24 weeks of age (Figure 6.3A). A significant main effect ($P < 0.03$) of carbohydrate treatment was present at both ages, which indicated that gilts in the C+ treatment had higher levels of plasma NO than gilts in the C- treatment (Figure 6.3B). Arginine treatment did not significantly ($P > 0.05$) affect NO levels at either age (Figure 6.3C).

6.4.4 Osteochondrosis Prevalence

Osteochondrosis was found in the elbow joint on the bilateral homologues of the medial and lateral humeral condyle, and on the anconeal process only on the left elbow joint; in the hock joint on the bilateral homologues of the medial and lateral trochlea of the talus; and in the knee joint on the bilateral homologues of the medial and lateral femoral condyle. The prevalence of OC in the elbow joint was relatively low (< 8%; Table 6.3) and the majority of OC was found on the lateral humeral condyle (77.8% of the total prevalence of OC scores greater than 0). Osteochondrosis in the hock joint was predominantly found on the medial trochlea

Figure 6.3A-C. Levels of nitric oxide (NO) in gilts at 10 (n=117) and 24 weeks of age (n=34). Treatments consisted of a 2x2 factorial design of carbohydrates and arginine. C+ = high carbohydrate diet; C- = low carbohydrate diet; A+ = diets supplemented with arginine; A- = diets supplemented without arginine. The levels of NO are shown for each treatment group (A), for each main effect of carbohydrate treatment (B), and for each main effect of arginine treatment (C). Significant differences are indicated with symbols and specify the following significant differences were no common superscript is found: * indicates C+^{a}, C-^{b}.

of the talus (69.8% of the total prevalence of OC scores greater than 0). Osteochondrosis in the knee joint was predominantly found on the medial femoral condyle (97.3% of the total prevalence of OC scores greater than 0). The prevalence of severe OC lesions (scores 3 and 4) was low in all joints assessed (7.2% in the elbow joint; 1.5% in the hock joint; 2.9% in the knee joint; Table 6.3).

Descriptive statistics at the animal level indicate that gilts in the C+ treatment showed a lower prevalence of OC compared with gilts in the C- treatment (55.2% versus 68.0 % respectively) and gilts in the A+ treatment showed a lower prevalence of OC compared with gilts in the A- treatment (59.6% versus 63.5%). The order of the treatments groups from lowest to highest OC prevalence was C+A+ (50.0 %), C+A- (60.4%), C-A- (66.7%), and C-A+ (69.2%). Significant effects of treatments were not present ($P > 0.05$) for the hock joint and the animal level (the elbow joint was not statistically assessed due to low prevalence of OC). Only a significant ($P = 0.01$) main effect of carbohydrate treatment was found at the knee joint and indicated that gilts in the C- treatment had significantly higher odds (OR = 2.05, CI: 1.18-3.58) to be affected by OC in the knee joint than gilts in the C+ treatment. A tendency was found for arginine treatment and suggested that gilts in the A+ treatment had a lower prevalence of OC in the knee joint ($P = 0.07$) and at the animal level ($P = 0.07$) than gilts in the A- treatment. When BW at slaughter was added to the models as a covariate, treatment effects were no longer present ($P > 1.3$). However, BW at slaughter became significant at the knee joint and the animal level ($P < 0.01$), which indicated that each 10 kg increase in BW at slaughter resulted in higher odds to be affected with OC at both the knee joint (OR=1.33, CI=1.11-1.6) and the animal level (OR=1.17, CI=1.05-1.31).

6.5 DISCUSSION

The aim of this study was to investigate whether dietary carbohydrate and arginine levels have an effect on OC prevalence during the rearing period of breeding gilts.

6.5.1 Bodyweight, Feed Residuals, and Feed Efficiency

Feed residuals for all treatments were high the first week after administering the dietary treatments and may represent habituation to the new feed. After the first week, feed residuals steadily declined and approached 0% at around

Table 6.3. Prevalence of osteochondrosis (OC) scores for the total number of locations assessed (n_{loc})[1] and for gilts with the greatest OC score present (n_{gi} and % total number of gilts)[2] per treatment[3]

	C+			C-			A+			A-		
	n_{loc}	n_{gi}	%	n_{loc}	n_{gi}	%	n_{loc}	n_{gi}	%	n_{loc}	n_{gi}	%
Elbow												
0	516	98	93.3	506	94	91.3	513	98	94.2	509	94	90.4
1	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0
2	0	0	0.0	1	1	1.0	0	0	0.0	1	1	1.0
3	4	3	2.9	3	3	2.9	4	3	2.9	3	3	2.9
4	5	4	3.8	5	5	4.9	3	3	2.9	7	6	5.8
Total[4]	**9**	**7**	**6.7**	**9**	**9**	**8.7**	**7**	**6**	**5.8**	**11**	**10**	**9.6**
Hock												
0	374	71	67.6	372	72	69.9	375	71	68.3	371	72	69.2
1	27	18	17.1	23	17	16.5	20	15	14.4	30	20	19.2
2	18	15	14.3	15	12	11.7	19	16	15.4	14	11	10.6
3	0	0	0.0	2	2	1.94	1	1	1.0	1	1	1.0
4	1	1	1.0	0	0	0.0	1	1	1.0	0	0	0.0
Total	**46**	**34**	**32.4**	**40**	**31**	**30.1**	**40**	**33**	**31.7**	**45**	**32**	**30.8**
Knee												
0	377	76	72.4	340	51	49.5	369	69	66.4	348	58	55.8
1	35	23	21.9	55	37	35.9	36	25	24.0	54	35	33.7
2	4	3	2.9	11	9	8.7	7	6	5.8	8	6	5.8
3	2	2	1.9	3	3	2.9	2	2	1.9	3	3	2.9
4	2	1	1.0	3	3	2.9	2	2	1.9	3	2	1.9
Total	**43**	**29**	**27.6**	**72**	**52**	**50.5**	**47**	**35**	**33.7**	**68**	**46**	**44.2**
Animal[5]												
0	1267	47	44.8	1218	33	32.0	1257	42	40.4	1228	38	36.5
1	62	31	29.5	78	36	35.0	56	30	28.9	84	37	35.6
2	22	16	15.2	27	20	19.4	26	20	19.2	23	16	15.4
3	6	5	4.8	8	7	6.8	7	6	5.8	7	6	5.8
4	8	6	5.7	8	7	6.8	6	6	5.8	10	7	6.7
Total	**98**	**58**	**55.2**	**121**	**70**	**68.0**	**95**	**62**	**59.6**	**124**	**66**	**63.5**

[1] OC prevalence expressed as number of OC scores present for total number of locations assessed for the elbow joint, hock joint, knee joint, and animal level. *(Continued on the next page)*

Table 6.3 ***(continued).***

	C+A+			C+A-			C-A+			C-A-			Overall[4]		
	n_{loc}	n_{gi}	%	n_{loc}	n_{gi}	%	n_{loc}	n_{gi}	%	n_{loc}	n_{gi}	%	n_{loc}	n_{gi}	%
Elbow															
0	255	48	92.3	261	50	94.3	258	50	96.2	248	44	86.3	1022	192	92.3
1	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0	0	0	0.0
2	0	0	0.0	0	0	0.0	0	0	0.0	1	1	2.0	1	1	0.5
3	3	2	3.9	1	1	1.9	1	1	1.9	2	2	3.9	7	6	2.9
4	2	2	3.9	3	2	3.8	1	1	1.9	4	4	7.8	10	9	4.3
Total[4]	**5**	**4**	**7.7**	**4**	**3**	**5.7**	**2**	**2**	**3.8**	**7**	**7**	**13.7**	**18**	**16**	**7.7**
Hock															
0	189	37	71.2	185	34	64.2	186	34	65.4	186	38	74.5	746	143	68.8
1	8	6	11.5	19	12	22.6	12	9	17.3	11	8	15.7	50	35	16.8
2	10	8	15.4	8	7	13.2	9	8	15.4	6	4	7.8	33	27	13.0
3	0	0	0.0	0	0	0.0	1	1	1.9	1	1	2.0	2	2	1.0
4	1	1	1.9	0	0	0.0	0	0	0.0	0	0	0.0	1	1	0.5
Total	**19**	**15**	**28.8**	**27**	**19**	**35.8**	**22**	**18**	**34.6**	**18**	**13**	**25.5**	**86**	**65**	**31.3**
Knee															
0	196	43	82.7	181	33	62.3	173	26	50.0	173	25	49.0	717	127	61.1
1	11	8	15.4	24	15	28.3	25	17	32.7	25	20	39.2	90	60	28.8
2	1	1	1.9	3	2	3.8	6	5	9.6	6	4	7.8	15	12	5.8
3	0	0	0.0	2	2	3.8	2	2	3.9	2	1	2.0	5	5	2.4
4	0	0	0.0	2	1	1.9	2	2	3.9	2	1	2.0	5	4	0.5
Total	**12**	**9**	**17.3**	**31**	**20**	**37.7**	**35**	**26**	**50.0**	**35**	**26**	**51.0**	**115**	**81**	**38.9**
Animal[5]															
0	640	26	50.0	627	21	39.6	617	16	30.8	601	17	33.3	2485	80	38.5
1	19	13	25.0	43	18	34.0	41	17	32.7	41	19	37.3	140	67	32.2
2	11	8	15.4	11	8	15.1	12	12	23.1	12	8	15.7	49	36	17.3
3	3	2	3.9	3	3	5.7	4	4	7.7	4	3	5.9	14	12	5.8
4	3	3	5.8	5	3	5.7	5	3	5.8	5	4	7.8	16	13	6.3
Total	**36**	**26**	**50.0**	**62**	**32**	**60.4**	**62**	**36**	**69.2**	**62**	**34**	**66.7**	**219**	**128**	**61.5**

[2] Prevalence of OC scores expressed as the number of gilts with the greatest OC score present and as the percentage of the total number of animals per treatment.

[3] Treatments consisted of a 2x2 factorial design of carbohydrates and arginine. C+ = high carbohydrate diet; C- = low carbohydrate diet; A+ = arginine supplemented diets; A- = diets supplemented without arginine. ***(Continued on the next page)***

80 days of age, except for gilts in the C+A+ treatment. Body weight differences were present between treatments and were especially evident in the C+A+ treatment. Gilts in the C+A+ treatment showed a higher overall prevalence of feed residuals throughout the experiment, which is reflected by the smaller increase in BW compared with the other treatments. Although no significant interaction effect of carbohydrate and arginine treatments were found for feed residuals, the high proportion of feed residuals by gilts in the C+A+ treatment may have influenced the significant main effects found of arginine and carbohydrate treatment on feed residuals. The reason for higher feed residuals present in gilts in the C+A+ treatment is unknown. Previous studies using arginine have reported decreased (Southern and Baker, 1982; Anderson et al., 1984), increased (Mateo et al., 2008; Hernandez et al., 2009; Tan et al., 2009; He et al., 2011; Yao et al., 2011; Chen et al., 2013), or no significant effects on weight gain (He et al., 2009; Ma et al., 2010). However, the studies that do not find an effect of arginine on BW started treatments at 40+ kg, while the studies that found effects applied arginine supplementation shortly after weaning for (less than) a month. Perhaps effects of arginine on BW are only evident in a short time frame immediately after weaning. The mechanism by which a prolonged period of high carbohydrate and arginine supplementation in the diet results in negative consequences on BW gain are, as of yet, unknown. After approximately 144 days of age, feed residuals were slightly higher in the C+A- treatment, which may explain the slightly lower BW of this treatment at the end of the experiment compared with the C-A- treatment. Previously, we showed that growing gilts show a quick reaction in BW when feed intake is adjusted (de Koning et al., 2013). Therefore, minor differences in feed intake at the end of the experiment may explain the minor BW differences between the C+A- treatment and the C-A- treatment. However, when looking at the feed efficiency, the gilts in the C+ treatment were the most inefficient group of gilts in terms of gaining BW and were 17% to 20% less efficient than the gilts in the C- treatment. This could also explain the differences in BW and might be explained by the differences found in metabolic parameters (see below). The amount of the feed

Table 6.3 ***(continued)***

[4] 'Total' indicates the total number of animals affected with any form of OC. 'Overall' indicates the total prevalence of an OC score for all gilts.

[5] 'Animal' is the number of OC lesions for all joints combined.

provided could not be further decreased, as 3 treatment groups (C+A-, C-A+, and C-A-) had relatively few feed residuals during the major part of the experiment. Further lowering the feed likely would have caused unrest and agitation in the treatment groups that showed a relatively good feed intake, which could result in differences in overall activity of the animals and hence affect OC prevalence on its own as shown in a previous study (de Koning et al., 2014).

6.5.2 Treatment Effects on Glucose, Insulin, Insulin-Like Growth Factor-1, and Nitric Oxide

Dietary treatments affected several plasma components measured in the current study. Significant effects were only found for the carbohydrate treatment, whereas arginine treatment did not show any significant effects on any of the plasma components analyzed. Insulin and glucose profiles showed that although gilts in the C+ treatment presented with (numerically) higher responses shortly after a meal (short term response), gilts in the C- treatment showed a higher level of insulin and glucose before feeding and at longer term (more than 2 h after feeding). These higher insulin and glucose levels in the carbohydrate treatment coincide with higher IGF-1 levels in gilts receiving the C- treatment. Previous experiments from literature with insulin stimulating diets (high carbohydrates) report similar results for glucose and insulin on the short term (van den Brand et al., 1998, 2000; Wientjes et al., 2012, 2013). The increase in glucose on the longer term for gilts in the C- treatment may be explained by the available energy source used for ATP production. To obtain energy, an animal must have lipogenic and glucogenic compounds to run the citric acid cycle, yielding energy in the form of ATP (van Knegsel et al., 2005). The gilts in the C- treatment will have enough lipogenic compounds, but may have less availability of glucogenic compounds from the diet. Possibly, the increase in glucose levels seen in the C- treatment over time reflects glycogenolysis, which results in glucose (Consoli et al., 1987; Jin et al., 2005; Satapi et al., 2012; Jin et al., 2013) needed for the citric acid cycle.

The associations of insulin and IGF-1 responses between treatments are confirmed by associations found between insulin and IGF-1 in other studies (van den Brand et al., 2001; Wientjes et al., 2013; and reviewed by Thissen et al., 2004). However, the same studies report that dietary treatments did not significantly affect IGF-1 levels. The previously mentioned studies applied dietary treatments during a

short time (lactation or weaning to estrous interval) in adult sows, whereas the current study applied dietary treatments for a much longer time in much younger gilts, which might explain these differences. In humans it is reported that (prolonged) dietary fat is associated with higher IGF-1 levels (Kaklamani et al., 1999; Gunnell et al., 2003; Heald et al., 2003), as is the case in the current study. Considering that dietary fat is associated with higher IGF-1 levels, one may wonder whether this effect also explains the differences in growth curves found in the gilts and their feed efficiency. It has been indicated that IGF-1 reduces protein breakdown (Asakawa et al., 1992; Hussain et al., 1993; Cioffi et al., 1994; Ling et al., 1995; Thissen et al., 2004) and increases protein synthesis (Fang et al., 1997; Fang et al., 1998; and reviewed by Humbel, 1990; Laron, 2001; Schiaffino et al., 2013). Possibly, the gilts in the C- treatment, that have higher IGF-1 levels than gilts in the C+ treatment, can more efficiently grow due to the protein synthesis effects of IGF-1. However, this does not fully explain why gilts in the C+A+ treatment showed a much lower BW gain. Studies have shown that arginine decreases protein turnover by decreasing protein synthesis and breakdown (i.e. turnover) during fetal growth in sheep (de Boo et al., 2005), or increases both protein synthesis and breakdown during and after endotoxemia in pigs (Bruins et al., 2002). Perhaps this could mean that the effect of lower IGF-1 in C+ treatments on protein synthesis is attenuated when arginine is presented in the diet, although the catabolic state as seen in endotoxemia is likely not comparable to the situation of growing animals.

Although NO is a product of arginine metabolism (Wu and Morris Jr., 1998), this study was unable to find significant differences in plasma NO in the arginine treatment. Others were able to find effects of arginine treatment on NO production, but those were often localized to areas of hypoxia at tissue level (Schwarzacher et al., 1997; Duan et al., 2000ab). Therefore, in the current study, even when NO production would have been affected in osteochondrotic areas, it may have been impossible to detect such an effect at systemic level. Such a local effect on NO may even be likely indeed, as there was a tendency for reduced OC prevalence in gilts with arginine supplementation (see below). Gilts in the C- treatment had significantly lower levels of NO. A high fat diet (C- treatment) has consistently been shown to reduce NO availability by reducing NO synthase activity or increasing reactive oxygen species limiting NO present in the body

(Duan et al., 2000a; Roberts et al., 2000; Bender et al., 2007; Yang et al., 2007; Huang et al., 2011), which seems to have occurred also in the current study. This NO reducing effect of a fatty diet may have masked the effects of arginine treatment on NO levels. It can be concluded at any rate that the dietary treatments used were effective in eliciting a difference in metabolic responses.

6.5.3 Osteochondrosis Prevalence

Prevalence of gilts affected with OC (OC score > 0) was approximately 61%, which is roughly equal to the prevalence found in our earlier studies using the same line of gilts (de Koning et al., 2013, 2014) and is comparable with other studies (Lundeheim, 1987; Jørgensen, 1995, 2000; Jørgensen et al., 1995; Stern et al., 1995; Jørgensen and Andersen, 2000; Kadarmideen et al., 2004; Ytrehus et al., 2004c; Jørgensen and Nielsen, 2005; Luther et al., 2007; Busch and Wachmann, 2011; van Grevenhof et al., 2011). However, the prevalence of severe lesions (OC score 3 and 4) was low (12% in the current study) when compared with our previous studies (ranging from 22 to 28%). Especially the hock joint and the knee joint showed a low prevalence of severe lesions. The elbow joint had a low prevalence of any OC score, which is similar to our previous studies. In contrast, the prevalence of minor lesions (OC scores 1 and 2) was higher in the current study (49.5%) compared with our previous studies (30% to 40%). Feed intake alone does not explain the differences, as animals that are continuously restricted (80% of ad libitum) in feed intake (de Koning et al., 2013) showed a lower overall prevalence of OC when compared with gilts with the lowest feed intake in the current study in the C+A+ treatment (44% versus 50%, respectively), but a higher prevalence of severe lesions of OC (13.5% versus 9.6%, respectively). The relatively low prevalence of severe lesions compared with our previous experiments and the high incidence of minor lesions of OC in the current study cannot be readily explained at this moment.

6.5.4 Treatment Effects on Osteochondrosis

This study was only able to find significant effects of carbohydrate treatment on OC prevalence in the knee joint. Gilts in the C+ treatment had lower odds to be affected with OC than gilts in the C- treatment. Considering that there are indications that gilts in the C- treatment had higher overall levels of glucose,

insulin, and IGF-1 compared with gilts in the C+ treatment, these results seem consistent with other studies in horses that indicate higher levels of these metabolic parameters in association with OC dissecans (Ralston, 1996; Pagan, 2001; Semevolos et al., 2001; Verwilghen et al., 2009). However, the mechanism behind it remains unclear. Osteochondrosis involves the formation of necrotic cartilage due to vascular disruption within the growth cartilage at young age in pigs, after which reparative attempts by viable chondrocytes and vasculature ensue that may or may not be successful and, together with the level of lesion formation, determine final clinical outcome (Carlson et al., 1986, 1989, 1991, 1995; Woodard et al., 1987ab; Ekman et al., 1990; Wegener and Heje, 1992; Henson et al., 1997b; Ytrehus et al., 2004abc; Olstad et al., 2007; 2008abc, 2011; and reviewed by Ytrehus et al., 2007; Laverty and Girard, 2013; McCoy et al., 2013; Olstad et al., 2015). For insulin and IGF-1 it has been shown that they affect growth cartilage chondrocytes and act as proliferating or survival signals (Böhme et al., 1992; Ballock and Reddi, 1994; Hunziker et al., 1994; Alini et al., 1996; Henson et al., 1997a). As discussed above, dietary carbohydrate level affects insulin, glucose, and IGF-1 levels. We therefore reasoned that a difference in dietary carbohydrate level would influence OC prevalence through the actions of insulin, glucose, and IGF-1 affecting chondrocyte functioning and possibly enhancing reparative attempts. However, this does not seem to have influenced OC prevalence in this way in the current study, as higher levels of glucose, insulin, and IGF-1 found in the treatments were not associated with less OC. Additionally, studies have indicated that arginine affects formation of blood vessels during hypoxic conditions likely through actions mediated by NO and vascular endothelial growth factor (Schwarzacher et al., 1997; Murohara et al., 1998; Duan et al., 2000ab; Dulak et al., 2000; Zhang et al., 2003; Milkiewicz et al., 2005; Hazeleger et al., 2007). Local hypoxia within the growth cartilage is expected when OC develops. Furthermore, increased vascular endothelial growth factor immunostaining in chondrocytes near necrotic cartilage has been reported (Ytrehus et al., 2004d). Therefore, dietary supplementation with arginine might affect OC prevalence as well by enhancing formation of vasculature. We were only able to find tendencies for arginine supplementation to reduce OC prevalence. The higher NO levels found in the C+ treatment would be fitting in that NO is necessary for vascular growth to occur, which is necessary for reparative responses to occur and may, therefore, also have

had a reducing effect on OC prevalence.

One may wonder whether the significant carbohydrate effect and the tendency of the arginine effect is determined by the BW difference, which was a consequence of the C+A+ treatment. Indeed, descriptive statistics show that gilts in the C+A+ treatment had the lowest prevalence of OC in the knee joint and at the animal level. However, no significant interaction effect between carbohydrate and arginine treatments could be found. The effect of BW at slaughter was significantly associated with OC prevalence, while treatment effects became non-significant. This may indicate that BW has a higher explanatory value than each of the treatments. The effect of BW at slaughter seemed to be independent of treatments, as interaction effects could not be found. In contrast to studies that could not find associations of BW with OC (Woodard et al., 1987b; Jørgensen, 1995; Ytrehus et al., 2004ac), the results of BW association with OC in the current study agrees with other studies (Carlson et al., 1988; van Grevenhof et al., 2012; de Koning et al., 2013).

A complicating factor in this study is the relatively low prevalence of severe OC as mentioned above. The effects of dietary carbohydrates and arginine may only be evident during the development of severe lesions of OC where a more prominent role for reparative responses undertaken by chondrocytes and vasculature would be expected. Although it would be beneficial to undertake a study in a group of animals that develop severe lesions, this is likely impossible as one cannot predict beforehand whether a group of gilts selected at 4 weeks of age will develop a sufficient prevalence of severe OC lesions.

6.6 CONCLUSION

Dietary carbohydrate levels, but not arginine supplementation, during the rearing period were found to affect metabolic parameters at approximately 24 weeks of age. Low carbohydrate (fatty) diets increased insulin, glucose, and IGF-1 levels compared with carbohydrate rich diets. Carbohydrate rich diets increased NO levels at 10 and 24 weeks of age. This study could not find significant effects of arginine supplementation during the rearing period of gilts on OC prevalence at approximately 25 weeks of age. Dietary carbohydrate levels were significantly associated with OC in the knee joint. However, results indicate that body weight at slaughter supersedes treatment effects and may be a more important explanatory

parameter for OC status in which heavier gilts at slaughter have a higher odds to be affected with OC. Therefore, the manipulation of dietary carbohydrate or arginine to directly influence OC prevalence in practice through dietary measures, at this point, seems to be of little benefit. Likely, dietary treatments affected OC prevalence indirectly by affecting BW development.

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CHAPTER 7

General discussion

7.1 INTRODUCTION

Osteochondrosis (OC) concerns an aberrant condition in the process of bone formation (endochondral ossification) causing deformation of the articular cartilage. Osteochondrosis has previously been suggested to be associated with lameness and its related welfare issues (Jørgensen and Andersen, 2000; de Koning et al., 2012), and an increased risk for culling of sows (Yazdi et al., 2000). Therefore, reduction of OC in breeding gilts would be important as they will have to last several parities in which they produce fattening pigs. The measures to be taken in order to affect OC prevalence in practice may in fact not be straight forward. Many factors have been associated with OC in the past, indicating that OC is a complex multifactorial problem. However, some environmental factors have been identified to affect OC prevalence and will be discussed later in this chapter. The question remains at what age environmental factors have an effect on OC and under which conditions. In this chapter, results of our experiments are further discussed. First, to refresh what OC is and how it develops, a very brief recap of the introduction on OC development will be presented. For further details of OC development, the reader is referred to the general introduction (Chapter 1). Second, the time dependency of OC development will be discussed in relation to the time point in the treatment switches performed in the dietary restriction experiment (Chapter 2) and floor type experiment (Chapter 3). Third, the manner in which OC can be affected with respect to dietary restriction (Chapter2), floor type (Chapter 3), and dietary composition (Chapter 6) will be further discussed. Fourth, several possibilities in detecting OC in vivo will be discussed involving conformation and locomotive characteristics (Chapter 4) and natural (auto-) antibodies (Chapter 5). Fifth, the possible implication of OC on animal welfare will be discussed, including new data of OC prevalence in practice in Dutch commercial husbandry. Finally, concluding remarks are presented.

7.2 OSTEOCHONDROSIS DEVELOPMENT

Osteochondrosis develops at young age in pigs and concerns the formation of necrotic growth cartilage within bone. Proliferating and hypertrophying chondrocytes within the growth cartilage produce an extracellular matrix consisting of, amongst others, collagens and proteoglycans that give support to the structure (reviewed by Martel-Pelletier et al., 2008; Kheir and Shaw, 2009; Garcia-Carvajal

et al., 2013; Gao et al., 2014). The extracellular matrix is eventually ossified near the subchondral bone (the ossification front) resulting in growth of bone (Carlson et al., 1985; Ekman et al., 1990; Henson et al., 1997b; and reviewed by Hunziker, 1994; Mackie et al., 2008; Ytrehus et al., 2007). Chondrocyte functioning is supported by blood vessels contained in cartilage canals and naturally regress (chondrification) with aging (Ytrehus et al., 2004ab). A disruption of vasculature near the ossification front may occur, due to loading of joints, when blood vessels from the growth cartilage 'merge' with blood vessels of the subchondral bone (anastomosis), which results in the chondrocytes not being able to sustain themselves and become necrotic (Ytrehus et al., 2004ab; reviewed by Ytrehus et al., 2007; Laverty and Girard, 2013; McCoy et al., 2013; Olstad et al., 2015). Necrotic growth cartilage is not ossified and is retained in the subchondral bone that may fracture due to loading of the joint, leading to joint surface abnormalities (Ytrehus et al., 2004ab; and reviewed by Ytrehus et al., 2007). Necrotic growth cartilage may be repaired by viable chondrocytes and vasculature that proliferate towards the necrotic cartilage, and may be repaired within the subchondral bone through intramembranous ossification (Carlson et al., 1986; Woodard et al., 1987; Ekman et al., 1990; Wegener and Heje, 1992; Henson et al., 1997b; Ytrehus et al., 2004bd; Olstad et al., 2007; 2008ac; and reviewed by Ytrehus et al., 2007; Olstad et al., 2015).

7.2.1 Timeframe of Osteochondrosis Development

A key aspect for OC to develop is the presence of vasculature as only vascular disruption, and not chondrification of vessels, is associated with OC development and without vasculature new lesions of OC cannot develop (Carlson et al., 1991; Carlson et al., 1995; Ytrehus et al., 2004a; Ytrehus et al., 2004b). Studies by Ytrehus et al. (2004abd) indirectly indicate that the majority of vascular regression and, therefore, the peak of the development of new OC lesions lies between 6 and 16 weeks of age. However, studies by Olstad et al. (2014ab) suggest that pigs at an age up to 22 weeks of age still show OC lesions developing and resolving, although at a smaller rate than at younger age. Therefore, the entire time frame for the development and resolution of OC is likely longer than 16 weeks of age. In 2 of our experiments concerning dietary restriction (Chapter 2) and floor type (Chapter 3), we set a switch in treatments at 10 weeks of age based on the

studies by Ytrehus et al. (2004abd). In this manner we hoped to affect OC prevalence by having gilts encounter a certain treatment / environment in the first and second half of the main time frame of OC development. However, the recent studies by Olstad et al. (2014ab) would suggest that the time frame of OC development is longer than we assumed and it could be possible that a more optimal time point to switch treatments is at a slightly older age due to the longer timeframe of OC development and resolution indicated by these studies. However, our switch at 10 weeks of age is still in the time frame where many blood vessels are still present, allowing for a higher chance of affecting OC development than, for example, at 16 to 20 weeks of age when the number of blood vessels should be greatly reduced. This is in concordance with Olstad et al. (2014ab) who suggested a decreasing rate of OC development up to 22 weeks of age. Therefore, our switch in treatments were likely still affecting the main initiating and ending stages of OC development.

7.3 AFFECTING OSTEOCHONDROSIS

Now that we have recapped the basics of OC in pigs, one may wonder what factors affect development of OC. What would make some pigs more susceptible for OC than others? This is unfortunately a difficult question to answer. Over the last decades, prevalence of OC has been associated with factors such as dietary restriction and housing (Chapter 2; Chapter3; van Grevenhof et al., 2011; Etterlin et al., 2014), growth rate (Stern et al., 1995; Busch and Wachmann, 2011; van Grevenhof et al., 2012), genetic background (Lundeheim, 1987; van der Wal et al., 1987; Ytrehus et al., 2004c; Jørgensen and Nielsen, 2005), a variety of hormones, plasma components (growth- and, parathyroid hormones, calcium, etc.), and collagen breakdown products (Sloet van Oldruitenborgh-Oosterbaan et al., 1999; Billinghurst et al., 2004; Gangl et al., 2007; de Grauw et al., 2011), and sex differences (Lundeheim, 1987; Stern et al., 1995; Ytrehus et al., 2004c; Busch and Wachmann, 2011; van Grevenhof et al., 2011). In other words, OC is a multifactorial condition, which makes it difficult to develop an effective strategy to try and reduce the prevalence of OC in practice. Discussing each factor separately would be beyond the scope of this discussion, but one common factor might be related to some of the mentioned factors and have a promising association with OC. Several studies have previously suggested that the occurrence or development

of OC is associated with loading of joints (Nakano and Aherne, 1988; Carlson et al., 1991; Ytrehus et al., 2004b; Ytrehus et al., 2004c) and seems to be a logical contributing factor considering the etiology of OC in vascular disruption of anastomoses and the fracturing of necrotic cartilage.

7.3.1 Dietary Restriction and Growth Rate

One seemingly straightforward method of influencing the loading on joints is through the application of different amounts of feed available to the animals, which affects their weight development and, consequently, relative loading on joints. We have found effects of dietary restriction applied to gilts, but the effects were not as straightforward as one might think (Chapter 2). Gilts that were continuously fed restricted or had ad libitum feed available from weaning until slaughter did not differ significantly in the prevalence of OC on the animal level. However, in gilts fed restricted before 10 weeks of age and switched to ad libitum feeding after 10 weeks of age, a significantly higher prevalence of OC was present compared with gilts fed continuously restricted or gilts switched from ad libitum to restricted at 10 weeks of age. Body weight at slaughter was higher for the gilts fed continuously ad libitum compared with gilts switched to ad libitum at 10 weeks of age, but they did not differ significantly in OC status. We concluded from this that the loading of the joints, per se, may not be detrimental when loading is gradually and consistently increased throughout life and indicates that the early life development of the gilts, i.e. environment encountered, is important for OC status. More specifically, the environment encountered in early life (before 10 weeks of age) interacts with the environment encountered later in life (after 10 weeks of age). Gilts that were switched from restricted to ad libitum feeding showed the highest prevalence of OC. There was no significant positive effect on OC prevalence at the animal level of restricted feeding after 10 weeks of age when gilts were fed ad libitum or restricted before 10 weeks of age. This indicates that only a high feeding level significantly affects OC development at the animal level when gilts started on a lower feeding level. A rise in body weight will likely affect relative loading experienced on the joints. When body weight and, therefore, loading on the joints is increased dramatically or in a short time frame, as would be expected when gilts are switched from dietary restriction to ad libitum feeding, joints may not be able to adapt fast enough to the increasing stress that the

environment is placing on them. We speculated that this may have to do with the development of the extracellular cartilage matrix that is able to gradually increase its strength over time, dependent on loading experienced, due to a higher incorporation of proteoglycan within its matrix (Buschmann et al., 1995; Little and Ghosh, 1997; Quinn et al., 1999; Spiteri et al., 2010). When the matrix is not adapted to higher loading experienced on the joints, the matrix fails in its support, stressing chondrocytes and vascular elements, and increasing the risk to develop OC. Several studies indicate that there might be a short time frame for when body weight gain may have an effect on OC in horses (van Weeren et al., 1999; Donabédian et al., 2006; Lepeule et al., 2009) and pigs (Lundeheim, 1987; Busch and Wachmann, 2011; van Grevenhof et al., 2012). For example, van Weeren et al. (1999) indicated that foals that had OC in the knee joint grew significantly faster in the third and fifth month. Busch and Wachmann (2011) indicated that OC was higher in pigs with a higher growth rate in the weaning period, finishing period, and growth rate from birth to slaughter. The result that growth rate from birth to slaughter is associated with OC is in contrast to the statement made above that body weight gain may have a short time frame to affect OC development. In contrast, Ytrehus et al. (2004ac) was not able to show an effect of growth rate or body weight in pigs from birth to slaughter or in specific age intervals on OC manifesta lesions.

To assess if growth rates are associated with OC close to slaughter (24 weeks of age) from our studies, we analyzed average daily growth rates for every 4 weeks from birth until 24 weeks of age (on average 3.8 [0.3 SD], 7.8 [0.3 SD], 11.8 [0.4 SD], 15.8 [0.3 SD], 19.8 [0.3 SD], 23.7 [0.5 SD] weeks of age) within each experiment. Analyses were performed only for gilts that were continuously on the same treatment throughout an experiment to exclude possible environmental (treatment) influences on OC status. In other words, associations were assessed separately for gilts receiving continuously ad libitum (AA treatment) or restricted feeding (80% of ad libitum; RR treatment) in the dietary restriction experiment (Chapter 2), for gilts kept continuously on a concrete partially slatted floor (CC treatment) or on a deep litter system using wood shavings (WW treatment) in the floor type experiment (Chapter 3), and for gilts from 1 of 4 treatment groups of the 2x2 factorial treatment design of carbohydrates (high carbohydrates [C+] consisting of 12.5% cornstarch and 12.5% dextrose versus low carbohydrates [C-]

in which cornstarch and dextrose were replaced with 8.9% soya bean oil) and arginine supplementation (0.8% arginine supplementation [A+] versus 1.6% alanine supplementation [A-] as the isonitrogenous control) in the carbohydrate and arginine experiment (Chapter 6). Within each treatment group, the gilts were classified into 3 OC categories at the animal level: no OC (OC score 0), minor OC (OC scores 1 and 2), and severe OC (OC scores 3 and 4). This was not performed for each joint separately as prevalence of severe lesions was too low for sensible analyses (Chapter 2, 3, and 6). The classification of gilts in the OC categories was based on the maximum OC score present. For example, if a gilt was scored with an OC score 1 in the knee joint, 4 in the hock joint, and 3 in the elbow joint, then that gilt would be classified in the severe category as the maximum OC score present is OC score 4. The associations of growth rates with OC were assessed in a mixed linear repeated measurement analysis using PROC MIXED in SAS 9.2 in which growth rates served as the dependent variable. The model included the class effects of the departments and pens in which the gilts were kept (4 departments; 8 pens) to account for environmental variation, the fixed class effect of the time period for which growth rates were calculated (4 week intervals from birth to 24 weeks of age), the fixed class effect of the OC severity classifications (no OC, minor OC, severe OC), the fixed class effect of the interaction between the time period and OC classifications to assess whether OC classifications are associated with growth rates at different 4 week intervals, and the random effects of dams (38 to 43 dams). As mentioned, a repeated measurement analysis was performed with gilts as the subject to account for the fact that growth rates within one animal are correlated over time per pen (experimental unit). A first-order ante-dependent variance-covariance structure was applied to this repeated structure, which was determined to fit to the data best according to the corrected Akaike's information criterion from analyses on the dietary restriction experiment. The results are indicated as the least squares means with accompanying SE and presented in Figure 7.1. Very limited associations were found between OC and growth rates. A significant association ($P < 0.05$) was found only for the overall growth rate of gilts in the WW treatment from the floor type experiment and indicated that gilts with minor OC lesions had a higher growth rate (0.688 [0.014 SE] kg/d) than gilts with no OC (0.657 [0.009] kg/d; $P = 0.03$) or with severe OC (0.653 [0.009 SE] kg/d; $P = 0.01$). Osteochondrosis tended ($P = 0.06$) to be associated with the growth rates of

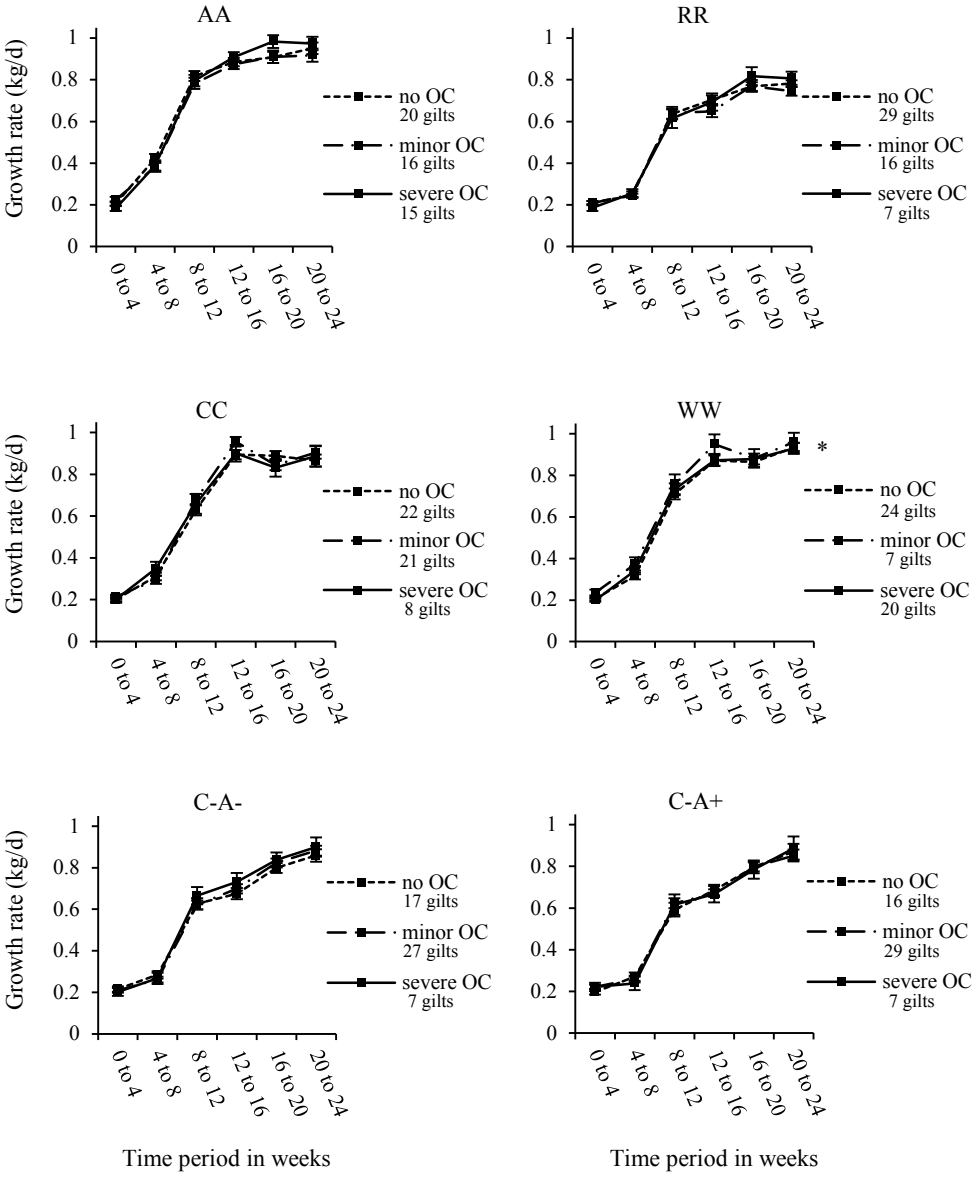

Figure 7.1. Associations of osteochondrosis (OC) with average daily growth rates for each 4 week period from birth to 24 weeks of age (close to slaughter). Osteochondrosis was classified as no OC (OC score 0), minor OC (OC score 1 and 2), and severe OC (OC score 3 and 4). Classification of OC was based on the maximum OC score present within a gilt at the animal level (elbow, hock, and knee joints combined). Associations with growth rates were analysed for gilts within 1 treatment group from 3 separate experiments. From the dietary restriction experiment (Chapter 2): AA = ad libitum feeding from weaning until slaughter; RR = continuously restricted feeding from weaning until slaughter. From the floor type experiment (Chapter 3): CC = conventional floor from weaning until slaughter; WW = wood shavings as bedding from weaning until slaughter. *(continued on the next page)*

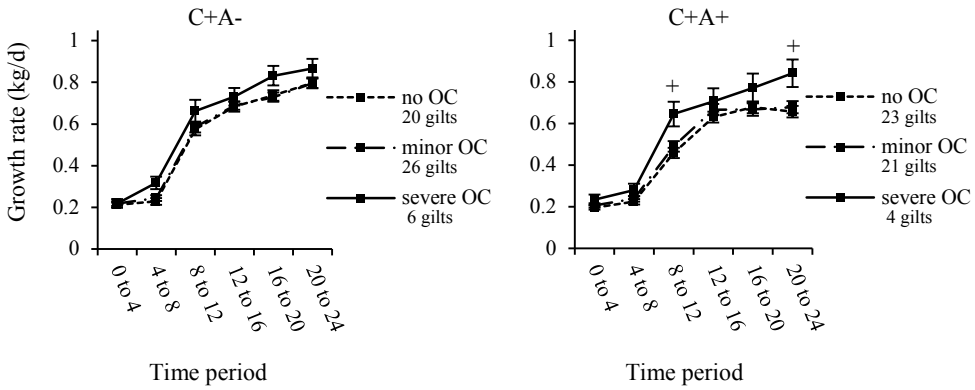

Figure 7.1. ***(continued).*** From the dietary carbohydrate and arginine experiment: C+ = high carbohydrate diet; C- = low carbohydrate diet; A+ = diets supplemented with arginine; A- = diets supplemented without arginine (2x2 factorial design). The number of gilts for each treatment and OC classification is depicted in the figures. Symbols indicate the following differences: * indicates overall significant (P<0.05) effects of minor lesions associated with a higher growth rate than no lesions or severe lesions in the WW treatment; + indicates a tendency (P=0.06) effect for severe OC lesions associated with a higher growth rate compared with no or minor lesions.

gilts in the C+A+ treatment in the carbohydrate and arginine experiment when dam effects were excluded from the model (model would not run otherwise). This association indicated that in the period of 8 to 12 weeks of age and 20 to 24 weeks of age, gilts with severe OC had a higher growth rate (0.646 [0.059] kg/d and 0.842 [0.067] kg/d, respectively) compared with gilts with no OC (0.458 [0.025] kg/d and 0.657 [0.028] kg/d, respectively) and minor OC (0.490 [0.026] kg/d and 0.679 [0.029] kg/d, respectively). A complicating factor in these analyses is the classification of OC in different categories. This classification of OC in categories does not take the number of lesions into account. To analyze whether the number of lesions per gilt is associated with growth rates, the number of OC lesions present per gilt were classified as no OC lesions present, one OC lesion present, and 2 or more OC lesions present. Similar statistical models were performed with the association of growth rates and OC severity classifications, but replacing the OC severity classification by the number of OC lesions classification. Only one significant association was found for gilts in the dietary restriction experiment receiving the AA treatment (P = 0.04). This significant association indicated that in the period of 0 to 4 weeks of age, gilts with 2 or more lesions had a significantly

lower growth rate (0.19 [0.01] kg/d) than gilts with one lesions (0.24 [0.02] kg/d). Additionally, gilts with 2 or more lesions had a significantly lower growth rate in the period from 8 to 12 weeks of age (0.76 [0.02] kg/d) compared with gilts with 1 lesions (0.86 [0.03] kg/d) and gilts with no lesions (0.82 [0.02] kg/d). The reason why minor lesions in the WW treatment was associated with a higher growth rate and 2 or more lesions was associated with a lower growth rate in the AA treatment cannot currently be explained. One would expect that an increase in growth rates would result in an increase in number and severity of OC lesions as that would increase the loading experienced on the joints. These limited and contradictory associations of OC with growth rates may be due to small contrasts in growth rates between OC affected gilts within 1 treatment group (Figure 7.1). Considering that there were clear effects of dietary restriction on body weight (and therefore also growth rates) and OC prevalence (Chapter 2) between treatment groups, effects of growth rates on OC may only be apparent with large contrasts in growth rates. Possibly, in our study, the number of severe lesions present at such a young age were limited and still in their minor to moderate stage. Associations between growth rates and OC may depend on the severity of lesions. Lundeheim (1987) followed the growth rate of piglets after 30 kg of body weight during 4 periods of approximately 14 days each. They indicated that although pigs with minor to moderate OC lesions grew faster during each period, the pigs with severe OC lesions had a lower growth rate in the fourth period. They attributed this to an increase in lameness that was possibly related to OC, resulting in pigs becoming uncomfortable and expressing itself in a lower intake of food. Thus, growth rate and body weight attained are likely associated with each other when contrasts in growth rates are large or when severe lesions of OC are developing. The associations are, however, not absolute. Carlson et al. (1988) indicated that given time, OC lesions might regress naturally. They indicated that the prevalence of OC, as scored through histology and radiography, does not differ between pigs either fed restricted or ad libitum when the pigs were slaughtered at the same age (approximately 29 weeks of age). When pigs were fed restricted and slaughtered at the same body weight as the ad libitum fed animals (approximately 39 weeks of age), the restricted animals had a lower prevalence of OC and was less severe. They contributed their results to the fact that many OC lesions in the restricted fed older pigs will have had a chance to resolve when compared with the restricted fed

younger pigs (Carlson et al., 1988). This time dependence of resolution complicates associations of environmental factors that influence body weight with OC, because they might depend on when OC is assessed. Even more complicating is the fact that the effects of dietary restriction on OC is especially true for pigs kept on conventional floors as compared with pigs kept on a deep litter type system (van Grevenhof et al., 2011). General continuous (gradual) loading of the joints through body weight may be insufficient in explaining the prevalence of OC by itself. Perhaps irregular or variating loading pressures experienced on joints explain the prevalence of OC better, which can be influenced by the floor that gilts are kept on.

7.3.2 Floor Type

Considering that van Grevenhof et al. (2011) found effects of floor type, one may wonder whether it is impact loading that is important in the development of OC rather than a continuous higher loading. In that respect, compared with a deep litter type system, a conventional concrete floor could yield a higher impact load experienced on the joints as well as being more slippery, leading to more traumatic events. However, we found contrary evidence for effects of floor type on OC when we kept gilts on a concrete partially slatted floor compared with a deep litter type system using wood shavings as bedding with or without a switch in floor type at 10 weeks of age (Chapter 3). Gilts kept on a deep litter type system before or after 10 weeks of age or both, had significantly a higher prevalence of severe lesions of OC compared with gilts on a concrete partially slatted floor, without affecting total prevalence of OC. This is in agreement with other studies who also noted a higher prevalence of severe lesions of OC in pigs kept on a deep litter type system or free range system (van Grevenhof et al., 2011; Etterlin et al., 2014). This was puzzling to us until we focused on the behavior that was performed by the animals. Not surprisingly, the gilts kept on a deep litter type system showed more play behavior compared with gilts kept on a concrete floor and is in concordance with other studies (Lay Jr. et al., 2000; Bolhuis et al., 2005, 2006). It, therefore, could mean that although impact loading overall was less in the deep litter type gilts, the sudden movements made during play behaviors could have stressed the joints to an extent that existing minor lesions would be aggravated into severe lesions of OC. In this sense, one would conclude that irregular and sudden loading

is more important in the development of OC as partially suggested by others (Etterlin et al., 2014). This is not in agreement with observations (not quantified) made in our dietary restriction experiment (Chapter 2). In that experiment, gilts fed restricted were observed to be very agitated, aroused, and active around feeding in the morning and in the afternoon, especially in anticipation of the food. Much like play behaviors that are short bouts of activity, this type of anticipatory arousal and activity also included short bouts of activity such as running, mounting the pen and other gilts, and jumping. The gilts fed restricted after 10 weeks of age had the lowest prevalence of OC and would suggest that high activity levels are not causative of OC. However, one of the differences between the dietary restricted experiment and the floor type experiment was that gilts in the floor type experiment were fed ad libitum. The difference in feeding strategy could mean that if pigs have a lower body weight (restricted feeding) that activity may not be detrimental for OC to develop, whereas activity is detrimental for OC development on a high feeding level leading to a higher impact loading due to a higher body weight. Therefore, activity alone does not seem to be the main factor important in the development of OC. Such contradictory results are also found in horses where both indoor stable housing (less exercise) and irregular exercise compared to free daily exercise with or without a rough and/or large terrain has been shown to increase (severe) OC (van Weeren and Barneveld, 1999; Lepeule et al., 2009; Lepeule et al., 2013; Praud et al., 2013; Vander Heyden et al., 2013).

It seems that neither dietary restriction, floor type, or activity alone is able to predict with high accuracy that the animals will develop OC. Most likely it is a combination of factors, reflecting the multifactorial facet of OC. Animals with a high body weight have a high loading on joints that could be aggravated by sudden movements as expected in play behavior in deep litter type systems, resulting in high impact loading on the joints. An animal is then especially at risk to develop OC when loading of the joints increases drastically during a short period within the major time frame of OC development, which is likely around 10 to 16 weeks of age as mentioned before.

7.3.3 Resolving by Diet

The previous section elaborated on environmental factors that may increase OC development. As was noted in the beginning of this discussion, viable

chondrocytes and vasculature within the growth cartilage are reported to undertake reparative attempts against OC lesions. Additionally, OC manifesta lesions may be resolved in the subchondral bone. To the authors' knowledge, there are no studies that try to affect OC resolution by enhancing the reparative attempts undertaken by chondrocytes and vasculature. In an attempt to affect these processes, we performed a study on the effects of dietary carbohydrate and arginine (Chapter 6). Dietary carbohydrates are known to affect insulin, glucose, and insulin like growth factor-1 (IGF-1) responses. In general, insulin and IGF-1 have shown to increase proliferation and survivability of chondrocytes (Böhme et al., 1992; Ballock and Reddi, 1994; Hunziker et al., 1994; Alini et al., 1996; Henson et al., 1997a). With a lower IGF-1 concentration one can envision that the chondrocyte are less stimulated to proliferate due to a reduction in the growth stimulating aspects of IGF-1 (Thorp et al., 1995). Moreover, higher IGF-1 responses could potentially promote reparative processes undertaken by viable chondrocytes. In contradiction, higher glucose, insulin, and IGF-1 levels have been associated with a higher prevalence of OC in horses (Ralston, 1996; Pagan, 2001; Semevolos et al., 2001; Verwilghen et al., 2009). In an attempt to affect vascularization within the growth cartilage, we supplemented gilts either with or without arginine, which has been shown to affect vascularization / angiogenesis in hypoxic conditions (Schwarzacher et al., 1997; Murohara et al., 1998; Duan et al., 2000; Dulak et al., 2000; Hazeleger et al., 2007). Hypoxia can be expected during OC development as vascular elements are disrupted. In addition, a recent study suggests that OC in pigs seems to be related with genes associated with angiogenesis (Rangkasenee et al., 2013).

Our results indicated that gilts on a high carbohydrate diet significantly had a lower prevalence of OC than gilts on a low carbohydrate diet (55% versus 68%, respectively). Gilts receiving arginine supplementation tended to have a lower prevalence of OC (59% versus 63%, respectively). However, several confounding factors may underlie this result. First, the low carbohydrate diet had carbohydrates replaced with soya bean oil as a fat source to meet energetic requirements. As fat has a higher energy density than carbohydrates, the amount of feed given to the gilts has to be adjusted in order to feed them isocaloric. In other words, gilts receiving high carbohydrates will receive a higher amount of feed when compared with gilts receiving a lower amount of carbohydrates. This meant in our study that

the gilts on the high carbohydrate diet were seen (observational only) to be less aroused and agitated before feeding than the animals on the low carbohydrate diet, which often finished the feed well before gilts in the high carbohydrate diet. Considering that activity / behavior likely has an effect on OC development, we cannot conclusively say that dietary carbohydrates affect OC by affecting chondrocyte functioning. Second, feed refusals were high in the gilts receiving the diet with a high carbohydrate level and supplemented with arginine. This resulted in body weight differences that may have affected the development of OC in a manner similar to loading distribution as discussed in the previous paragraph. The effect of body weight differences at slaughter was significantly associated with OC prevalence and superseded carbohydrate and arginine effects. It therefore seems that our attempts to affect OC development through dietary composition may have been mediated through body weight gain. These results, however, provide further indications for body weight as an important factor affecting OC prevalence.

7.4 DETECTING INSTEAD OF AFFECTING

Rather than focusing on the factors involved in the development or prevalence of OC for an entire group of animals regardless of their OC status, one may wonder whether detection at an early age is possible. Such information would give potential to exclude those animals from the breeding population or to take preventive measures for those animals so that it doesn't exacerbate. Although this would be an interesting avenue, the reason that it is not widely applied is because it is difficult to assess a large number of animals in a relatively short time frame in vivo. Methods such as X-ray imaging, magnetic resonance imaging, and computed tomography scanning need a relatively calm, partially or completely sedated animal to obtain clear pictures of the joints (Dik et al., 1999; Aasmundstad et al., 2013; Toth et al., 2013; Olstad et al., 2014ab). Such methods, therefore, are laborious and difficult in terms of practicality on a large scale in a short time frame, but are likely more accurate, as indicated for computed tomography scanning (Olstad et al., 2014ab), than indirect measurements. Indirect measurements of detecting OC include in vivo measurements on an animal other than direct evaluation of the joints such as plasma components or the constitution of an animal. In the study presented in Chapter 4, we focused on the constitution of a gilt by scoring conformation and locomotive characteristics (CLC). In the study

presented in Chapter 5, we focused on a plasma component concerning natural (auto-) antibodies (N[A]Ab). However, both factors had limitations in their associations with OC.

7.4.1 Conformation and Locomotive Characteristics

It was previously stated that CLC have an association with OC (Jørgensen, 1995, 2000; Jørgensen et al., 1995; Stern et al., 1995; Jørgensen and Andersen, 2000; Luther et al., 2007; Kirk et al., 2008; de Koning et al., 2012). However, when we assessed CLC several times during the rearing period of breeding gilts, consistent associations between CLC and OC were not clearly evident (Chapter 4). Partially, this is because severe deviations from normal conformation were not rigorously present. Additionally, relatively low correlations were found between CLC assessments for several CLCs, indicating that CLC are not consistent throughout time (Chapter 4; van Steenbergen et al., 1990; Stavrakakis et al., 2014). When the data were analyzed based on the moment a deviation from normal CLC occurred and persisted towards the end of the experiment, it resulted frequently in less than 10 animals in the different categories in which animals consistently were deviating in the same direction. Thus, CLC were determined as being variable over time as is corroborated by others (van Steenbergen et al., 1990; Meijer et al., 2014a). Stavrakakis et al. (2014) indicated that pigs with abnormal leg conformation show a difference in walking kinematics compared with pigs with OC lesions, to which the authors indicated that CLC may not be a good predictor of OC considering the difference in walking kinematics between the 2 conditions. Such a result could be an underlying difficulty in our efforts to associate CLC with OC. Additionally, considering that OC development is also dynamic in time, associations between CLC and OC are difficult to assess. Some CLC that may cause OC through local overloading at some point in time, may not cause a detrimental local overload at a different time point due to less vasculature being present. Conversely, some CLC may be present before OC development but change several weeks afterwards. This complicates at what age CLC should be assessed to predict what the OC status of the animals is. The limitation of our study was that OC was not determined at the same time that CLC were assessed. The way CLC affect OC, or vice versa, remain speculative and uncertain. Furthermore, breed and sex differences exist in the associations of CLC with OC, complicating

comparisons between studies (Lundeheim, 1987; van der Wal et al., 1987; Jørgensen and Andersen, 2000). Therefore, CLC may not be a clear indicator of OC status or not sensitive enough. Studies using pressure plate or motion capture analysis show subtle differences in gait characteristics in lame and sound pigs and might be better suited to assess lameness in animals that could then be associated with OC (Meijer et al., 2014ab; Stavrakakis et al., 2014; 2015).

7.4.2 Plasma Components

Perhaps it is necessary to focus research on biomarkers within plasma. However, the question then arises on which biomarker the focus should lie. We have indicated a component of the immune system to be associated with OC (Chapter 5) consisting of naturally occurring (auto-) antibodies (N[A]Ab). The function of N(A)Ab revolves around maintaining homeostasis of the body and prevention of disease by clearance of damaged cells, intracellular components, and cell waste products (reviewed by Ochsenbein and Zinkernagel, 2000; Zelenay et al., 2007; Lutz et al., 2009; Ehrenstein and Notley, 2010; Binder, 2012; Bouhlal and Kaveri, 2012; Elkon and Silverman, 2012; Grönwall et al., 2012; Santos-Argumedo, 2012; Avrameas et al., 2007). Considering that OC development revolves around the formation of necrotic growth cartilage, one might expect that waste products from this necrosis are recognized and reacted to by N(A)Ab. Our results indicated that the odds of a gilt to have OC was higher for gilts with predominantly higher levels of IgM N(A)Ab measured at 6 weeks of age against chondroitin sulfate A, actin, and albumin (Chapter 5). However, associations were not very high and may indicate that they are less suitable to reliably predict OC status in pigs in practice, although much research remains to be done in the association of N(A)Ab and OC. Other components that may function as a biomarker for OC include cartilage products such as type I and II collagen pro-peptides or breakdown products or indicators of aggrecan (proteoglycan) synthesis to assess cartilage turnover (Semevolos et al., 2001; Billinghurst et al., 2003; Billinghurst et al., 2004; Frantz et al., 2010). However, this again is difficult as it has been noted that associations can be reversed depending on the age that markers are assessed (Billinghurst et al., 2003; Billinghurst et al., 2004) or with severity of OC lesions (Frantz et al., 2010). Other markers may lie in hormonal function considering that chondrocyte functioning is hormonally regulated by, amongst

others, growth hormone, IGF-1, thyroid hormones, and insulin (Böhme et al., 1992; Ballock and Reddi, 1994; Hunziker et al., 1994; Alini et al., 1996; Henson et al., 1997a; Sloet van Oldruitenborgh-Oosterbaan et al., 1999; Semevolos et al., 2001). In studies taking repeated samples throughout growth of the animals, it has been noted that associations between OC and IGF-1 are not always significant for the months in which blood samples are taken (Sloet van Oldruitenborgh-Oosterbaan et al., 1999). This is likely due to the fact that OC development is highly dynamic with lesions developing and resolving. If a lesion has developed, the cartilage responds by attempting reparative processes and then associations of biomarkers and OC are likely to be found. However, when samples are taken in which OC lesions just by chance are not there or are stable, associations become difficult to ascertain. Furthermore, components measured in plasma (such as GH, IGF-1, glucose, insulin, and N[A]Ab) often have general functions and do not only function on chondrocytes within the growth cartilage, but act on many other cells in other organs as well. For example, IGF-1 has many mitogenic properties in different phases, which may rise during puberty and due to sex hormones (reviewed by Humbel, 1990). Cartilage tissue is abundant in the body and degradative products from cartilage on a systemic level may not fully reflect what is happening in the growth cartilage. This may make it difficult to associate a plasma component that has systemic effects on a very localized problem such as OC. Additionally, in our experiments, OC lesions predominantly consisted of OC scores 1 and 2 (minor OC lesions). It is possible that associations between plasma components and severe OC lesions are more apparent because there is more tissue damage taking place that allow for more cellular components to react with constituents of the body such as N(A)Ab. To conclude, relying on indirect in vivo measurements of either plasma components or CLC are, at this point, not viable for accurate assessment of OC on a large scale in a short time frame.

7.5 IMPLICATIONS OF OSTEOCHONDROSIS

Now that we have indicated what factors affect OC in pigs, one may wonder what the implications of OC are in practice. Osteochondrosis is suggested to be involved in the occurrence of lameness and reduced longevity, but the exact extent is not known. Differences in OC prevalence between studies exist and can range from 10% to 80% depending on the joints or breeds assessed (Jørgensen et

al., 1995; Jørgensen and Andersen, 2000; Ytrehus et al., 2004c; Luther et al., 2007; Busch and Wachmann, 2011; van Grevenhof et al., 2011). In our studies with breeding gilts (Chapters 2, 3, and 6) we noted that the prevalence of severe lesions of OC is lower compared with minor lesions of OC. Figure 7.2 shows the prevalence of OC on the animal level (elbow, hock, and knee joints combined) at roughly 6 months of age in Topigs 20 breeding gilts throughout the 3 experiments. The proportion of gilts having an OC score greater than 0 often consists for a large part of OC scores 1 and 2 (minor lesions), especially in the dietary carbohydrate and arginine experiment. Additionally, it was noted that the elbow joint was hardly affected by OC, followed by the hock joint and the knee joint. Such a relatively high prevalence of minor lesions of OC makes it difficult to assess the implications of OC for animal welfare. Considering that no gilts were removed from the experiment due to severe lameness related to OC, OC may not be a very important health and welfare risk at 6 months of age in this line of gilts. Possibly in our experiments we were too early in slaughtering the animals at 6 months of age to see the effects of OC on lameness and animal welfare. One may speculate that existing lesions may be aggravated beyond 6 months of age due to increasing loading pressures experienced on the joint as the animal increases in weight. Crenshaw et al. (2013) have indicated that OC prevalence in parities 0 to 7 in 1 herd undergoing a 'planned depopulation' does not differ between parities in the elbow joint (75% OC prevalence). However, that study had a limited number of sows per parity (on average 8 per parity) and might limit the comparisons that can be made, especially considering that severe OC lesions in this thesis in a population of 200+ animals was relatively lower compared with minor lesions. New data on OC prevalence in Dutch commercial husbandry sows may provide some further insight into the prevalence of OC in sows over several parities and culling reasons.

7.5.1 Osteochondrosis Prevalence in Dutch Commercial Husbandry

Recently, data on OC prevalence from Dutch commercial husbandry sows from the left hock and knee joints were collected on Topigs 20 F1 sows, randomly selected at a slaughterhouse from March 2014 to October 2014 (raw data supplied by van Grevenhof et al., unpublished data). Farmers decided which sows were sent to be culled according to standard commercial practices and agreed to collaborate to keep track of reasons for culling per sow. Eight farms participated with a total of

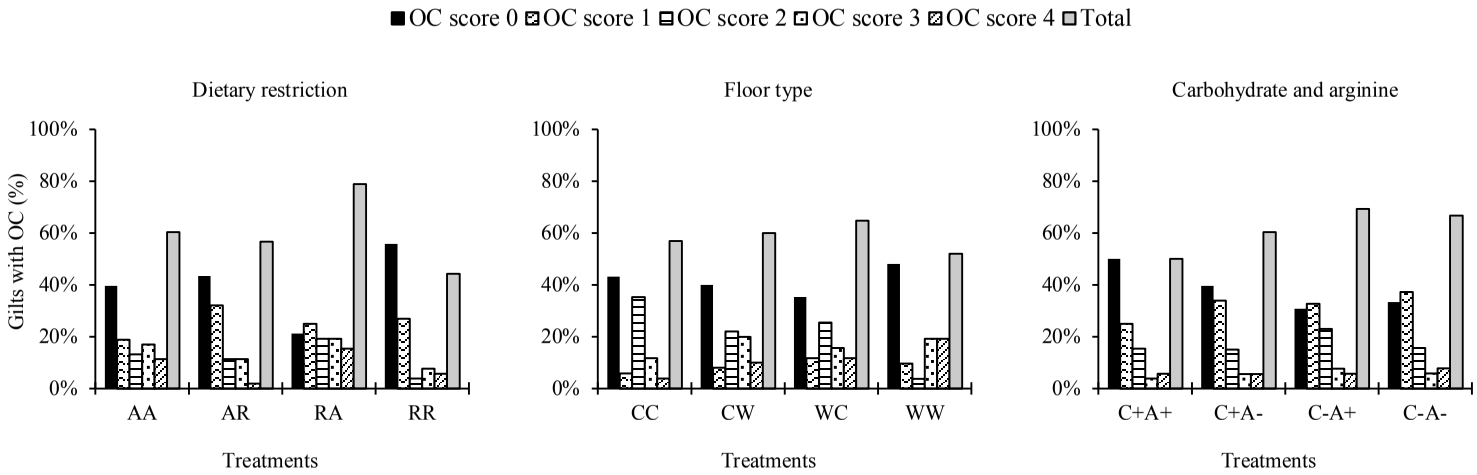

Figure 7.2. Percentage of gilts affected with osteochondrosis (OC) at approximately 6 months of age within the dietary restriction experiment, floor type experiment, and carbohydrate and arginine experiment. For the dietary restriction experiment treatments are: AA = ad libitum feeding from weaning until slaughter (53 gilts); AR = ad libitum feeding up to 10 weeks of age, after which feeding was switched to restricted (80% of ad libitum uptake; 53 gilts); RA = restricted feeding up to 10 weeks of age, after which feeding was switched to ad libitum (52 gilts); RR = continuously restricted feeding from weaning until slaughter (52 gilts). For the floor type experiment treatments are: CC = conventional floor from weaning until slaughter (51 gilts); CW = conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding (50 gilts); WC = wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a conventional floor (51 gilts); WW = wood shavings as bedding from weaning until slaughter (52 gilts). For the carbohydrate and arginine experiment treatments consisted of a 2x2 factorial design of carbohydrates and arginine: C+ = high carbohydrate diet; C- = low carbohydrate diet; A+ = diets supplemented with arginine; A- = diets supplemented without arginine (C+A+ 52 gilts; C+A- 53 gilts; C-A+ 52 gilts; C-A- 51 gilts). For each treatment, the percentage of gilts are displayed with the highest OC score present. For example, if a gilt had an OC score 0 in the elbow joint, an OC score 2 in the hock joint, and an OC score 4 in the knee joint, then that gilt is counted in the OC score 4 category as it is the highest score present. 'Total' is the total percentage of gilts with any form of OC higher than score 0.

540 sows culled. Due to practical circumstances, information on parity and culling reasons were not present for all sows slaughtered. The number of sows for which information on parity and culling reason were present are shown in Table 7.1. The left hind legs were collected after slaughter and stored at -20°C before use. The legs were thawed 2 days before dissection of the hock and knee joints. After dissection, the surface of the knee and hock joint were macroscopically scored on several locations (see Chapter 2 Table 2.1) for irregularities indicative of OC on a scale of 0 to 4, with 0 indicating no OC and 4 indicating severe irregularities (van Weeren and Barneveld, 1999). Osteochondrosis was scored by 1 veterinarian specialized in orthopedics and experienced in judging OC.

The data of a random population of culled sows (termed commercial sows from here on out) showed a higher rate of total OC lesions (Figure 7.3A-B) compared with our experiments (Figure 7.2). Locations affected by OC in the hock joint were the lateral and medial trochlea of the talus, and the lateral and medial tibial cochlea. Locations affected by OC in the knee joint were the medial femoral trochlea and the lateral and medial femoral condyle. Of the total prevalence of OC scores greater than 0 in the knee joint, the majority were found in the medial femoral condyle (97%). Of the total prevalence of OC scores greater than 0 in the hock joint, the majority were found in the medial trochlea of the talus (54%), followed by the medial tibial cochlea (27%) and the lateral tibial cochlea (17%). Total prevalence of OC lesions ranged up to 90% at the animal level. However, the

Table 7.1. Number of sows[1] send for culling per parity and culling reason from commercial husbandry.

Culling reason	Parity					
	0 to 2	3 to 5	6 to 8	9 to 11	Total	Total regardless of parity
Fertility	7	10	14	8	39	64
Lameness	9	10	4	1	24	39
Udder	1	6	7	0	14	23
Age	0	0	35	10	45	76
Condition	0	1	1	0	2	4
Other	6	5	8	0	19	35
Total	23	32	69	19	143	241
Total regardless of culling reason	23	32	71	19	145	

[1] The number of sows for which both parity and culling reasons were present (centre block) were lower than for sows when only taking either parity (=total regardless of culling reason) or culling reason (=total regardless of parity) into account. (Raw data supplied by van Grevenhof et al, unpublished data)

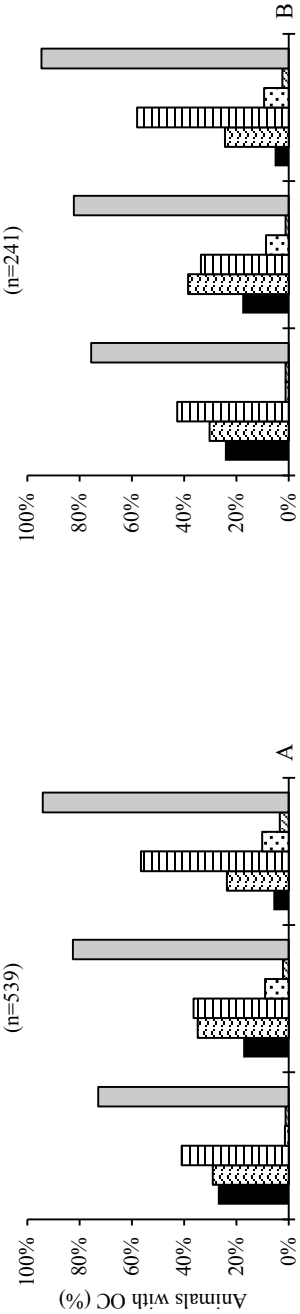

Figure 7.3A-B. Osteochondrosis (OC) prevalence in commercial husbandry sows. Osteochondrosis was assessed in the left hock and knee joint and at the animal level (hock and knee joint combined). (A) The percentage of animals with OC for all animals slaughtered and (B) for which culling reasons are known. Within each graph, the percentage of animals are displayed with the highest OC score present. For example at the animal level, if a sow had an OC score 0 in the hock joint and an OC score 4 in the knee joint, then that sow is counted in the OC score 4 category as it is the highest score present. 'Total' is the total percentage of sows with any form of OC (higher than score 0). (Raw data supplied by van Grevenhof et al, unpublished data)

total prevalence was again made up of predominantly OC scores 1 and 2 as in our own experiments. Because OC was scored in all 4 legs from each gilt in our experiments and only 1 hind leg was scored from each commercial sow, comparisons between the two are somewhat difficult. The prevalence of severe OC lesions (OC score 3 and 4) was 13.7% of sows affected at the animal level in the commercial sows. In our dietary restriction and floor type experiments, the prevalence of severe lesions of OC differed depending on which number of legs were scored. For gilts that were switched from restricted to ad libitum feeding (Chapter 2), prevalence of severe OC was 34.6% when all 4 legs were assessed, but decreases to 9% (left hind leg) or 11% (right hind leg) when only taking 1 hind leg into account. For gilts that were kept continuously on a deep litter type system using wood shavings (Chapter 3), prevalence of severe OC was 38.4% when all 4 legs were assessed, but decreases to 27% (left hind leg) or 29% (right hind leg) when only taking 1 hind leg into account. These experimental groups do not generally represent housing or feeding conditions used in the Dutch commercial husbandry, which therefore may explain the difference in severe OC lesions with that of the commercial sows. Gilts that received continuously an ad libitum feeding level (Chapter 2) showed a prevalence of 28% severe OC lesions when all 4 legs were assessed, but decreases to 13% (left hind leg) or 7% (right hind leg) when taking only 1 hind leg into account. When taking all 4 legs into account, the prevalence of severe lesions in the commercial sows is similar to gilts receiving a restricted diet after 10 weeks of age (Chapter 2; approximately 13% prevalence of severe OC) and gilts continuously kept on partially slatted flooring (Chapter 3; approximately 15% prevalence of sever OC). However, when assessing only 1 hind leg in gilts continuously fed restricted, the prevalence of severe OC decreases from 13% to 0% (left hind leg) or 5% (right hind leg). Similarly, when assessing only 1 hind leg in gilts continuously kept on a partially slatted floor, the prevalence of severe OC decreases from 15% to 2% (left hind leg) or 9% (right hind leg). Thus, depending on the rearing conditions, number of legs assessed, and from which side (left or right) legs were assessed, prevalence of severe OC in the experimental gilts compared with the commercial sows can be larger, similar, or smaller. Data on the exact rearing conditions of the commercial sows is unavailable. It is a possibility that the prevalence of severe lesions in the commercial sows was higher when all 4 legs would have been assessed, but it is unknown whether this is the case.

When looking at the reasons for culling (due to practical circumstances not all culling reasons were known for all animals) and prevalence of OC within each reason (Figure 7.4A-C), lameness is not the category with the highest prevalence of total or severe OC lesions. Rather, the prevalence of OC seems relatively equally divided over the different reasons for culling. With increasing parity (due to practical circumstances, parities were not known for all animals) one can see a slight increase of total prevalence of OC but not for severe lesions of OC (Figure 7.4D-F). The data were analyzed in PROC GLIMMIX in SAS 9.2 with a binary logistic regression analysis in which OC scores were grouped in a binary variable consisting of 0 (OC score 0, no OC) and 1 (OC score greater than 0). Ordinal logistic regression was not possible due to too few number of each OC scores with parity class and culling reasons. The locations on which OC was scored served as the repeated measurement element with animals as the subject. Parity class, culling reasons, and their interaction were entered in the model as fixed class effects. Farm and culling date were entered in the models as random variables. However, the model for the effect of culling reason on OC in the knee joint was not convergent and could therefore not be tested. No significant effects could be found for either parity class or culling reason ($P>0.05$) when farm and culling date were not in the model as random variables. When farm and culling date were in the model as random variables, only parity had a significant effect on OC prevalence only in the hock joint and indicated that sows in parity class 3 to 5 (81.3%) had a higher OC prevalence than sows in parity class 0 to 2 (60.9%) and 9 to 11 (68.4%). The relatively few OC scores 0 could possibly complicate assessments with culling reasons and parity. The analyses were performed again by taking the mild lesion OC score 1 into the 0 category of the binary OC variable in statistical analyses. Significant parity effects were not present ($P>0.05$). Culling reasons showed a significant ($P=0.05$) effect only at the hock joint when farm and culling date were excluded from models, and specified that sows culled for udder reasons (65.1%) had a significantly ($P<0.05$) higher prevalence of OC (OC score higher than 1) compared with the culling reasons for lameness (46.2%), age (40.8%), and other reasons (37.1%).

The results from the commercial sows indicate that OC prevalence does not significantly increase with parity as supported by Crenshaw et al. (2013), which could indicate that OC does not worsen with increasing age with respect to severe

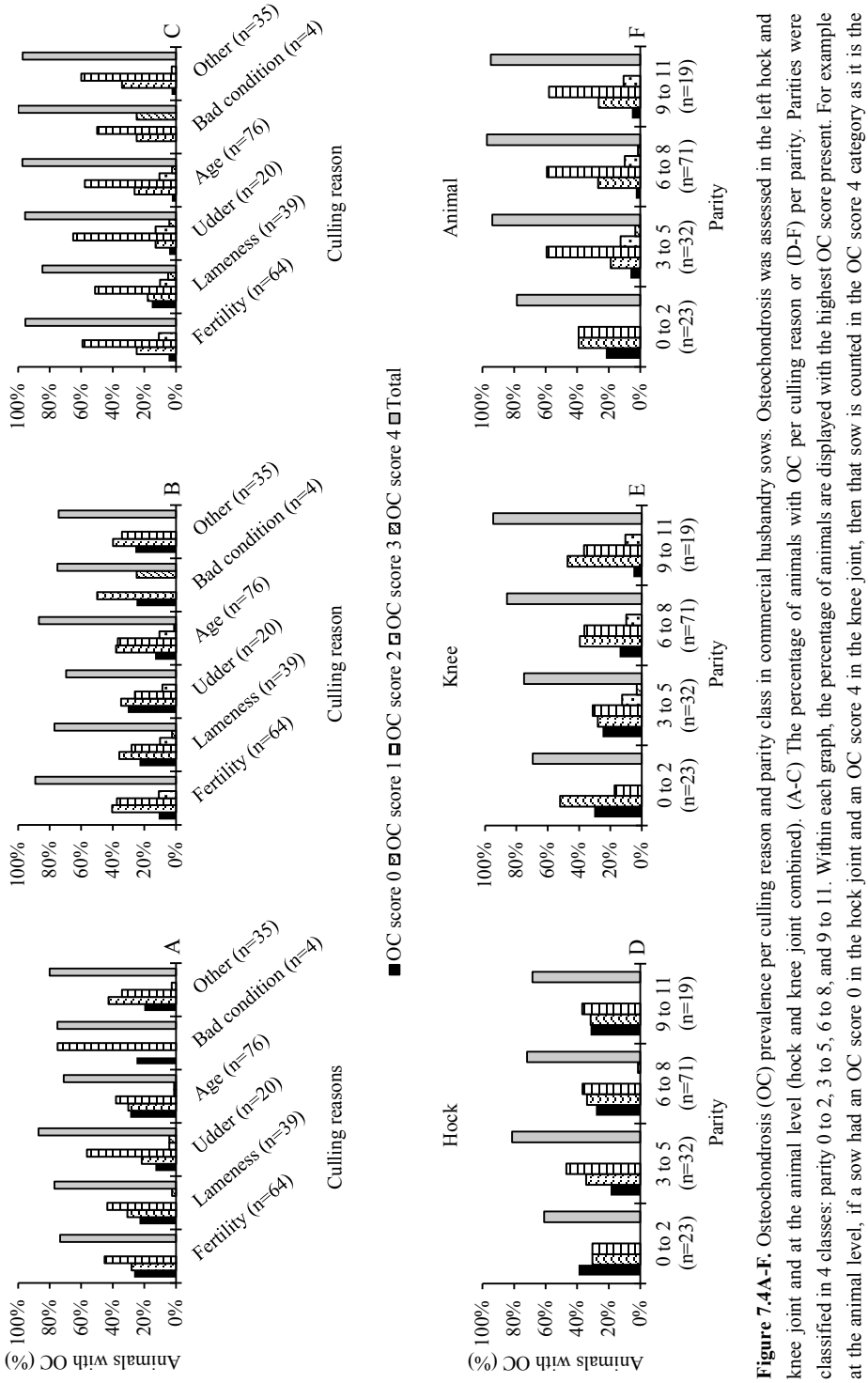

Figure 7.4A-F. Osteochondrosis (OC) prevalence per culling reason and parity class in commercial husbandry sows. Osteochondrosis was assessed in the left hock and knee joint and at the animal level (hock and knee joint combined). (A-C) The percentage of animals with OC per culling reason or (D-F) per parity. Parities were classified in 4 classes: parity 0 to 2, 3 to 5, 6 to 8, and 9 to 11. Within each graph, the percentage of animals are displayed with the highest OC score present. For example at the animal level, if a sow had an OC score 0 in the hock joint and an OC score 4 in the knee joint, then that sow is counted in the OC score 4 category as it is the highest score present. 'Total' is the total percentage of sows with any form of OC (higher than score 0). (Raw data supplied by van Grevenhof et al, unpublished data)

lesions. Considering these results from practice with the prevalence of OC in the experiments in young gilts described in this thesis (Figure 7.2), it would seem that especially the prevalence of minor lesions increases with age. Additionally, the lack of a significant effect of lameness as a culling reason on OC in the sows indicates that lameness is not the predominant factor associated with OC, at least in this dataset from practice. A complicating factor in this dataset is that only the left hind leg was assessed for OC, while a sow could have been lame in another leg affected by a severe lesion of OC when only a mild lesion was scored in the left hind leg. Complete associations of OC with lameness are then difficult to make.

Although the data of the randomly selected commercial sows (van Grevenhof et al., unpublished data) indicate that the overall OC prevalence is relatively high in a randomly selected population of crossbred sows that is culled, the exact implications of OC on animal welfare cannot be fully assessed. The random population of sows were specifically send to slaughter and data on OC status of remaining sows that are present at the farms are lacking. In addition, the number of minor OC lesions (OC score 1 and 2) were relatively large and it is unknown whether these types of lesions actually have presented or would likely present welfare issues directly related from OC to such an extent that the farmer decided to send the sows for culling. The problem in assessing welfare complications of OC also lies in the correct assessment of a welfare complication due to OC. As noted by Quinn et al. (2015), lameness resulting from OC can be difficult to detect when OC is bilaterally symmetrical. Any attempts of the pig to redistribute its weight away from a joint with a painful OC lesion is difficult when both the bilateral joints are affected with OC (Quinn et al., 2015). Additionally, Stavrakakis et al. (2014) showed that OC affected pigs visually scored as normal locomotion, have slight abnormal walking kinematics when assessed with motion capture methods, indicating the difficulty of visually assessing an animal with an abnormal gait. If such a case would present itself, then the animal is experiencing welfare complications that may be reflected in productive traits (reviewed by Heinonen et al., 2013), resulting in the observer incorrectly determining the reason for culling. Such subjective observations for culling reasons as performed for the commercial sows, therefore, might have limited value to determine effects of OC on animal welfare.

7.5.2 To Be or Not To Be Osteochondrosis

Actual prevalence of OC may have been larger in the current studies presented in this thesis (Chapter 2, 3, and 6). We assessed joint surface irregularities that are indicative of OC. It is possible that there were sub-articular cartilage lesions that were not detected, likely of the OC manifesta type (Ytrehus et al., 2004abcd). Articular cartilage surface irregularities have been found to be correlated well with the occurrence of fissures in the sub-articular cartilage in pigs (Busch and Wachmann, 2011) that are indicative of OC. Busch and Wachmann (2011) indicated that joints with underlying fissures were found to be related with humeral condyle surface irregularities in 77% of the cases, whereas no humeral condyle irregularities were visible when no underlying fissures were present in 80% of the cases. These results would indicate that 23% of the cases would show no irregularities on the articular cartilage surface when there are underlying fissures and 20% would show irregularities when there are no underlying fissures, indicating that some lesions may have been missed or misclassified in this thesis. Jørgensen and Nielsen (2005) showed that phenotypic correlations of elbow joint surface irregularities with cartilage thickness and subchondral lesions indicative of OC were 0.49 and 0.65, respectively, whereas genetic correlations were 0.75 and 0.97, respectively. Again, this indicates that relationships between articular surface irregularities and OC do exist and can be quite high, but they are not absolute. This does not take away any importance of the results described for the small percentage of irregularities unrelated to OC, as an articular cartilage surface irregularity might be able to induce lameness if severe enough as suggested by others (Lundeheim, 1987; Goedegebuure et al., 1988; Jørgensen, 1995), regardless of whether it was caused by typical OC lesions. Additionally, the lesions that were missed are questionable for the practical relevance. Several studies have indicated that sub-articular OC lesions (of the OC latens and manifesta type) can be resolved in several months (Carlson et al., 1988; Dik et al., 1999; van Weeren and Barneveld, 1999; Olstad et al., 2007; 2008a; 2014a). It would therefore seem beneficial to focus on OC lesions that have affected the articular surface as they are likely to be of more practical relevance than underlying lesions for which it is unknown whether they will progress to affect the articular surface. Partially, however, this may explain the difference in OC prevalence from the experiments performed in this thesis (Figure 7.2) and the OC prevalence from the commercial husbandry

sows (Figure 7.3). If at 6 months of age in our experiments (Figure 7.2) necrotic growth cartilage was present that had not affected the joint surface, and therefore was not scored, then that necrotic growth cartilage could (slightly) fracture with increasing age and body weight, affecting the articular surface at older age where it can be macroscopically scored. This could explain why in the commercial sows the prevalence of OC was higher (which constituted mainly OC scores 1 and 2).

7.5.3 Welfare Implications

The main factors that seem to be associated with OC are loading of joints through body weight or through activity of the animal. What type of implications does this have for practice? There is an increasing demand from society for husbandry systems that increase welfare of pigs. These include group housing systems of sows and piglets, increasing space available per animal, and environmental enrichment (EU Council Directives 2001/88/EC and 2008/120/EC; van Nieuwamerongen et al., 2014). One consequence of these systems is that the animals are stimulated more in their natural behaviors that could lead to higher activity levels. For example, animals in group housing systems will have more encounters with each other allowing for a higher degree of playing behaviors or aggressive conflicts, especially at introduction of animals into a group. Animals in systems with much environmental enrichment are stimulated to be more active (Morgan et al., 1998; Lay Jr. et al., 2000; Bolhuis et al., 2005, 2006; Scott et al., 2006). These factors obviously have beneficial effects for welfare, but are they negatively affecting the constitution of the animals? It is a disappointing result to find that an environmental enrichment such as deep litter (Chapter 3) or free range type housing (Etterlin et al., 2014) increases the incidence of severe lesions of OC in gilts. Clearly such systems have beneficial effects for the behavioral development of young pigs as they can perform a larger part of their ethological repertoire (such as foraging behavior). Additionally, there are clear indications that a deep litter type system increases welfare experienced by the gilts as they perform a higher degree of play behaviors (Lay Jr. et al., 2000; Bolhuis et al., 2005, 2006), which would only be performed when the animal is comfortable in its surroundings with a large part of the ethological repertoire being satisfied (reviewed by Fraser and Duncan, 1998; Boissy et al., 2007; Held and Spinka, 2011). One then has to determine whether possible long term negative effects of a deep litter type system

(increase in severe OC) outweigh the benefits of increased welfare at a young age and a better behavioral development of the animals. Perhaps an intermediate is possible. As mentioned, gilts kept continuously on a deep litter type system had a higher prevalence of severe lesions of OC. It might be a possibility that if gilts are kept on a conventional floor to 16 or 20 weeks of age (more on the outer edges of the time frame of OC development) and then switched to a deep litter type system, that the effects on severe OC are less present. These intermediate solutions may also be possible for other systems that stimulate activity or weight gain. Perhaps it is necessary to have a somewhat limiting environment at young age in order to reduce the risk for the development of OC.

The previously mentioned option of limiting the environment during rearing should only be optional when it is clear that OC has clear negative welfare effects at later age. The exact implications for animal welfare are still unknown. The results of OC prevalence in the commercial husbandry sows per culling reason (Figure 7.3) indicate that 16.2% of the sows culled were for lameness reasons of which 84.6% showed signs of OC. However, only 6 sows culled for lameness had severe lesions of OC (OC score 3 and 4) at the animal level (5 sows at the knee joint and only 1 sow in the hock joint). In contrast, a total of 23 sows with severe OC lesions were culled for other reasons. One may wonder what the implications are for animal welfare. Obviously, the animals culled for lameness are likely experiencing reduced welfare, but whether this is strongly associated with OC is uncertain. As mentioned before, studies indicated that only severe lesions of OC are able to induce lameness, but it is not an absolute relationship (Lundeheim, 1987; Goedegebuure et al., 1988) and severe clinical lameness related to OC is not noted in other studies as well (Carlson et al., 1986; Jørgensen, 1995; Jørgensen et al., 1995; van Weeren and Barneveld, 1999; Arnbjerg, 2007; Quinn et al., 2015). Jørgensen (1995) indicated that a lack of strong correlations between CLC and OC likely are a result of a lack of a high prevalence of severe OC lesions. Stavrakakis et al. (2014) showed that pigs with OC have detectable changes in walking kinematics, but clinical lameness prevalence was lower than OC prevalence. In our studies (Chapter 4; de Koning et al., 2012), we also indicated that a slower and stiffer gait is associated with higher odds to have OC as is corroborated by other studies (Jørgensen, 2000; Jørgensen and Andersen, 2000; Kirk et al., 2008), but this relationship was also not absolute. Apparently, some animals have OC but are

not affected by it through lameness or do not show signs of lameness. Lundeheim (1987) indicated that the relationship between OC and lameness may be influenced by an animals' perception of pain. Lundeheim suggested that this difference in perception towards pain may result in some animals not showing clinical lameness even though severe lesions of OC are present. To the authors' knowledge, it is unknown at which severity an OC lesion becomes painful to a pig and what distribution there is of pain perception with respect to OC. Osteochondrosis is likely associated to some degree with negative animal welfare resulting from lameness, but other factors contribute to lameness as well (reviewed by Jensen and Toft, 2009). Therefore, the exact implications of OC on animal welfare are not fully understood and may be a result from difficulties in studying OC. The multifactorial aspect of OC complicates assessments to be made for the effect that factors have on OC and what the welfare implications are of OC.

7.5.4 Difficulties in Assessing Effects

Many studies assessing treatment effects assess other factors confounded in the main treatment effects of interest. As discussed above, when we assessed the effect of dietary restriction (Chapter 2), floor type (Chapter 3), or dietary carbohydrate content (Chapter 6) we implicitly caused differences in the behaviors performed by the animals, which, in turn, can lead to differences in OC prevalence. As discussed by Etterlin et al. (2014), the higher prevalence of OC found for free range pigs compared with indoor conventional pigs is confounded with differences in number of animals in a pen and the area available. When assessing floor types (Jørgensen, 2003; Scott et al., 2006), activity will be influenced and, if slatted flooring is present, the stance of the legs or claws will also be influenced with differing size of the slats between studies. This may cause local overload within the joints that can be exacerbated by activity. Additionally, studies use different genetic lines or sexes. Breed differences exist in OC and even differences in sex within one breed exist (Goedegebuure et al., 1980; Lundeheim, 1987; van der Wal et al., 1987; Jørgensen and Andersen, 2000). It is therefore difficult to translate the results of one study to the results of other studies and comparing studies in their differences would be a never ending story. In our studies described in this thesis another complicating factor exists. Variables were measured (such as body weight, N(A)Ab, behavior, etc.) throughout the rearing period of gilts and related to OC at

6 months of age (end point measurement) at the end of an experiment. Such associations of variables throughout the rearing period associated with an end-point measurement can be difficult, as other non-measured factors in the time between measured variables could have influenced the end-point measurement of a dynamic condition such as OC. Partially, some of the issues could be resolved by the choice of the study subject. Studies and drastic measures as limiting the environment at young age should, according to the authors' opinion, only focus on breeding gilts. Fattening pigs are likely to develop OC, but as they are slaughtered at around 100 kg of BW, the likelihood of them experiencing negative welfare effects due to OC may actually not be very likely. Especially since several studies indicate that no severe clinical lameness due to OC is seen (Carlson et al., 1986; Jørgensen, 1995; Jørgensen et al., 1995; van Weeren and Barneveld, 1999; Arnbjerg, 2007; Quinn et al., 2015). Effects found in fattening pigs and boars cannot readily be translated to breeding gilts that are of higher (economic) interest to industry due to the reported sex and breed differences for OC prevalence (Goedegebuure et al., 1980; Lundeheim, 1987; van der Wal et al., 1987; Jørgensen and Andersen, 2000). By reducing research on fattening pigs and boars, it is likely that a bigger advance can be made on which factors contribute or are associated with OC. In order to assess any effect encountered at young age in breeding gilts on the prevalence of OC at later life and assess its welfare implications, a long term study from birth to culling would be beneficial in order to determine if OC lesions have implications for welfare to such an extent that sows need to be sent for premature culling. It would be of benefit to relate measured variables with OC at the time they were measured and not with much time in between. Ideally, one would like to serially monitor gilts during the rearing period to assess OC status (either through serial slaughtering or imaging) so that measured variables can be more directly related to the current OC status of a gilt. Following the progression of OC lesions would not only be beneficial during the rearing period, but also during later life to assess whether lesions of OC present at 6 months of age can be exacerbated by the continuing increase in body weight as a sow ages. Throughout each imaging or slaughtering time point, one could measure various traits such as CLC, body weight, or various plasma components including N(A)Ab, IGF-1, insulin, glucose, and cartilage breakdown products. Prospects on how serial OC imaging can be accomplished without sacrificing animals likely lie in computed tomography, magnetic resonance

imaging, radiographic analysis, motion capture, and pressure plate analysis (Dik et al., 1999; Aasmundstad et al., 2013; Toth et al., 2013; Meijer et al., 2014a; Olstad et al., 2014a, b; Stavrakakis et al., 2014). In order to ensure that gilts will develop OC severe enough that it might lead to lameness at a later age necessary to assess welfare implications of OC, one could use a rearing strategy that increases the odds to develop OC. Such a rearing strategy should at least involve dietary restriction up to 10 weeks of age and then switched to ad libitum feeding after 10 weeks of age (Chapter 2). Whether it is necessary that the gilts would be kept on a deep litter type system is uncertain. A deep litter type system increased the prevalence of severe lesions but not total lesions (Chapter 3), while dietary restriction with a switch to ad libitum feeding increased both (Chapter 2). It would therefore seem that initial dietary restriction combined with ad libitum feeding later on is sufficient in itself to increase the odds to develop OC. It would be interesting, however, to see whether the detrimental effects of a sudden and fast weight gain are exacerbated by sudden movements / impact loading due to play behaviors on deep litter type systems. This type of (large) experiment could lead to a better understanding of which factors are associated with OC and ultimately lead to a better understanding of how animal welfare is affected by OC. Only then will we be better able to indicate what the implications of OC are for practice.

7.6 CONCLUSION

This thesis focused on early life factors encountered by breeding gilts to determine if they influence prevalence of OC. In the studies performed, a myriad of factors were found to be associated with OC to some degree, i.e. dietary restriction, floor types, conformation and locomotive characteristics, natural (auto-) antibodies, and carbohydrates levels. From these studies, we suggest that the odds to develop OC is predominantly influenced by an (rapid) increase in body weight affecting joint loading, as is the case for gilts switched from restricted to ad libitum feeding after 10 weeks of age compared with gilts fed restricted after 10 weeks of age (Chapter 2) or in gilts receiving a low carbohydrate diet compared with a high carbohydrate diet (Chapter 6). Additionally, we suggest that irregular loading of the joints due to, for example, playing behaviors aggravates existing minor lesions to severe lesions, as evidenced from a higher prevalence of severe lesions of OC in gilts on a deep litter type system compared with a partially slatted concrete floor.

Although OC has been associated with various CLC such as lameness, we could not find a strong and consistent association between CLC and OC (Chapter 4). A similar conclusion could be made for higher levels of N(A)Ab, which we showed to be associated with higher odds to have OC (Chapter 5). At this moment, these in vivo measurements to predict risk of OC remain unreliable for use in commercial husbandry. Finally, we showed that Dutch commercial husbandry sows have a high total prevalence of OC (up to 90%; Chapter 7), but consisted for the majority of scores 1 and 2. However, lameness as a culling reason was not the dominant factor associated with OC. Although these studies provide some clues in the factors that are associated with OC prevalence in breeding gilts, OC is still a complex multifactorial problem and much research will be needed to develop strategies to reduce prevalence of OC in practice when needed. What needs to be taken into account in future studies is that OC is time dependent with a relatively short time frame of development at a young age. Additionally, focus should be made on the key animals that are of interest for the reduction of OC prevalence, which are gilts / sows that have to last several parities and are therefore more likely to experience negative welfare effects due to OC. Finally, a long term study from birth to slaughter is needed with a focus on the relationship between OC and severe lameness associated with premature culling, as at this moment it is uncertain what the full extent of OC is on animal welfare.

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Summary

Osteochondrosis (OC) involves the development of defects in growth of bones. Growth of bones in young pigs is accomplished by growth cartilage that is situated at the ends of long bones, termed the epiphysis, that is involved in joint movement. More specifically, growth cartilage resides, amongst others, just below the surface of an epiphysis between the articular cartilage (overlying cartilage) and the subchondral bone (underlying bone). Chondrocytes (cartilage cells) proliferate (divide) and undergo hypertrophy (includes enlargement of the cell) while producing an extracellular matrix that provides strength to the entire structure. The extracellular matrix is eventually converted into bone tissue (ossification) resulting in growth of bone. Osteochondrosis involves the formation of necrotic growth cartilage due to vascular disruption that can take place due to loading of joints. Necrotic growth cartilage is not converted into bone tissue (ossification) and remains as a weak site in the underlying bone. This necrotic cartilage may fracture due to loading of joints, causing irregularities at the surface of a joint. Such joint surface irregularities may impair normal joint movement, causing lameness in the animals. Considering that breeding gilts will have to last several parities to produce fattening piglets, these animals may be at risk to develop OC induced lameness, decreasing animal welfare and increasing the risk for premature culling. Important to note is that OC develops at a very young age in gilts at around 10 weeks of age. Thus, if one wants to take measures to reduce OC prevalence in gilts, one needs to start early. Additionally, the early time frame of OC development might indicate that measures undertaken have time dependent effects on OC prevalence. The aim of this study was 1) to assess possible time dependent effects on OC prevalence of early life environmental conditions consisting of either dietary restriction (Chapter 2) or floor type (Chapter 3); 2) to assess whether early in vivo characteristics are associated with OC prevalence at a later age and consisted of either conformation and locomotive characteristics (Chapter 4) or natural (auto-) antibodies (Chapter 5); and 3) to assess whether OC prevalence is affected by dietary composition consisting of a difference in dietary carbohydrate and arginine level (Chapter 6).

In Chapter 2 we assessed time dependent effects of dietary restriction on OC prevalence in 211 Topigs 20 (Dutch Large White x Dutch Landrace) breeding gilts acquired after weaning (4 weeks of age). The underlying mechanism behind dietary restriction affecting OC prevalence was that dietary restriction affects body weight

development and, in turn, affects loading on the joints. Gilts received 1 of 4 of the following treatments: ad libitum feeding continuously from weaning until slaughter (AA); restricted feeding continuously from weaning until slaughter (RR); ad libitum feeding from weaning until 10 weeks of age, after which gilts were switched to restricted feeding (AR); or restricted feeding from weaning until 10 weeks of age, after which gilts were switched to ad libitum feeding (RA). Restricted feeding was calculated as 80% of the ad libitum uptake of the preceding week. At 26 weeks of age, gilts were slaughtered and the elbow, hock, and knee joint were dissected and macroscopically assessed for irregularities of the joint surface indicative of OC. Results indicated that the gilts responded as expected in body weight gain to the treatments. Gilts in the AA treatment were overall heavier than the other treatments, gilts in the RR treatment were overall less heavier than the other treatments, and gilts in the RA and AR treatments showed a higher and lower increase in body weight, respectively, after 10 weeks of age compared to before 10 weeks of age. Differences in OC prevalence were found between treatments at the animal level (all joints combined) and indicated that gilts in the RA treatment had significantly ($P < 0.05$) higher odds of being affected by OC than gilts in the RR and AR treatments (OR = 2.5 and OR = 1.9, respectively). We speculated that these results are due to a rapid increase in body weight after 10 weeks of age when gilts are switched from restricted to ad libitum feeding and thereby increases loading experienced on the joint. Possibly, the growth cartilage within the joint is sensitive for this rapid increase in body weight and might be damaged, leading to an increased risk for OC development.

In Chapter 3 we assessed time dependent effects of floor type on OC prevalence in 212 Topigs 20 (Dutch Large White x Dutch Landrace) breeding gilts acquired after weaning (4 weeks of age). The underlying mechanism behind floor type affecting OC prevalence was that the type of floor may affect impact loading or slipping of the gilts that could result in higher impact loading or traumatic events experienced on the joints. Gilts received 1 of 4 of the following treatments: a conventional floor from weaning until slaughter (CC); wood shavings as bedding from weaning until slaughter (WW); a conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding (CW); or wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a

conventional floor (WC). Indications of behavioral activity of the gilts were assessed at 8, 10 (2 days after the treatment switch) and 17 weeks of age. At 24 weeks of age, gilts were slaughtered and the elbow, hock, and knee joint were dissected and macroscopically assessed for irregularities of the joint surface indicative of OC. Results indicated that gilts kept on wood shavings after 10 weeks of age showed a higher degree of play behaviors as compared to gilts kept on a conventional floor. No effect of treatments on overall prevalence of OC was found. At the animal level, however, gilts had greater odds to have severe OC lesions ($P \leq 0.05$) in the CW treatment (OR = 2.3), WC treatment (OR = 2.6), and WW treatment (OR = 3.7) compared with gilts in the CC treatment. We speculated that the negative effect of a deep litter type system using wood shavings may be mediated through differences in play behaviors recorded between treatments that place more stress on the joints to cope with the irregular loading patterns likely associated with play behaviors. A higher degree of play behavior could then increase the risk to aggravate the existing minor lesions to severe lesions of OC.

Considering that OC is suggested to affect, amongst others, lameness of the animals, we assessed whether conformation and locomotive characteristics (CLC) throughout the rearing period of a gilt have associations with OC at slaughter in Chapter 4. If strong and persistent associations exist, then CLC may be viable indicators of OC in vivo. The CLC were measured in the gilts used in the experiments described in Chapters 2 and 3. Gilts were subjectively assessed for CLC at, on average, 4, 9, 11, 16, and 22 (Chapter 3) or 24 (Chapter 2) weeks of age. Assessment of CLC included 10 conformation and 2 locomotive characteristics using a 9-point grading scale by 2 observers. The CLC included stiff or slow gait, swaying hindquarters, X- or O-shaped front and hind legs, size of the inner and outer claw of the front and hind legs, steep or weak pasterns of the front and hind legs, sickled or buckled front legs, straight or sickled hock, and straight or bowed hind legs. Several CLC were associated with OC at several ages. The CLC most frequently associated with OC ($P < 0.05$) were O shape or X shape of the hind legs, straight or bowed hind legs, and straight or sickled hock. However, associations were not consistently present throughout every assessment within an entire experiment or were not consistently present between experiments. For a large part, these inconsistencies are likely due to the fact that a gilt was not

consistently affected by the same CLC at each assessment. Considering that both OC and CLC are dynamic over time, where a condition can appear but also disappear, the viability of using CLC to predict risk for OC is, at this point, limited.

In another attempt to associate an in vivo measured characteristic in the rearing period of gilts with OC at slaughter, we focused on natural (auto-) antibodies (N[A]Ab) in Chapter 5. The function of N(A)Ab revolves around the maintenance of homeostasis and prevention of disease by clearance of damaged cells, intracellular components, and cell waste products. Considering that OC involves the formation of necrotic cartilage, one could envision that N(A)Ab would be able to recognize waste products resulting from this necrotic cartilage formation. To assess if such an association exists, blood samples were collected from the gilts used in the experiment described in Chapter 3 at 6, 10, and 24 weeks of age and were analyzed for N(A)Ab titers against 11 (auto-) antigens using ELISA. Results indicated that several N(A)Ab at 6 weeks of age were associated with OC at 24 weeks of age. In general, a higher odds ($P < 0.05$) to have OC at the animal level was associated with higher levels of IgM N(A)Ab measured at 6 weeks of age against chondroitin sulfate A (OR=1.5) and actin (OR=1.3), at 24 weeks of age with thyroglobulin (OR=1.3) and actin (OR=1.3), and of IgG N(A)Ab measured at 6 weeks of age against insulin (OR=1.4). Differences in OC affected animals and OC unaffected gilts were in general less than half a titer point and indicate that associations may not be very strong nor suitable for reliable prediction of risk to have OC. Further research will require the expansion of the antigen repertoire recognized by N(A)Ab that are possibly associated with OC to a higher degree.

In Chapter 6 we assessed whether dietary composition has an effect on OC prevalence in 212 Topigs 20 (Dutch Large White x Dutch Landrace) breeding gilts acquired after weaning (4 weeks of age). Necrotic growth cartilage has been suggested to be associated with chondrocytes and vasculature proliferating towards the necrotic growth cartilage in reparative attempts. In an attempt to affect these processes, we fed gilts diets differing in carbohydrate and arginine content. Carbohydrates affect metabolic parameters such as glucose, insulin, and insulin-like growth factor-1 (IGF-1) which all can have an effect on chondrocyte functioning. Arginine can have an effect on vascularization under hypoxic

conditions as can be expected during OC development. Considering that OC is associated with reparative attempts undertaken by blood vessels and chondrocytes, we hypothesized that carbohydrates and arginine may affect these processes. Gilts were subjected to a 2x2 factorial treatment design of dietary carbohydrate and arginine level. Carbohydrate level consisted of 12.5% cornstarch and 12.5% dextrose added to a basal diet (C+ treatment) versus an isocaloric diet in which cornstarch and dextrose were replaced with 8.9% soya bean oil (C- treatment). Arginine supplementation consisted of 0.8% arginine supplemented to a basal diet (A+ treatment) versus 1.64% alanine as the isonitrogenous control (A- treatment). At 24 weeks of age, blood samples of 34 randomly selected gilts around feeding were taken and assessed for insulin, glucose, IGF-1, and NO levels. After slaughter at 25 weeks of age, the elbow, hock, and knee joint were dissected and macroscopically assessed for irregularities of the joint surface indicative of OC. Arginine supplementation tended to decrease OC prevalence ($P = 0.07$) at the animal level (all joints combined) and the knee joint. Carbohydrate treatment affected prevalence of OC only in the knee joint ($P < 0.05$) in which gilts in the C- treatment had higher odds to have OC (OR = 2.05) than gilts in the C+ treatment. These effects of dietary treatment were likely mediated through the diets affecting body weight development. Gilts in the C+A+ treatment had a lower body weight than gilts in the other treatments. Possibly, the treatment effects on OC prevalence were influenced by this differences in body weight attained. Indeed, when assessing body weight at slaughter in statistical models, body weight was significant ($P < 0.05$) while treatment effects became non-significant. Through this experiment, we again have indications that loading of joints, mediated through body weight changes, can affect OC prevalence.

Chapter 7 discussed, amongst others, the relationship between OC and growth rates. Additionally, new data on OC prevalence in the Dutch commercial husbandry was presented. The relationship between growth rates and OC in the gilts used in the experiments described in this thesis (Chapter 2, 3, and 6) was assessed to study whether growth rates have a strong association with OC. These associations were assessed within treatments for gilts that did not receive a switch in treatments. Results indicated limited associations with OC, which might be caused by insufficient contrasts of growth rates within a treatment. Considering

that there were clear effects of dietary restriction on body weight (and therefore also growth rates) and OC prevalence (Chapter 2) between treatment groups, effects of growth rates on OC may only be apparent with large contrasts in growth rates. New data on OC prevalence from left hind legs in Dutch commercial husbandry sows send for premature culling indicated a 90% prevalence of OC. However, the majority of OC scores were minor lesions (OC score 1 and 2). Depending on the number of legs assessed, the prevalence of OC in the commercial sows compared to our experimental gilts was lower, similar, or higher. An increase in parity was not associated with a significant increase in OC and lameness as a culling reason was not the main factor associated with OC. This data was not able to clearly indicate welfare implications of OC. Considering that in our experiments no gilts were removed for severe lameness related to OC, one may wonder what the exact welfare implications of OC for sows are. A long term study in sows from weaning to culling will be necessary, so that individual sows can be monitored throughout life for OC and lameness incidence. Such a study will provide a better understanding of the welfare implications of OC.

In conclusion, we found that dietary restriction and floor type during young age in the rearing period affect OC prevalence in Topigs 20 breeding gilts. Conformation and locomotive characteristics were associated with OC at several ages during the rearing period in the gilts, but were not consistently associated with OC at every age or between ages. Natural (auto-) antibodies measured as early as 6 weeks of age were associated with OC, but results were, at this point, not strong enough to be viably used in practice. Finally, a high dietary carbohydrate level decreased the prevalence of OC, but this effect was likely mediated through body weight changes resulting from the dietary treatments. We speculated that loading of joints through behavioral activity, such as play behavior, and body weight changes affect OC prevalence and it is therefore important not to stimulate a rapid high body weight gain during the rearing period. When studying OC, one must realize that OC development is time dependent and, therefore, (environmental) factors may affect OC prevalence differently at different ages during the rearing period of breeding gilts.

Acknowledgements

Much has changed in the last few years (including moving of Zodiac and Carus) and quite a bit of work has been done concerning this PhD thesis. Of course, I cannot take full credit for all this work and have some people to thank for making it a success.

Let us start with the first one on the list, Bjorge Laurenssen. I think any PhD candidate would be lucky to have you in their project, I know I felt very lucky. You spend a great deal of time (sometimes I made you stay a bit too long, sorry) in my research (and other research as well) during and after regular work hours, for which I'm sure your family is not grateful to me. Your practical experience in performing experiments (in general) and pigs were of high value. I'm specifically mentioning performing experiments as a separate entity as your knowledge is not only limited to pigs. Many times (especially in the beginning) I found myself overlooking aspects that you pointed out to me and many times I found myself not knowing how to proceed and discussing the matter with you helped me to proceed. From start to end, you helped and taught me how to perform an experiment including ordering, acquiring, and tagging piglets, organizing transport of the piglets, organizing and taking measurements on the piglets, pen design, organizing slaughter of piglets, collecting blood samples, dissecting legs, when to treat pigs, what to look for in pig health, cannulation of pigs, strategically dealing with people, building those side boards to the pens, and the list goes on and on. The work performed here would not have come to such a good end without your help. Thank you!!

I want to thank my supervisors Bas Kemp, Wouter Hazeleger, and Ilse van Grevenhof. Ilse, thank you for sharing your knowledge on osteochondrosis and experiments, supervision, and your time for reading my manuscripts and the detailed comments on them. Bas, thank you for your insights and comments on my work, which helped me a great deal when I was stuck in my work or when I was using too many words to describe a small aspect in my article (as I generally seem to do). Additionally, you gave suggestions on other types of statistical analyses that take account of other variables or to look at a statistical problem from another angle, which, much to my frustration, showed new insights that I wasn't able to think of. Even though your job as a professor keeps you very busy, you're an open person to which I could always talk to about my problems. Thank you! Wouter, thank you for your supervision. I know I wasn't the easiest person to work with at

many times, as I can be a tad bit stubborn. Due to your vast experience in running experiments with much applied work, I could always talk to you about what step to take next, especially when measurements weren't going the way they were planned. I think especially both you and Bjorge are the ones that helped me complete experiments successfully. Your comments on my articles and analyses, whether they were statistical or laboratory, were of great value. Your vast experience in guiding and helping students (and all their problems) must have been a good source to advise and guide me in all the problems I had, whether this was in supervision of thesis students, dealing with other people, or problems with my own supervision. Thank you!

In the performance of large experiments, you will need the help of people building the enclosures, taking care of the animals, making sure that the climate is optimal, medically treating animals, etc. Those people are the employees of Carus. Many thanks to all employees of Carus that work on various experiments. In my case, specific thanks go to Ries Verkerk, Rinie Ernste, and Ben van den Top. Ries, it has been an extraordinary busy number of years. The move to the new Carus facility and rebuilding some of the new enclosures has been challenging. On top of that, you had this new PhD candidate (me!) nagging you on things such as pig enclosures, feed, and caretaking. In spite of that, you were always open for suggestions and questions, and quickly came with solutions to my problems. You were the go to person to get things organized at Carus and making sure everything runs smoothly. Thank you for the incredible amount of time you put into Carus in general, but also for the time you put into my experiments including caretaking of pigs! Ben and Rinie, you were the main caretakers of the pigs in my experiments. Thank you for all your hard work. As mentioned before, my experiments were quite large and required quite some time and effort on your behalf to take care of the pigs. You were always willing to think with me for solutions to problems and to take actions. Your concern for the pigs' health and welfare, and experimental needs were always high. Thank you for all your hard work!

In general, the group that I work in is a group that help each other out where possible. Much administrative work exists concerning costs of experiments, planning attendance at conferences, student work, and, basically, just work in general. I'm fortunate enough to work in a group with a fantastic secretarial team, Lora Bor-van der Kleijn and Nanette van Hapert. Lora and Nanette, both of you

have a very open door policy. No matter what anyone is concerned with, you lend a listening ear and are very willing to help. The support you give to a PhD candidate (and of course others as well) are very valuable and makes our work easier and more fun. Thank you for all your support! A lot of the work and my knowledge on statistical analyses would not have been possible without people to discuss it with. Bart Ducro, Liesbeth Bolhuis, Nicoline Soede, and Ariette van Knegsel, you helped me a great deal in understanding what statistical analyses are possible and how to perform them whenever I had questions, for which you have my gratitude. Monique Ooms and Fleur Bartels, thank you for helping me in the experiments such as with setting up the cameras, helping with dissecting joints, and guiding students involved in my experiments where possible. Mike Nieuwland, Joop Arts, and Henk Parmentier, thank you for helping me with (understanding) the ELISA procedure, interpretation of the results, guidance of my students, putting up with me in the lab, the many (so many) drinks we had, and (at least for Henk) helping with writing our N(A)Ab article. Rudie Koopmanschap, thank you for helping me with the various lab procedures, the cannulation procedure, various never ever ending anecdotes, and blood sampling. Ger de Vries Reilingh and Ilona van den Anker, thank you for helping me with dissecting joints and understanding or performing laboratory analyses in my work. Jan Wijnen and Inonge Reimert, thank you for helping me with dissecting joints and/or blood sampling. Thank you to the students that worked with me over the past years: Willemijn Duijsters, Shaoyang Xu, Linda Mergner, Guus Diepenmaat, Anne-Marieke Smid, Ellen Damen, Manon Pippel, and Saffira Koorn. I hope you're all doing well after your studies. Thank you to my roommates in the office who make the office work more enjoyable: CONNY!!!! Maatjens and CAROL!!!! Lima Alvares da Silva (my post-it target practice), Novi 'tricky fingers' Mayasari, Handsome Talented man (Juncai Chen), Sofie 'nerves of steel' van Nieuwamerongen, Tom 'second coming of Henk' Berghof, and Ampai 'I don't think this is spicy' Nangsuay. Adding to this list is a previous (promoted) roommate with whom I could share my PhD difficulties with, Anne Wientjes. You are all very open, sweet, kind, certainly very weird and crazy, and very fun to be around with. Many of you also helped me with my experiments, for which you have my gratitude.

One of the biggest and surprising events during my PhD was that I found (or actually was found by) someone that is able to put up with me in my personal

life, my girlfriend Kristina. Thank you for all your support during my PhD and talking me out of quitting my job. Your (German) quirks keep surprising me. I love you and can't wait to see what the future holds for us.

Everybody in our group, including the above mentioned people, also provide with a valuable daily asset: laughter is almost always heard throughout the day. Such a thing I cannot express enough gratitude for, as it makes the work environment very enjoyable. Additionally, the regular drinks after work often with Mike, Joop, Henk (my beer buddies!!!), Juncai and Caroline (my newly found beer musketeers!!!) also provides much relief from a busy week. I hope I can contribute to this great atmosphere in the coming year. Thank you to everybody in ADP!!!!!

About the Author

Curriculum Vitae

Publications

WIAS Training and Supervisions Plan

CURRICULUM VITAE

Daniël (Danny) Benjamin de Koning was born on the 18th of March 1985 in Venray, The Netherlands. Always interested in everything concerning animals, he was keen on studying the matter further (all studies performed in The Netherlands). After graduating from high school Raayland College in Venray, he went to study animal caretaking at AOC community college in Horst, acquired a Bachelor degree in Animal Husbandry and Animal Healthcare at HAS University of Applied Sciences, and finally acquired a Master degree in Animal Health and Behavior at Wageningen University and Research center from which he graduated *cum laude*. At Wageningen University and Research center he specialized in adaptation physiology and behavior. For the specialization adaptation physiology, he performed a minor thesis concerning a feasibility study towards feed levels effects on ovarian follicle quality in sows. For his behavior/adaptation physiology specialization, he performed a major thesis concerning the effects of early life immune activation on feather pecking damage in laying hens at later life. After graduation, he started a PhD research entitled 'the identification of genetic and environmental risk factors determining the prevalence and the impact on welfare of osteochondrosis (OC) in pigs'. More specifically, he focused on the environmental risk factors affecting OC prevalence in breeding gilts and consisted of dietary restriction, floor types, and dietary composition. The results of this research are presented in this thesis.

PUBLICATIONS

Refereed Scientific Journals

D.B. de Koning, E.M. van Grevenhof, B.F.A. Laurenssen, B.J. Ducro, H.C.M. Heuven, P.N. de Groot, W. Hazeleger, and B. Kemp. 2012. Associations between osteochondrosis and conformation and locomotive characteristics in pigs. Journal of Animal Science 90:4752-4763.

D.B. de Koning, E.M. van Grevenhof, B.F.A. Laurenssen, P.R. van Weeren, W. Hazeleger, and B. Kemp. 2013. The influence of dietary restriction before and after 10 weeks of age on osteochondrosis in growing gilts. Journal of Animal Science 91:5167-5176.

D.B. de Koning, E.M. van Grevenhof, B.F.A. Laurenssen, P.R. van Weeren, W. Hazeleger, and B. Kemp. 2014. The influence of floor type before and after 10 weeks of age on osteochondrosis in growing gilts. Journal of Animal Science 92:3338-3347.

D.B. de Koning, E.M. van Grevenhof, B.F.A. Laurenssen, W. Hazeleger, and B. Kemp. 2015. Associations of conformation and locomotive characteristics in growing gilts with osteochondrosis at slaughter. Journal of Animal Science 93:93-106.

D.B. de Koning, E.P.C.W. Damen, M.G.B. Nieuwland, E.M. van Grevenhof, W. Hazeleger, B. Kemp, and H.K. Parmentier. 2015. Association of natural (auto-) antibodies in young gilts with osteochondrosis at slaughter. Livestock Science 176:152-160.

Conference Book of Abstracts

D.B. de Koning, E.M. van Grevenhof, B.F.A. Laurenssen, P.R. van Weeren, W. Hazeleger, and B. Kemp. 2013. The influence of feeding levels before and after 10 weeks of age on osteochondrosis in growing gilts. Book of abstracts of the 64th annual meeting of the European federation of animal science, Nantes, France, 26-30 August, 2013, p. 448.

D.B. de Koning, E.M. van Grevenhof, B.F.A. Laurenssen, P.R. van Weeren, W. Hazeleger, and B. Kemp. 2014. The influence of flooring type before and after 10 weeks of age on osteochondrosis in gilts. Book of abstracts of the 65th annual meeting of the European federation of animal science, Copenhagen, Denmark, 25-29 August, 2014, p. 381.

Other Publications

J. Lamers, D.B. de Koning, E.M. van Grevenhof. 2014. Overbelast groeikraakbeen kan hele varkensleven een handicap blijven; Schokbreker sparen. Varkens 2014 (maart): 22-23.

WIAS Training and Supervision Plan[1]

Description	Year
The Basic Package (3.0 ECTS)	
WIAS Introduction Course	2011
WGS course Ethics and Philosophy in Life Sciences	2011
International Conferences (3.0 ECTS)	
64th Annual Meeting of the European Federation of Animal Science (EAAP), Nantes, France	2013
65th Annual Meeting of the European Federation of Animal Science (EAAP), Copenhagen, Denmark	2014
Seminars and Workshops (1.3 ECTS)	
WIAS Science Day, Wageningen, The Netherlands	2012-2015
WIAS seminar Aspects of Sow and Piglet Performance, Wageningen, The Netherlands	2013
Presentations (7 ECTS)	
CAWA Oral presentation, Wageningen, The Netherlands	2012, 2013, 2015
EAAP Poster presentation, Nantes, France	2013
WIAS Science Day poster presentation, Wageningen, The Netherlands	2013, 2014
EAAP Oral presentation, Copenhagen, Denmark	2014
WIAS Science Day oral presentation, Wageningen, The Netherlands	2015
Disciplinary and Interdisciplinary Courses (3.4 ECTS)	
VLAG / WIAS course Epigenesis and Epigenetics, Wageningen, The Netherlands	2011, 2014
WA course Gut Health in Pigs and Poultry, Wageningen, The Netherlands	2014
UU GSLS course Advanced Immunology, Utrecht, The Netherlands	2015
Advanced Statistics Courses (5.1 ECTS)	
WIAS course Statistics for the Life Sciences, Wageningen, The Netherlands	2011
WIAS course Advanced Statistics: Design of Experiments, Wageningen, The Netherlands	2011

PE&RC course Generalized Linear Models, Wageningen, The Netherlands	2011
PE&RC course Multivariate Analysis, Wageningen, The Netherlands	2011
Statutory Courses (1.4 ECTS)	
Laboratory Use of Isotopes, Wageningen, The Netherlands	2012
Professional Skills Support Courses (3.6 ECTS)	
WGS course Techniques for Writing and Presenting a Scientific Paper, Wageningen, The Netherlands	2013
ESD course Teaching and Supervising Thesis Students, Wageningen, The Netherlands	2014
WGS course Career Perspectives, Wageningen, The Netherlands	2014
Didactic Skills (19.2 ECTS)	
Supervising project groups Introduction to the Animal Sciences	2011
Supervising practical Behavior and Environment	2011
Supervising practical Behavior and Hormones	2011, 2012
Supervising practical Reproduction and Fertility	2011, 2012, 2014
Supervising project groups Adaptation and Physiology II	2013, 2014
Supervising project groups Integration Course Pigs and Poultry	2014
Reviewing RMC proposals	2014
Supervising 2 BSc and 5 MSc thesis students	2011-2015
Education and Training Total	**47 ECTS**

[1] 1 ECTS credit equals a study load of approximately 28 hours

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Osteochondrosis (OC) involves the development of defects in growth of bones. Growth of bones in young pigs is accomplished by growth cartilage that is situated at the ends of long bones, termed the epiphysis, that is involved in joint movement. More specifically, growth cartilage resides, amongst others, just below the surface of an epiphysis between the articular cartilage (overlying cartilage) and the subchondral bone (underlying bone). Chondrocytes (cartilage cells) proliferate (divide) and undergo hypertrophy (includes enlargement of the cell) while producing an extracellular matrix that provides strength to the entire structure. The extracellular matrix is eventually converted into bone tissue (ossification) resulting in growth of bone. Osteochondrosis involves the formation of necrotic growth cartilage due to vascular disruption that can take place due to loading of joints. Necrotic growth cartilage is not converted into bone tissue (ossification) and remains as a weak site in the underlying bone. This necrotic cartilage may fracture due to loading of joints, causing irregularities at the surface of a joint. Such joint surface irregularities may impair normal joint movement, causing lameness in the animals. Considering that breeding gilts will have to last several parities to produce fattening piglets, these animals may be at risk to develop OC induced lameness, decreasing animal welfare and increasing the risk for premature culling. Important to note is that OC develops at a very young age in gilts at around 10 weeks of age. Thus, if one wants to take measures to reduce OC prevalence in gilts, one needs to start early. Additionally, the early time frame of OC development might indicate that measures undertaken have time dependent effects on OC prevalence. The aim of this study was 1) to assess possible time dependent effects on OC prevalence of early life environmental conditions consisting of either dietary restriction (Chapter 2) or floor type (Chapter 3); 2) to assess whether early in vivo characteristics are associated with OC prevalence at a later age and consisted of either conformation and locomotive characteristics (Chapter 4) or natural (auto-) antibodies (Chapter 5); and 3) to assess whether OC prevalence is affected by dietary composition consisting of a difference in dietary carbohydrate and arginine level (Chapter 6). In Chapter 2 we assessed time dependent effects of dietary restriction on OC prevalence in 211 Topigs 20 (Dutch Large White x Dutch Landrace) breeding gilts acquired after weaning (4 weeks of age). The underlying mechanism behind dietary restriction affecting OC prevalence was that dietary restriction affects body weight development and, in turn, affects loading on the joints. Gilts received 1 of 4 of the following treatments: ad libitum feeding continuously from weaning until slaughter (AA); restricted feeding continuously from weaning until slaughter (RR); ad libitum feeding from weaning until 10 weeks of age, after which gilts were switched to restricted feeding (AR); or restricted feeding from weaning until 10 weeks of age, after which gilts were switched to ad libitum feeding (RA). Restricted feeding was calculated as 80% of the ad libitum uptake of the preceding week. At 26 weeks of age, gilts were slaughtered and the elbow, hock, and knee joint were dissected and macroscopically assessed for irregularities of the joint surface indicative of OC. Results indicated that the gilts responded as expected in body weight gain to the treatments. Gilts in the AA treatment were overall heavier than the other treatments, gilts in the RR treatment were overall less heavier than the other treatments, and gilts in the RA and AR treatments showed a higher and lower increase in body weight, respectively, after 10 weeks of age compared to before 10 weeks of age. Differences in OC prevalence were found between treatments at the animal level (all joints combined) and indicated that gilts in the RA treatment had significantly ($P < 0.05$) higher odds of being affected by OC than gilts in the RR and AR treatments (OR = 2.5 and OR = 1.9, respectively). We speculated that these results are due to a rapid increase in body weight after 10 weeks of age when gilts are switched from restricted to ad libitum feeding and thereby increases loading experienced on the joint. Possibly, the growth cartilage within the joint is sensitive for this rapid increase in body weight and might be damaged, leading to an increased risk for OC development. In Chapter 3 we assessed time dependent effects of floor type on OC prevalence in 212 Topigs 20 (Dutch Large White x Dutch Landrace) breeding gilts acquired after weaning (4 weeks of age). The underlying mechanism behind floor type affecting OC prevalence was that the type of floor may affect impact loading or slipping of the gilts that could result in higher impact loading or traumatic events experienced on the joints. Gilts received 1 of 4 of the following treatments: a conventional floor from weaning until slaughter (CC); wood shavings as bedding from weaning until slaughter (WW); a conventional floor from weaning until 10 weeks of age, after which gilts were switched to wood shavings as bedding (CW); or wood shavings as bedding from weaning until 10 weeks of age, after which gilts were switched to a conventional floor (WC). Indications of behavioral activity of the gilts were assessed at 8, 10 (2 days after the treatment switch) and 17 weeks of age. At 24 weeks of age, gilts were slaughtered and the elbow, hock, and knee joint were dissected and macroscopically assessed for irregularities of the joint surface indicative of OC. Results indicated that gilts kept on wood shavings after 10 weeks of age showed a higher degree of play behaviors as compared to gilts kept on a conventional floor. No effect of treatments on overall prevalence of OC was found. At the animal level, however, gilts had greater odds to have severe OC lesions ($P \leq 0.05$) in the CW treatment (OR = 2.3), WC treatment (OR = 2.6), and WW treatment (OR = 3.7) compared with gilts in the CC treatment. We speculated that the negative effect of a deep litter type system using wood shavings may be mediated through differences in play behaviors recorded between treatments that place more stress on the joints to cope with the irregular loading patterns likely associated with play behaviors. A higher degree of play behavior could then increase the risk to aggravate the existing minor lesions to severe lesions of OC. Considering that OC is suggested to affect, amongst others, lameness of the animals, we assessed whether conformation and locomotive characteristics (CLC) throughout the rearing period of a gilt have associations with OC at slaughter in Chapter 4. If strong and persistent associations exist, then CLC may be viable indicators of OC in vivo. The CLC were measured in the gilts used in the experiments described in Chapters 2 and 3. Gilts were subjectively assessed for CLC at, on average, 4, 9, 11, 16, and 22 (Chapter 3) or 24 (Chapter 2) weeks of age. Assessment of CLC included 10 conformation and 2 locomotive characteristics using a 9-point grading scale by 2 observers. The CLC included stiff or slow gait, swaying hindquarters, X- or O-shaped front and hind legs, size of the inner and outer claw of the front and hind legs, steep or weak pasterns of the front and hind legs, sickled or buckled front legs, straight or sickled hock, and straight or bowed hind legs. Several CLC were associated with OC at several ages. The CLC most frequently associated with OC ($P < 0.05$) were O shape or X shape of the hind legs, straight or bowed hind legs, and straight or sickled hock. However, associations were not consistently present throughout every assessment within an entire experiment or were not consistently present between experiments. For a large part, these inconsistencies are likely due to the fact that a gilt was not consistently affected by the same CLC at each assessment. Considering that both OC and CLC are dynamic over time, where a condition can appear but also disappear, the viability of using CLC to predict risk for OC is, at this point, limited. In another attempt to associate an in vivo measured characteristic in the rearing period of gilts with OC at slaughter, we focused on natural (auto-) antibodies (N[A]Ab) in Chapter 5. The function of N(A)Ab revolves around the maintenance of homeostasis and prevention of disease by clearance of damaged cells, intracellular components, and cell waste products. Considering that OC involves the formation of necrotic cartilage, one could envision that N(A)Ab would be able to recognize waste products resulting from this necrotic cartilage formation. To assess if such an association exists, blood samples were collected from the gilts used in the experiment described in Chapter 3 at 6, 10, and 24 weeks of age and were analyzed for N(A)Ab titers against 11 (auto-) antigens using ELISA. Results indicated that several N(A)Ab at 6 weeks of age were associated with OC at 24 weeks of age. In general, a higher odds ($P < 0.05$) to have OC at the animal level was associated with higher levels of IgM N(A)Ab measured at 6 weeks of age against chondroitin sulfate A (OR=1.5) and actin (OR=1.3), at 24 weeks of age with thyroglobulin (OR=1.3) and actin (OR=1.3), and of IgG N(A)Ab measured at 6 weeks of age against insulin (OR=1.4). Differences in OC affected animals and OC unaffected gilts were in general less than half a tiger point and indicate that associations may not be very strong nor suitable for reliable prediction of risk to have OC. Further research will require the expansion of the antigen repertoire recognized by N(A)Ab that are possibly associated with OC to a higher degree. In Chapter 6 we assessed whether dietary composition has an effect on OC prevalence in 212 Topigs 20 (Dutch Large White x Dutch Landrace) breeding gilts acquired after weaning (4 weeks of age). Necrotic growth cartilage has been suggested to be associated with chondrocytes and vasculature proliferating towards the necrotic growth cartilage in reparative attempts. In an attempt to affect these processes, we fed gilts diets differing in carbohydrate and arginine content. Carbohydrates affect metabolic parameters such as glucose, insulin, and insulin-like growth factor-1 (IGF-1) which all can have an effect on chondrocyte functioning. Arginine can have an effect on vascularization under hypoxic conditions as can be expected during OC development. Considering that OC is associated with reparative attempts undertaken by blood vessels and chondrocytes, we hypothesized that carbohydrates and arginine may affect these processes. Gilts were subjected to a 2x2 factorial treatment design of dietary carbohydrate and arginine level. Carbohydrate level consisted of 12.5% cornstarch and 12.5% dextrose added to a basal diet (C+ treatment) versus an isocaloric diet in which cornstarch and dextrose were replaced with 8.9% soya bean oil (C- treatment). Arginine supplementation consisted of 0.8% arginine supplemented to a basal diet (A+ treatment) versus 1.64% alanine as the isonitrogenous control (A- treatment). At 24 weeks of age, blood samples of 34 randomly selected gilts around feeding were taken and assessed for insulin, glucose, IGF-1, and NO levels. After slaughter at 25 weeks of age, the elbow, hock, and knee joint were dissected and macroscopically assessed for irregularities of the joint surface indicative of OC. Arginine supplementation tended to decrease OC prevalence ($P = 0.07$) at the animal level (all joints combined) and the knee joint. Carbohydrate treatment affected prevalence of OC only in the knee joint ($P < 0.05$) in which gilts in the C- treatment had higher odds to have OC (OR = 2.05) than gilts in the C+ treatment. These effects of dietary treatment were likely mediated through the diets affecting body weight development. Gilts in the C+A+ treatment had a lower body weight than gilts in the other treatments. Possibly, the treatment effects on OC prevalence were influenced by this differences in body weight attained. Indeed, when assessing body weight at slaughter in statistical models, body weight was significant ($P < 0.05$) while treatment effects became non-significant. Through this experiment, we again have indications that loading of joints, mediated through body weight changes, can affect OC prevalence. Chapter 7 discussed, amongst others, the relationship between OC and growth rates. Additionally, new data on OC prevalence in the Dutch commercial husbandry was presented. The relationship between growth rates and OC in the gilts used in the experiments described in this thesis (Chapter 2, 3, and 6) was assessed to study whether growth rates have a strong association with OC. These associations were assessed within treatments for gilts that did not receive a switch in treatments. Results indicated limited associations with OC, which might be caused by insufficient contrasts of growth rates within a treatment. Considering that there were clear effects of dietary restriction on body weight (and therefore also growth rates) and OC prevalence (Chapter 2) between treatment groups, effects of growth rates on OC may only be apparent with large contrasts in growth rates. New data on OC prevalence from left hind legs in Dutch commercial husbandry sows send for premature culling indicated a 90% prevalence of OC. However, the majority of OC scores were minor lesions (OC score 1 and 2). Depending on the number of legs assessed, the prevalence of OC in the commercial sows compared to our experimental gilts was lower, similar, or higher. An increase in parity was not associated with a significant increase in OC and lameness as a culling reason was not the main factor associated with OC. This data was not able to clearly indicate welfare implications of OC. Considering that in our experiments no gilts were removed for severe lameness related to OC, one may wonder what the exact welfare implications of OC for sows are. A long term study in sows from weaning to culling will be necessary, so that individual sows can be monitored throughout life for OC and lameness incidence. Such a study will provide a better understanding of the welfare implications of OC. In conclusion, we found that dietary restriction and floor type during young age in the rearing period affect OC prevalence in Topigs 20 breeding gilts. Conformation and locomotive characteristics were associated with OC at several ages during the rearing period in the gilts, but were not consistently associated with OC at every age or between ages. Natural (auto-) antibodies measured as early as 6 weeks of age were associated with OC, but results were, at this point, not strong enough to be viably used in practice. Finally, a high dietary carbohydrate level decreased the prevalence of OC, but this effect was likely mediated through body weight changes resulting from the dietary treatments. We speculated that loading of joints through behavioral activity, such as play behavior, and body weight changes affect OC prevalence and it is therefore important not to stimulate a rapid high body weight gain during the rearing period. When studying OC, one must realize that OC development is time dependent and, therefore, (environmental) factors may affect OC prevalence differently at different ages during the rearing period of breeding gilts.