IMPACT OF DIETARY CALCIUM AND PHOSPHORUS ON SOW REPRODUCTIVE PERFORMANCE AND BONE DEVELOPMENT IN PIGLETS

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ABSTRACT

The concern for restricted movement for sows housed in stalls during gestation has prompted the swine industry to move towards group housing. Additionally, the emphasis on increasing sow productivity has led to a continuous need for re-evaluation of nutrient requirements for sows, including minerals. Unfortunately, the majority of studies that examined the role of dietary Ca and P for sows were older (early 1970s to 1990s), and may not be applicable to the modern, prolific sow, particularly those housed in group housing systems. Two studies were conducted to determine the effects of dietary Ca and P for gestating sows on reproductive performance, bone metabolism and fetal skeletal development. The objective of the first study was to determine if the recommended levels of dietary Ca and P are adequate for sows housed in groups, and thus have potential for increased mobility. A total of 180 multiparous sows and gilts were assigned to 1 of 6 treatments. Treatments, arranged as a 3 × 2 factorial, included main effects of dietary Ca:P; 0.70:0.55 (% as fed, Control); 0.60:0.47 (-15 % 1998 NRC); 0.81:0.63 (+ 15 % 1998 NRC) and housing; stalls or groups. Sows were fed 2.3 kg/d from wk 4 to 5 of gestation until 2 wk prior to farrowing when the allotment was increased to 3.0 kg/d. Serum samples were collected at the start of the trial and on d 100 of gestation, and both serum and milk samples were collected at mid-lactation and prior to weaning. Neither diet nor housing had an effect on total number of piglets born, ADG from birth to weaning, or weaning weight ($P > 0.10$). The number of piglets born live and birth weight were unaffected by diet ($P > 0.10$) but improved in group housing relative to stalls ($P < 0.05$). In late gestation, group-housed sows fed the low Ca diet had reduced serum Ca (diet × housing interaction; $P = 0.02$) and the greatest reduction in serum P level was also observed in group-housed sows fed the low Ca diet (diet × housing interaction; $P = 0.04$). Osteocalcin (OC), and pyridinoline (PYD) markers of bone formation and resorption respectively, were unaffected by diet or housing ($P > 0.10$). The second study was conducted to determine the influence of Ca and P intake by young, gestating sows on the growth and skeletal development of their developing piglets and if smaller birth-weight piglets are at greater risk from mineral insufficiency during gestation. A total of 30 sows were randomly assigned to 1 of the 3 dietary Ca:P treatments used in the first study. Sows were fed their daily rations in 1 allotment as described in Exp. 1. Only sows farrowing litters with 12 piglets or more remained on trial. At birth, the smallest and a normal-sized piglet from each litter were euthanized, and the

left femur extracted for peripheral quantitative computed tomography (pQCT) scanning. Serum samples were collected at birth and prior to weaning. Number of piglets born, body weight (BW) at 3 d of age, and piglet ADG were unaffected by treatment ($P > 0.10$). At birth, the highest serum Ca level was seen in the small piglets from sows fed a high Ca diet (diet × size interaction; $P = 0.04$) however, at weaning, this value had the smallest deviation from the initial value (diet × size interaction; $P = 0.02$). Femurs of piglets from sows fed the low Ca diet had the highest cortical density ($P = 0.03$) and piglet size had no effect on cortical density *(*$P > 0.10$*)*. Bone ash %, ash Ca %, ash P %, and serum bone markers were unaffected by diet or piglet size ($P > 0.10$). Results from these studies suggest that the recommended level of dietary Ca and P as prescribed by NRC 1998, and thus for NRC 2012, is adequate for high-producing sows of modern genetics, whether housed in stalls or groups. Moderate changes in Ca and P intake by young, gestating sows, does not negatively affect the growth or skeletal development of their piglets.

Key words: bone, calcium, gestation, group housing, milk, phosphorus, piglet

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LIST OF ABBREVIATIONS

°C	degree Celsius
1,25(OH)D_3	1,25-dihydroxyvitamin D
AD	apparent digestibility
ADFI	average daily feed intake
ADG	average daily gain
AIA	acid insoluble ash
AIC	Akaike Information Criterion
ANOVA	analysis of variance
AP	alkaline phosphatase
avg	average
BCS	body condition score
BW	body weight
Ca	calcium
cm^3	cubic centimeter (volume)
CP	crude protein
CRT_CNT	cortical content
CRT_DEN	cortical density
CTX	C-terminal telopeptide
CV	coefficient of variation
d	day
DM	dry matter
DPD	deoxypyridinoline
ELISA	enzyme-linked immunosorbent assay
Eq.	equation
Exp.	experiment
g	gram
g	static acceleration of gravity
GE	gross energy
h	hour
HCl	hydrochloric acid

IU	International Unit
kg	kilogram
m	meter
Mcal	megacalories
mg	milligram
mL	milliliter
mm	millimeter
mmol	millimole
N	Normal (concentration)
No.	number
NRC	National Research Council
NTX	N-terminal telopeptide
OC	osteocalcin
P	phosphorus
pQCT	peripheral quantitative computed tomography
PVE	partial volume effect
PYD	pyridinoline
r	simple correlation coefficient
R^2	multiple coefficient of determination
RCBD	randomized complete block design
RIA	radioimmunoassay
SAS	SAS Institute Inc.
SEM	standard error mean
SSI	stress-strength index
VIF	variance inflation factor
wk	week
yr	year

1 INTRODUCTION

In the swine industry, reproductive efficiency is closely related to profitability. The breeding herd plays a key role in determining the successful outcome and sustainability of the enterprise. Apart from their genetic potential, which is of primary importance, optimum nutrition is essential to enhance sow reproductive potential, longevity, and maximize their contribution to the productivity of the entire system. Intensification of pork production, confinement housing, and breeding management, all designed to increase productivity, increases the biological demand on the sow. Inadequate nutrition may reduce production and longevity. The sow must complete 3 to 4 parities to cover her replacement costs (Stalder et al., 2000; Stalder et al., 2003; Rodriguez-Zas et al., 2006).

Particular attention needs to be given to the nutrient requirements of the modern, hyperprolific sow. Genetic selection has increased the prolificacy of sows. In Canada, litter size has increased by approximately 1.8 piglets since 2005 (PigChamp, 2015). Improvements in sow productivity have raised the question of adequacy of nutrients, including minerals such as Ca and P. The role of Ca and P in development and maintenance of the skeletal system has been well defined. However, most of the work that was carried out to establish Ca and P requirements for sows was conducted between the 1970s and the early 1990s and may not be applicable to the modern, hyperprolific sow. Moreover, the Ca and P requirements were established with sows confined in stalls and have not been re-examined using the group housed sow. This is of concern, particularly since confinement offers no opportunity for movement and it has been proposed that movement is required to maintain bone strength and may affect requirements for Ca and P.

Production efficiency of the modern sow is based on the number of piglets born per litter, per year, and breeding lifetime. The higher prolificacy of sows presumes a higher nutritional demand to support the metabolic needs of sows and their fetuses. Incorrect maternal nutrition, including minerals, is associated with nutrient limitations to the developing fetuses, which is exacerbated in large litters. This may lead to fetal growth retardation and decreased within-litter uniformity. In humans, sufficient Ca and P are required to fully mineralize the skeleton of the fetuses before birth. Whether this information can be extrapolated to newborn piglets is not clearly defined, but a few studies cited comparable maturity at birth for piglets and infants, and between preterm piglets and preterm neonates (Cooper, 1975; Miller and Ullrey, 1987; Puiman

and Stoll, 2008; Eiby et al., 2013). The effects of maternal Ca intake on fetal growth and skeletal development however, are not clearly defined in most species, including swine.

We hypothesized that group-housed sows require higher levels of dietary Ca and P compared to sows in gestation stalls and the current recommended dietary Ca and P may not be adequate for optimal bone metabolism for sows in group housing. The recommended levels of Ca and P may also be insufficient for adequate fetal skeletal development, particularly for the smaller-birth weight piglets. This study involved two experiments. The first experiment was conducted to examine the effects of the current recommended levels of dietary Ca and P for gestating sows on sow performance and bone metabolism. This study also examined 2 gestation housing systems and determined whether there would be an interaction between dietary Ca and P and housing system. The second study was performed to determine the effects of the current recommended levels of dietary Ca and P during gestation on the skeletal development of the newborn piglets.

2 LITERATURE REVIEW

2.1 Introduction

Gestation and lactation increase the daily nutrient requirements of mammals, particularly during late gestation and early lactation. In pigs, 50 % of the minerals retained in fetal tissue are deposited in the last 15 d of gestation (Mahan et al., 2009). The sow must adapt to this demand to provide sufficient minerals, particularly Ca and P, to enable mineralization of the developing fetal skeleton. Studies reviewed by Close (2010) concluded that a litter of 12 piglets required approximately 73 g of Ca and 44 g of P during the rapid fetal developmental stage in the last 2 wk of gestation. The sow requirement for Ca and P as recommended by NRC (2012) is approximately 21 g/d and 16 g/d (Table 2.1). Due to the large demand for these minerals, it is likely that bone mineral loss occurs in sows during gestation, particularly at late gestation and prior to lactation when the demands for Ca and P are higher. Similarly, the demand for Ca for milk production during lactation requires the dam to employ some form of adaptive mechanisms to provide sufficient Ca and P in the milk, while maintaining the homeostatic regulation of these minerals in her own body. Kovacs (2005) suggested that in humans, the extent of the different adaptive mechanisms used by the dam to meet the demands for Ca and P differ between gestation and lactation.

Appropriate animal models, particularly pregnant rats, have been widely used to study Ca metabolism during pregnancy as the obvious ethical constraints prevents these studies to be performed on humans. However, the differences in litter size and gestational period between rats and humans suggest that the adaptive strategies used for regulation of Ca during pregnancy may also differ (Kovacs and Kronenberg, 1997). The gestational period in pigs is approximately 115 d as compared to 22 d in rats and 285 d in humans. Even so, the time spent in each phase of fetal development is proportionately the same for pigs and humans, making studies on fetal development in pigs more relevant to humans compared to rats. Furthermore, compiled studies by Miller and Ullrey (1987) showed similar hematological values at specific developmental points during the prenatal and postnatal development in pigs and human. Pigs however, have a diffuse, epitheliochorial type placenta as compared to rats and humans that have placenta that are similar in terms of gross shape (discoid) and histological structure (hemochorial) (Furukawa et

al., 2014). The different histological structure that separates the maternal and fetal blood in pigs compared to humans suggests differences in placental permeability which may modify the maternal transfer of nutrients to the fetus (Furukawa et al., 2014). Therefore, extrapolating information from pigs to humans requires caution as apart from the larger litters in pigs, differences in placenta, grossly and histologically, may reflect differences in adaptive strategies for Ca metabolism (Kovacs and Kronenberg, 1997; Furukawa et al., 2014). Despite this caution, similarities between rats, humans and pigs have been described where the rate of maternal to fetal Ca transfer increased dramatically in late pregnancy with fetal plasma Ca higher than maternal plasma Ca (Husain et al., 2011).

Based on the current available information in human and animal models such as rats and pigs, the following section will review the regulation of Ca and P metabolism, the role of these minerals in swine and skeletal development, the current understanding of maternal and fetal adaptations to maintain the extracellular levels of Ca during reproduction and the relevant information on sow housing and lameness as it relates to dietary minerals.

2.2 Calcium and Phosphorus

2.2.1 Role of calcium and phosphorus in swine

Calcium and P are the most abundant minerals in the body (Crenshaw, 2001). In pigs, 96 to 99 % of total Ca and 60 to 80 % of P is located in skeletal tissue. These 2 minerals form hydroxyapatite crystals, the mineral which strengthens the organic matrix, providing rigidity and strength to the bone (Maxson, 1985; Cromwell, 1995; Crenshaw, 2001; Bonjour, 2011). The bones also act as storage sites and are used for the continuous deposition and reabsorption of Ca and P that occurs during the bone modeling and remodeling processes, depending on the physiological demand for these minerals. These minerals accumulate in the bone tissue interdependently; one will not accumulate in the absence of the other (Crenshaw, 2001; Veum, 2010).

One to 4 % of total body Ca is found in cells, blood and fluid; 50 % of this is ionized Ca, 40 % is bound to protein and 10 % is complexed with ions such as citrate or phosphate (Crenshaw, 2001). These levels are maintained accurately and precisely (Bronner and Stein,

1995) in a narrow range to perform regulatory functions such as cell signaling, muscle contraction, nerve conduction (Carafoli, 1991) and have an involvement in blood clotting (Hurwitz, 1996). In contrast approximately 20 % of P is located in cells and tissues throughout the body. Apart from being a structural component of DNA and RNA, the phosphate group plays an essential role in cell membrane structure, energy metabolism and maintenance of acid-base balance (Suttle, 2010). These intracellular and extracellular roles of Ca and P are controlled and maintained by specific transporter and hormonal systems which are also involved in the regulation of both minerals for bone health (Bonjour, 2011).

In pigs, studies have been conducted to study the response to dietary Ca and P by assessing the mechanical properties of bone. The mechanical strength of the bone is a focus since the skeletal tissue provides major structural support (Crenshaw, 1986) and acts as levers for muscles in locomotion (Clarke, 2008). Older studies have examined leg weakness or structural soundness, particularly in breeding herds (Smith, 1966; Grondalen, 1974; Miller et al., 1976; Greer et al., 1977; Nakano et al., 1979; Kornegay and Thomas, 1981). Leg weakness can refer to different forms of lameness; infectious and non-infectious (Muirhead and Alexander, 1997). Heredity, environment, and nutritional deficiencies, particularly Ca and P, are contributing factors for non-infectious lameness (Craig, 2001). Studies in the past have implied that the dietary Ca and P levels for maximizing bone development are higher than the requirements for maximizing growth (Kornegay and Thomas, 1981; Crenshaw, 1986; Combs et al., 1991). While leg weakness is often seen in growing pigs, posterior paralysis is more often seen in reproducing sows, primarily due to Ca, P or vitamin D deficiency (NRC, 1998, 2012). This usually occurs in late gestation or during lactation when Ca demands for fetal skeletal development or milk production are the greatest (Maxson, 1985). As most of the literature is older, it may not be applicable to sows of modern genetics, and more studies are required to investigate the role of Ca and P in bone health in the modern, prolific sow.

2.2.2 Regulation of calcium metabolism

As discussed above, the majority of Ca and P is present in the bone while the remainder is distributed within the soft tissue (intracellular compartment) and extracellular fluid space which includes blood (Crenshaw, 2001; Favus et al., 2006). The metabolism of these minerals is

closely linked through regulation that maintains distribution of Ca and P within the cells and the extracellular fluid space, while still providing sufficient Ca to maintain skeletal tissue. This mineral homeostasis is achieved by a coordinated interaction between intestinal Ca absorption, deposition of Ca in bone and renal Ca excretion. The physical-chemical properties of these minerals and the involvement of 1,25-dihydroxyvitamin D ($1,25(OH)D_3$) and hormones such as parathyroid hormone (PTH), and calcitonin regulate the mineral fluxes across the intestine, kidney and bone (Crenshaw, 2001).

Calcium is absorbed through both active and passive mechanisms. The active transport is a saturable, transcellular mechanism regulated by $1,25(OH)D_3$ through a Ca-binding protein in the proximal intestine, while passive transport is a paracellular mechanism in the lower small intestine and colon (Bronner, 1987; Crenshaw, 2001; Favus et al., 2006). Kornegay (1985) reported that in pigs, the absorption and retention of Ca is influenced by the Ca intake, ratio of Ca to P, age and physical demand. Higher rates of absorption are seen during active growth, and during pregnancy and lactation when requirements increase (Favus et al., 2006). Absorption efficiency is increased if the Ca level in the diet is low and studies have shown that Ca intake exceeding the requirement may result in decreased intestinal Ca absorption (Hurwitz and Bar, 1969; Pointillart et al., 1989; Soares, 1995). It has been suggested that when active transport is suppressed, passive paracellular transport is enhanced, leading to subsequent increases in Ca excretion to maintain Ca concentration in the body (Pointillart et al., 1989). A review by Crenshaw (2001) stated that the ratio of Ca to P is even more critical for absorption when dietary Ca is low. The amount of Ca and P excreted in the feces is linearly related to dietary mineral levels whereas the relationship between Ca absorption and Ca intake can be described as curvilinear (Crenshaw, 2001).

Ionized or complexed Ca which comprise about 90 % of the serum total Ca is freely filtered through the glomerulus in the kidney and a majority will be reabsorbed to maintain Ca balance by controlling the amount being excreted (Favus et al., 2006). Factors affecting the level of reabsorption include the plasma Ca level, hormonal influences and dietary mineral levels, among others. In pigs and rats, similar routes of excretion have been reported and urinary Ca is typically only about 10 % of endogenous fecal Ca (Besancon and Gueguen, 1969).

Ca and P in bones is stored as hydroxyapatite crystals of Ca-phosphate and accumulate in bone ash in a fixed ratio of 2.2:1 (Crenshaw, 2001). Ca regulation involves the bone remodeling

process of cell activation and resorption, followed by a reversal phase that completes the resorption process and initiates the formation process, (Raisz, 1999; Rucci, 2008). High demand for Ca and P in the extracellular fluid is met by Ca efflux and osteoclastic resorption leading to increased serum Ca. The fast response of Ca efflux from the bone to meet the high demands of extracellular fluid cannot be explained by bone resorption or absorption mechanisms; suggesting possible involvement of hormones or bone cell signaling (Parfitt, 1996; Bronner, 1998). In summary, the hormonal regulation of Ca and P in the body is a complex homeostatic mechanism, which is achieved by a coordinated interaction among intestinal absorption, renal excretion and mineral deposition in the bone.

2.2.3 Calcium economy in reproduction

In general, the physiological demand for Ca and P increases during gestation and lactation in proportion to requirements for fetal growth and skeletal development during gestation and milk production during lactation (NRC, 1998, 2012). Older studies in humans showed that 80 % of the total Ca that is transferred to the fetus, accumulates during the third trimester (Givens and Macy, 1933; Trotter and Hixon, 1974). The additional requirements for Ca and P during gestation and lactation in humans requires several maternal adaptations including increased mobilization of Ca from the maternal skeleton, increased intestinal absorption of Ca, decreased renal excretion of Ca and greater dietary intake (Kovacs and Kronenberg, 1997; Olausson et al., 2012). However, it is not clearly understood to what extent each of these maternal adaptations is a direct response to the increased demand of Ca during pregnancy and lactation.

2.2.3.1 Maternal bone mineral mobilization

Histomorphometric analyses have been conducted to study bone mineral mobilization in reproducing animals. Studies showed increased bone turnover in rats (Marie et al., 1986) and dogs (Fukuda and Iida, 1993) during pregnancy. Bone mineral content (BMC) however, remained unchanged in rats (Halloran and DeLuca, 1980; Miller et al., 1982; Marie et al., 1986). As histomorphometric data cannot be attained from humans, bone markers and imaging tools have been used to study the status of bone health in humans. Several sensitive RIA and ELISA

procedures have been developed for the detection of bone markers in the serum. These include bone formation markers; alkaline phosphatase (AP), osteocalcin (OC) and propeptides, and bone resorption markers in serum or urine such as N- and C-terminal telopeptides (NTX and CTX respectively), deoxypyridinoline (DPD) and pyridinoline (PYD) (Cross et al., 1995b; Allen, 2003). Although these methods were developed for humans, most of them are cross-reactive among animal species, including swine. Several studies have shown that concentrations of bone markers of resorption (Gallacher et al., 1994; Cross et al., 1995a; Yamaga et al., 1996) and formation (Seki et al., 1991; Karlsson et al., 1992; Cross et al., 1995a; Yamaga et al., 1996) were low at the beginning of pregnancy and steadily increased until the last trimester when Ca transfer to the fetus is at its highest and therefore, requiring maximal mobilization of maternal skeletal Ca reserves. In a review of studies in humans during lactation, Olausson et al. (2012) showed that bone turnover was low at the start of pregnancy and gradually increased during the last trimester and the onset of lactation. However, in studies with cows, sheep and sows, it was observed that bone formation decreased while bone resorption increased in late gestation and early lactation, suggesting an uncoupling of bone formation and resorption during this time of increased Ca demand. This would reduce bone formation to spare Ca for milk and to maintain serum Ca (Liesegang et al., 2000; Liesegang et al., 2005).

Several studies in humans have examined the possible influence of dietary Ca on maternal bone health; results however, were inconsistent, possibly due to confounding socio-economic or other factors (Olausson et al., 2012). Some studies concluded that dietary Ca did not influence the change in maternal bone mineral content during pregnancy (Sowers et al., 1991; Olausson et al., 2008) whereas others suggested that if maternal Ca intake was low, the significant changes in the rate of bone turnover during pregnancy and lactation will subsequently lead to bone loss (Aguado et al., 1998; Javaid et al., 2005; O'Brien et al., 2006). This is further supported by studies demonstrating the positive effect of Ca supplementation in reducing bone resorption in pregnant women with low daily Ca intake (Janakiraman et al., 2003; Liu et al., 2011). Similarly, in some studies, bone loss during lactation in young mothers appeared to be affected by dietary Ca (Chan et al., 1987), whereas other studies found no relationship between dietary Ca and bone mineral content during lactation (Sowers et al., 1995; López et al., 1996; Laskey et al., 1998) including studies in mothers that were accustomed to a low Ca diet and given Ca supplementation during lactation (Prentice et al., 1995; Prentice et al., 1998). These

results are in agreement with studies in rats where bone resorption during lactation appeared independent of Ca and P intake (Brommage and Aherne, 1986).

There have been very few studies conducted in recent years examining dietary Ca and P requirements for sows and the older literature may not be applicable to the modern, high-producing sow. Moreover, studies in sows examining the role of dietary Ca and P on bone health have yielded inconsistent results. A study by Kornegay et al. (1973) which examined sows for 5 parities, showed that the occurrence of femur fractures were higher when these sows consistently received low dietary Ca and P from their developmental period up to the completion of the 5 gestation-lactation cycles. This result agrees with work of Nimmo et al. (1981) which suggested that the gestational levels of Ca and P as suggested by National Research Council (NRC) Nutrient Requirements for Swine 1979 (Table 2.1) was not adequate to support maximum skeletal growth, particularly in gilts receiving diets with low levels of Ca and P when they were still growing. Subsequent studies based on requirements described in the NRC (1988) also supported these results where bones of young sows appeared weaker, less dense and more vulnerable to fractures than older sows (Giesemann et al., 1998). However, Maxson and Mahan (1986) stated that the Ca and P intake did not affect the skeletal reserves of these minerals as much as reproductive state (whether sows are reproducing or non-reproducing), litter size and parity. Utilizing NRC (1998) recommendations (Table 2.1), Mahan et al. (2009) evaluated mineral requirements of high-producing sows by determining the mineral content of the fetuses at different stages of gestation. The increased mineral content of fetal pigs at late gestation suggested that the sow might need to mobilize Ca from bones or from the diet to meet the fetal needs. This implies increased requirements for maternal Ca during late gestation, particularly in sows with large litters.

According to Mahan and Vallet (1997), inadequate dietary Ca and P in the sow will not affect the composition of these minerals in either the developing fetus or the milk. This is in agreement with an earlier study that indicated that unless the diet is severely deficient in Ca, homeostatic mechanisms of the sow can maintain the concentration of Ca and P in the milk resulting in no differences in the mineral contents of neonatal pigs (Mahan and Fetter, 1982). A separate study however, demonstrated greater bone loss (lower bone ash) in sows with larger litters and higher litter gain (Maxson and Mahan, 1986). Although sows with large litters had greater milk production, milk Ca and P concentration was unchanged, suggesting that the need to

mobilize Ca and P from skeletal reserves of the sows to meet the mineral requirements for milk production, is especially important for sows with large litters. The additional demands for Ca and P during reproduction, particularly in late gestation and early lactation, could be met through mobilization of Ca and P from the maternal skeleton. The different responses of dietary Ca and P on bone turnover observed in previous studies however, indicates the need for more research in this area, particularly for sows of modern genetics producing even larger litters.

2.2.3.2 Intestinal calcium absorption and renal excretion

In humans, longitudinal studies have shown that intestinal Ca absorption increases during gestation (Heaney and Skillman, 1971; Kent et al., 1991a; Cross et al., 1995a), while absorption gradually falls to the non-pregnant levels during lactation. The increased urinary Ca excretion during gestation reflects the increased intestinal Ca absorption, conversely renal Ca excretion decreases during lactation (Kovacs and Kronenberg, 1997; Olausson et al., 2012). The significant increase in Ca absorption, particularly during the second and third trimester, may be directly related to Ca intake (Cross et al., 1995a). However, even with a high rate of Ca absorption, a low Ca intake may not meet the maternal and fetal needs (Hacker et al., 2012). During pregnancy, the increased efficiency in Ca absorption is due to the increased expression of enterocyte Ca-binding protein in the small intestine (Cross et al., 1995a; Richie et al., 1998; Prentice, 2003b). A progressive increase in serum calcitriol observed in several studies may also influence Ca absorption (Cross et al., 1995a; Richie et al., 1998; Zeni et al., 2003). Overall, increased intestinal Ca absorption and glomerular filtration rate (GFR) results in the higher level of Ca excretion in pregnant women (Chef, 1969; Pitkin, 1985; Kent et al., 1991b; Prentice, 2003b). Similarly, studies in rats also showed increased intestinal Ca absorption during gestation (Delorme et al., 1979; Bruns et al., 1981; Delorme et al., 1982). The increased rate of Ca absorption starts in mid-gestation before the onset of fetal skeletal Ca accretion in late gestation (Quan-Sheng and Miller, 1989). This suggests that early Ca absorption allows for Ca storage before peak demand for Ca by the fetus in late pregnancy (Kovacs and Kronenberg, 1997).

During lactation, intestinal Ca absorption decreased to levels seen in early pregnancy (Cross et al., 1995a; Richie et al., 1998; Vargas Zapata et al., 2004) and a reduction in GFR reduces excretion of Ca into the urine (Kovacs, 2005). One study in women, reported that even though intestinal Ca absorption was increased during lactation, a more prominent effect was only

seen after weaning (Kalkward et al., 1996). In pigs, limited data is available on Ca metabolism in reproducing sows. A report by Kornegay (1985) concluded that the dietary Ca level, the ratio of Ca to P, pig age and the physiological status of the pig influenced the level of Ca being absorbed or excreted. Younger sows apparently retain more Ca and P during gestation compared to older sows, but retention of these minerals is limited during lactation due to lower feed intake in the younger sows during lactation relative to the older sows (Giesemann et al., 1998). In summary, increased Ca intake and absorption leads to increased renal Ca excretion. While studies in human and rats indicate intestinal Ca absorption as a major maternal adaptation during gestation, it is unclear whether this is the same for reproducing sows.

2.2.3.3 Fetal calcium and phosphorus accretion

The majority of fetal skeletal growth takes place from mid-pregnancy and reaches peak calcium accretion during the third trimester (Prentice, 2003a; Kovacs, 2006). Fetal mineral metabolism has been uniquely adapted to obtain sufficient Ca for skeletal mineralization and to maintain the extracellular Ca level. Fetal Ca regulation involves the placenta, kidney, bone and intestines (Kovacs, 2006). In the human, placental Ca transfer progressively increases from wk 12 and reaches its peak at wk 36 of gestation (Forbes, 1976; Koo et al., 1999; Jarjou et al., 2010) with 80 % of Ca accumulated during the third trimester (Kovacs and Kronenberg, 1997). Factors affecting placental Ca transfer are not clearly defined and observational studies on maternal Ca intake and fetal Ca accretion have been inconsistent. Although a positive relationship between maternal Ca intake and fetal bone outcomes have been reported in humans (Chang et al., 2003; Ganpule et al., 2006); a review of the literature by Kovacs and Kronenberg (1997) stated that the fetal skeleton will be mineralized and blood Ca levels maintained, regardless of the maternal blood Ca concentration. Studies in different mammalian species have reported that fetal blood Ca is maintained at a higher level than maternal blood Ca (Garel and Barlet, 1976; Schauberger and Pitkin, 1979; Pitkin et al., 1980; Wadsworth et al., 1982). In rats, this high level of Ca is maintained from early pregnancy even with low maternal Ca (Lima et al., 1993) or vitamin D intake (Miller et al., 1983). Other studies however, have shown that if this situation persists, the rapid fetal Ca accretion for skeletal mineralization in late pregnancy may be impaired (Gilbert et al., 1980; Garel and Gilbert, 1981).

In sows, there are few studies examining the impact of maternal Ca on fetal Ca accretion. Some older studies have reported results comparable to those seen in the human where the major factor affecting placental Ca transfer is the fetal demand for Ca which progressively increases as pregnancy advances (Hansard et al., 1966). However, in contrast to some human studies where a functional independence of the fetal-placental unit from the mother was suggested, Itoh et al. (1967) stated that maternal plasma Ca in the sow is probably the primary fetal Ca source. This implies that maternal Ca level will influence Ca transfer to the fetus.

2.2.4 Dietary calcium and phosphorus requirements in gestating sows

There have been several studies conducted on dietary Ca and P requirements in sows (Kornegay et al., 1973; Harmon et al., 1974; Nimmo et al., 1981; Mahan and Fetter, 1982; Arthur et al., 1983; Grandhi and Strain, 1983; Maxson and Mahan, 1986; Everts et al., 1998; Mahan et al., 2009). Most of these studies however, are older and may not be applicable to the modern, hyperprolific sow. More recent research has shown that the Ca and P requirements during gestation increase with fetal growth and development and reach a peak in late gestation (Mahan et al., 2009).

During lactation, milk yield is influenced by litter size, birth weight, parity, and genetics, among other factors (King'ori, 2012). The level of milk production determines the Ca and P requirement of lactating sows. Studies reported that sow reproductive performance was similar regardless of dietary Ca and P levels (Nimmo et al., 1981; Grandhi and Strain, 1983) and feeding higher than recommended levels of Ca and P in gilts did not improve subsequent reproductive performance or longevity (Kornegay et al., 1985). Other evidence suggests that Ca and P intake for skeletal growth and development was more critical in gilts compared to older parity sows (Giesemann et al., 1998). Another study suggested that reproductive state, litter size and parity affect the mineral skeletal reserves more than Ca and P intake (Maxson and Mahan, 1986). The concentration of these minerals in the bones allowed an estimation of mineral requirements by assessing the changes in skeletal storage. Hayes (1976) implied that although the concentration of bone ash Ca and P did not change with nutrient intake; the total amount of ash varied. Newer evidence shows that bone ash Ca (39 %) and P (18.9 %) is constant across parities, suggesting

that increasing dietary mineral supplementation with age is not necessary (Crenshaw et al., 2013).

Determining optimal Ca and P supplementation in all phases of pork production depends on the bioavailability of these minerals, the ratio of Ca to P and an adequate supply of vitamin D (Peo Jr., 1991). Little is known about Ca bioavailability in natural feed ingredients such as cereal grains and grasses, however, because of the low contribution from feedstuffs, phytic acid, which reduces Ca bioavailability, is of little concern (NRC, 2012). The majority of the Ca requirement in pigs, therefore, is supplied by inorganic sources (Crenshaw, 2001). Studies in poultry have shown that inorganic sources such as dicalcium phosphate, tricalcium phosphate, defluorinated phosphate and Ca gluconate or sulfate are highly available compared to Ca carbonate, the chief component of limestone (Baker, 1991; Soares, 1995). These inorganic sources are commonly used to meet the P requirements and a portion of the Ca requirement while the balance is usually supplied by limestone (Crenshaw, 2001). Phosphorus is bound to phytate and its bioavailability is variable (NRC, 1998). The presence of a natural phytase enzyme in wheat improves P availability compared to P in other cereal grains and grain by-products. To satisfy the P requirements for pigs, inorganic sources such as Ca phosphate (monocalcium or dicalcium) are typically used to augment the organic P in natural feed ingredients (Jongbloed et al., 1991).

It has been consistently shown that a wider Ca to P ratio leads to poor growth and bone calcification, especially in diets with low P (Peo et al., 1969; Vipperman Jr. et al., 1974; van Kempen et al., 1976; Reinhart and Mahan, 1986; Hall et al., 1991; Eeckhout et al., 1995; Qian et al., 1996). The NRC (1998) suggested that the ratio of total Ca to total P should be between 1:1 to 1.25:1 and this level has been maintained in the NRC 2012 (Table 2.1) The authors however, acknowledge that the requirements were left unchanged because of a lack of data to justify a change and state that research needs to focus on examining Ca and P requirements of the sow (NRC 2012) . Adequate vitamin D is also required for proper Ca metabolism and evidence suggests that insufficient vitamin D alters Ca and P metabolism and bone mineralization (Lauridsen et al., 2010). The requirement for Ca and P for sows in the recent NRC (2012) takes into consideration the sow's weight, anticipated litter size and stage of gestation (Fig. 2.1) or lactation (Fig. 2.2). This contrasts with the recommendations for these minerals in gestation and lactation in earlier versions, which listed only two levels, one for gestation and another for lactation (Table 2.1). A modeling approach was used to estimate the standardized total tract

digestibility (STTD) P requirements, which are calculated from feed intake, BW, weight gains of sows and conceptus, and P requirement for bone mineralization that is also dependent on parity. Due to limited recent data, the Ca requirements in NRC 2012 are primarily derived from the STTD P requirements using a fixed ratio between STTD P and total Ca (NRC, 2012). The contradictory results obtained from older studies and limited availability of data with the modern genetic lines calls for a re-evaluation of dietary Ca and P requirements as the modern sow may have different requirements than sows of older genetic lines (Stein, 2012).

2.2.5 Mineral interactions with Ca

Minerals are essential nutrients for swine. Feed ingredients do not have sufficient levels of these nutrients, and therefore, minerals are usually incorporated into feed as premixes. However, minerals interact and may directly or indirectly affect the metabolism of other minerals. As mentioned in the previous section, older studies (1970s to 1990s) have suggested the importance of a suitable Ca-to-P ratio in order to ensure adequate supply of these minerals to the pig (NRC 2012). However, studies on mineral interactions with Ca in laboratory animals and humans have produced conflicting results. Research has indicated that increasing Ca intake will reduce Zn bioavailability and decrease intestinal Mg, Mn and F absorption in laboratory animals; however, little or no effect was observed in humans (Miller and Groziak, 1997). In pigs, studies have suggested that excessive Ca will greatly reduce Zn bioavailability (Morgan et al., 1969) whereas Forsyth (1972) reported that there was no influence of maternal F intake on bone Ca in the young piglet. The limited studies available showed that high Ca intake reduces the retention of Cu and Fe in laboratory animals and humans, although the effects may be dose-dependent (Miller and Groziak, 1997). A study in pigs however, reported that in young piglets (d 3 or 4 of age) a high Ca diet fed for 2 wk, did not inhibit Fe absorption or alter Fe status in the body (Wauben and Atkinson, 1999). For these reasons, the recommended levels of minerals for pigs generally include a safety margin (Gaudré and Quiniou, 2009).

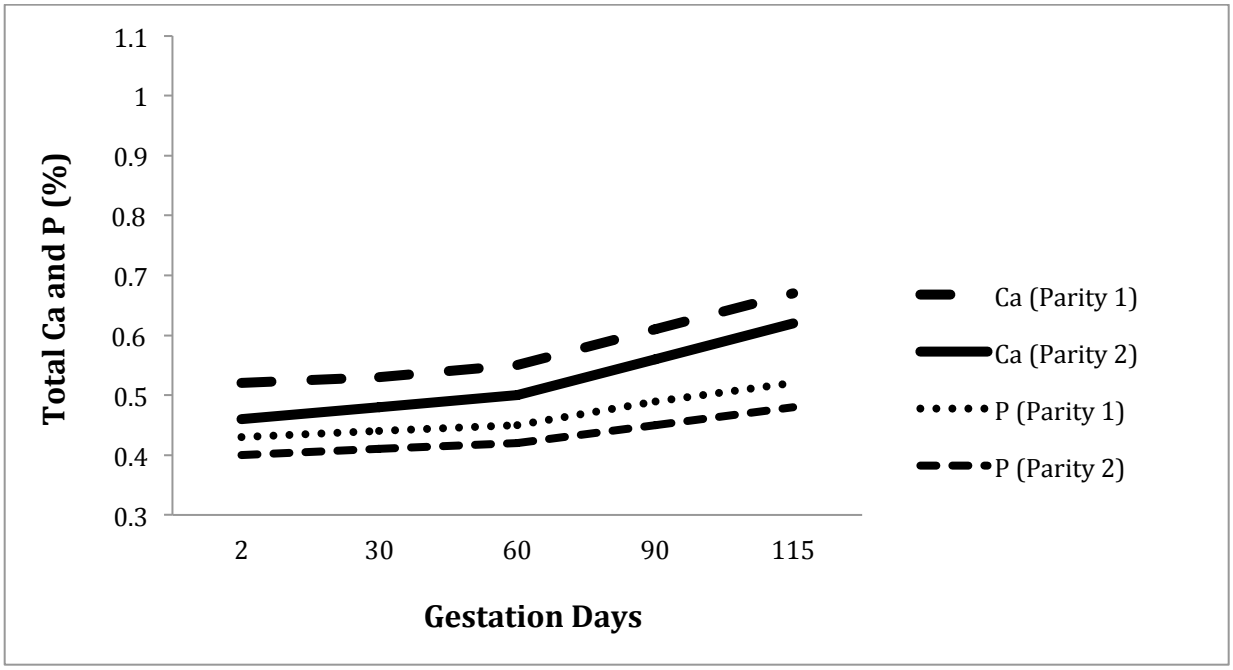


Fig. 2.1. Ca and P requirements (% of feed as fed) of gestating sows during parity 1 and parity 2 obtained from a simulation exercise using the NRC 2012 gestating sow model (NRC 2012). The sows were assumed to weight 180 and 210 kg in parity 1 and 2, respectively. Estimated gestation length of 115 d and anticipated litter size of 13.5 piglets.

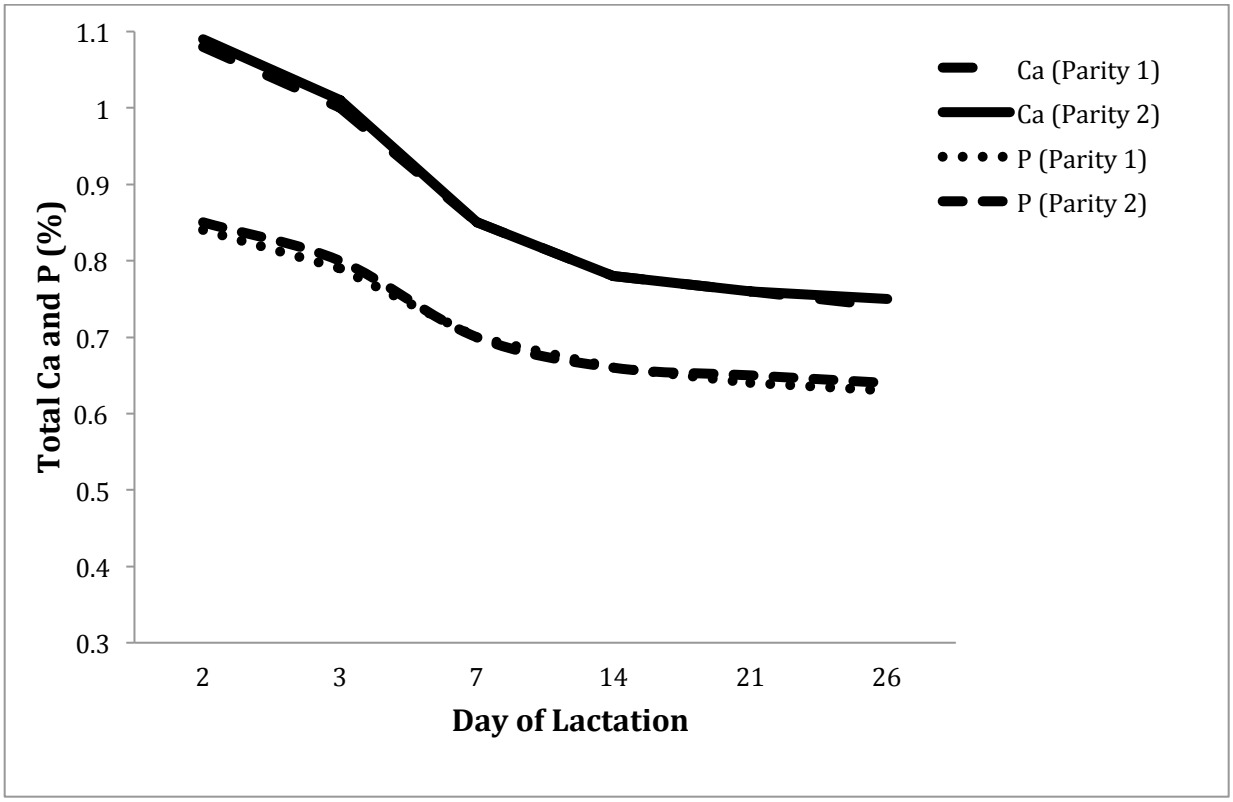


Fig. 2.2. Ca and P requirements (% of feed as fed) of lactating sows during parity 1 and 2 obtained from a simulation exercise using the NRC 2012 lactating sow model (NRC 2012). The sows were assumed to weight 200 and 230 kg for parity 1 and 2, respectively at the start of lactation. Sows were anticipated to nurse 12 piglets over a 26 d lactation.

Table 2.1. Dietary Ca and P requirements of gestating sows as recommended by National Research Council (NRC) for swine

	National Research Council (NRC) for swine					
	1968, 1973[1]	1979[1]	1988[2]	1998	2012[3]	
					≤ 90 d	≥ 90 d
Ca, g/d	15.0	13.5	14.2	13.9	13.0	20.9
P, g/d	10.0	10.8	11.4	11.1	10.4	15.7
Ca, %	0.83	0.74	0.75	0.75	0.61	0.83
P, %	0.55	0.59	0.60	0.60	0.49	0.62
Feed intake, kg/d	1.81	1.81	1.90	1.85	2.13	2.53
Ca:P	1.50	1.25	1.25	1.25	1.25	1.34

[1] Nimmo et al., 1981.
[2] Everts et al., 1998; Giesemann et al., 1998.
[3] Recommended levels for parity 1 sows at ≤ 90 d of gestation with assumption of 5 % feed wastage.

2.3 Formation and mineralization of the skeletal system

2.3.1. Basic concepts: Bone growth, modeling and remodeling

Generally, the skeleton of mammals serves a variety of roles, including protecting internal organs, providing structural support, facilitating movement and locomotion, and maintaining mineral homeostasis. The appendicular skeleton consists of the shoulder and pelvic girdle, and the bones of the limbs. Long bones make up the major skeleton of the limbs and this includes the humerus, radius, ulna, femur, tibia, fibula and metatarsals and metacarpals. The long bones are composed of the diaphysis, which is primarily dense cortical bone surrounding the marrow space on the shaft, while trabecular meshwork of the epiphysis and metaphysis constitute both ends of the bone. The ratio of cortical and trabecular bones differs at different skeletal sites, but on average, cortical bone represents 75 to 80 % of the total bone with the remaining comprised of trabecular bone (Gropper et al., 2009).

Three cell types are involved in bone growth, modeling and remodeling; osteoblasts, osteocytes and osteoclasts. Bone growth occurs at the epiphyseal plate where cartilage proliferates mitotically at the epiphysis and metaphysis while the chondrocytes at the diaphysis degenerate. This process occurs throughout the growth period (childhood and adolescence) until cartilage growth stops and osteoblasts, the bone-forming cells, form new mineralized matrix and subsequently, primary new bone. Bone modeling or reshaping is the process whereby bone adapts its structure in response to mechanical forces and physiological influences (Clarke, 2008). Bone may change size, shape or reshape by the independent action of osteoblasts or bone resorbing cells, the osteoclasts, hence, bone formation and bone resorption is not tightly coupled during this process.

Bone remodeling is an active process and bone is continuously renewed during life (before birth to death) to maintain a mechanically competent skeleton, to replace microdamage in bones caused by abnormal loadings (Burr et al., 1997; Rucci, 2008) and to maintain mineral homeostasis, particularly Ca and P (Rucci, 2008). This process relies on a balance between osteoclasts and osteoblasts, a tightly coupled group of bone cells that are involved in bone resorption and bone formation. The remodeling process begins with the activation phase where alteration in the microstructure or hormonal action on bone cells in response to systemic

changes, is detected by osteocytes and other factors in the bone microenvironment, triggering the osteoclastic differentiation and resorption phase (Rucci, 2008; Chowdhury, 2014). The reversal phase, where the transition from bone resorption to bone formation occurs, is followed by the release of growth factors that trigger osteoblasts to form new collagenous extracellular matrix. This proteinaceous matrix is then mineralized, leading to repair of the resorption site and completing the remodeling phase (Loveridge, 1999). Bone remodeling regulates the bone mineral density in an adult and therefore, a loss of the balance between the osteoclast-osteoblast coupling will lead to bone loss and skeletal pathology (Clarke, 2008).

2.3.2 Fetal skeletal development

The 3 main organs involved in Ca regulation are: the kidney, the bones and the intestine. Much attention has been given to intestinal and renal regulation of Ca. Bone however, may be of greater importance since a majority of Ca is stored in skeletal tissue (Crenshaw, 2001) with almost 97 % of Ca in skeletal tissue of neonates (Schneider et al., 1997). The primary response to dietary Ca and P therefore would preferentially be evaluated by examining the changes in skeletal tissue such as measurement of total ash content or bone mechanical properties (Crenshaw et al., 1981; Combs et al., 1991; Crenshaw, 2001).

In comparison with adults, there are obvious limitations to studies on mineral and bone homeostasis. In the developing fetus, fetal Ca metabolism is adapted to meets the needs during the developmental period through; 1) maintenance of sufficient Ca for skeletal mineralization and 2) extracellular Ca balance (Kovacs, 2006). Skeletal mineralization reaches a peak during late gestation and the importance of these adaptations becomes more evident as gestation advances. Comparable to adults, bone resorption may occur in the fetal skeleton to help maintain the Ca balance in the blood, which is probably controlled by fetal parathyroid hormone (PTH) and parathyroid hormone-related proteins (Kovacs and Kronenberg, 1997). Studies in sheep have shown that the fetal parathyroid gland is required by the developing skeleton (Aaron et al., 1989; Aaron et al., 1992). Several lines of evidence from animal models such as rats (Miller et al., 1983; Brommage and Deluca, 1984) and ewes (Lima et al., 1993) however, indicated that 1,25-dihydroxy vitamin D may not be required by the fetus for skeletal development. Fetal femur length and mineral ash content was reduced during maternal hypocalcemia (Chalon and Garel,

1985) suggesting a relationship between fetal skeletal development and maternal Ca. However, most of these studies were conducted in rats where differences in body size, gestation period, and litter size may be of concern if extrapolating to pigs. Older studies using radiochemical procedures to study placenta transfer in pigs suggested that sow requirements during gestation influenced Ca movement and transfer to the developing fetuses (Itoh et al., 1967) and selective deposition of Ca occurs at an incremental rate as gestation advances (Hansard et al., 1966). The limited studies in pigs showed fetal bone calcification was reduced in Ca-deficient sows (Itoh et al., 1967). This is consistent with evidence from ewe studies (Lima et al., 1993); however, in both cases, no effect was seen on fetal plasma Ca (Itoh et al., 1967; Lima et al., 1993). During lactation, sows apparently provide a buffer of Ca and P in milk hence, abnormalities in piglet skeletal development will not be seen during lactation even if sows receive inadequate Ca (Mahan and Fetter, 1982).

Since the mid-1900s, nutritionists have been using bone strength to assess the mineral requirements in swine and the bioavailability of the minerals (Crenshaw et al., 1981). The majority of the Ca and P is stored in skeletal tissue, thus changes in skeletal storage will provide information on dietary Ca and P requirements (Crenshaw, 2001). Several studies have shown that increasing dietary Ca and P will increase bone strength in piglets (Miller et al., 1962; Miller et al., 1964) and growing pigs (Libal et al., 1969; Cromwell et al., 1970; Nimmo et al., 1980; Crenshaw et al., 1981; Combs et al., 1991). These studies however, involved feeding diets with different Ca and P concentrations directly to the weanling or growing pigs. At present, there is no strong evidence showing that the sows' Ca and P status has an effect on fetal or newborn piglet bone development.

In pigs, the response to dietary Ca and P in skeletal tissue in the form of bone ash or mechanical properties depends on the bone used and the pig's age (Crenshaw et al., 1981). An earlier paper by Tanksley (1979) implied that the femur was a better indicator of bone status than the metacarpals whereas Crenshaw et al. (1981) showed that the femur, humerus and ribs were responsive to Ca and P in younger pigs (approximately 8 wk of age) in terms of bone mechanical properties, making them a better option to assess bone mineralization in young pigs compared to other bones such as the thoracic vertebra or metatarsals that were used in his study. Although results from both studies differ in the type of bone as being the better indicators of bone status,

both authors were in agreement that the type of bone used to assess Ca and P requirements may yield different results.

In summary, although the mechanisms associated with fetal skeletal mineralization are unclear; studies in humans, rats and pigs have suggested the possible influence of maternal bone metabolism in mediating fetal skeletal mineralization particularly at late gestation. Maternal Ca and P deficiencies, or conditions that alter the placenta Ca transfer may affect bone development of the fetuses in utero.

2.3.3 Effects of piglet birth weight and within-litter variation on bone health

Increasing litter size is one of the goals of swine production. Larger litters however are often associated with low piglet birth weight and piglet mortality (Rutherford et al., 2013). The large litter size of the modern sow means that there is a chance of a reduced proportion of heavy piglets, which have a higher chance of survival compared to the smaller birth-weight piglets (Boulot et al., 2008). Quesnel et al. (2008) reported that 20 % of the birth weight variation is due to litter size, parity, sow birth date and breeding season. Further investigation is required therefore, to identify other factors involved.

Maternal nutrition, which includes dietary energy, and protein, and amino acid ratios and feed intake is one possible factor that has been examined. A review of the literature by Campos et al. (2012) suggested that restrictions to sow feed and protein intake, and inadequate amino acid intake may have a profound effect on the developing fetus and consequently, affect piglet performance postnatally. The large increase in fetal Ca and P content during late gestation indicates a greater nutritional demand for these minerals especially in sows with larger litters (Mahan et al., 2009). As discussed by Lanham et al. (2011), nutrient restrictions in animal models such as rats and ewes during gestation have shown direct alteration to bone ossification (bone tissue formation) in growing fetuses, hence affecting the size, structure and strength of bones in the newborn. Incorrect maternal nutrition, may be associated with a limitation of sows to provide adequate nutrition to all the developing fetuses due to decreased uterine blood flow to each piglet as litter size increases (Père and Etienne, 2012) and this may subsequently lead to fetal growth retardation and decreased uniformity in piglets birth weight (Campos et al., 2012). Current available studies that evaluated maternal nutrition in relation to fetal development and

growth were mainly focused on dietary energy, protein, amino acid and feed intake during gestation (Campos et al., 2012). However, the question of the importance of low maternal Ca intake in this process and its influence on within-litter variation has not been properly addressed.

2.3.4 Other nutritional factors affecting bone development and remodeling

Heaney and Layman (2008) stated that the effect of protein on bone and Ca absorption is dependent on protein source and level, Ca intake and dietary acid/base balance. However, the role of protein in bone remodeling remains unclear. Bone quality may also be affected by dietary lipid content and the type of lipid; saturated or unsaturated. Human studies have found a negative impact of saturated fatty acids on bone density, which can be improved by increasing the dietary polyunsaturated fatty acid content of the diet (Corwin et al., 2006; van Riet et al., 2013).

Apart from Ca and P, Mg and Zn are also known to be important for bone formation. In humans, approximately 60 % of Mg and 30 % of Zn are found in the skeleton at birth (Prentice, 2003a). Although the currently available data on the role of Mg and Zn on bone development are limited, the adaptive mechanisms used to meet the demands of these minerals during gestation and lactation in humans, are comparable to the strategies used for Ca and P (Prentice, 2003a). Comparable to Ca and P, the extent of maternal intake of these minerals affecting bone metabolism and fetal growth are not fully established (Prentice 2003a, Bonjour et al., 2009). However, simultaneous supplementation of Zn and Fe for nursery pigs (d 31) increased bone strength and ash weight (Veum et al., 2009). Other microminerals, such as Cu, Mn and F have also been associated with adverse effects on bone remodeling if provided in excess or are deficient in the diet (van Riet et al., 2013). An older study reported a nonlinear effect of F levels in diets of gestating sows on humerus density of piglets at birth (Forsyth et al., 1972). The levels of F in the diets of sows however, did not affect the skeletal Ca and P content of the newborn piglets. A deficiency in Mn has been linked with gait abnormality and lameness in sows (McDowell, 2003).

Vitamin intake also impacts bone health. Studies in humans have suggested that improving maternal vitamin D status may have a positive effect on fetal skeletal development (Mahon et al., 2010; Young et al., 2012). A recent study in pigs suggested that adequate vitamin D supplementation of the sow helps to maintain Ca homeostasis during periods of high demand

such as late gestation and lactation, potentially sparing skeletal Ca reserves by reducing bone turnover (Weber et al., 2014). However, a separate study reported that bone mineralization in newborn piglets was not affected by maternal vitamin D intake (Flohr et al., 2014). In a book by Dryden, (2008), it is stated that severe deficiencies of B vitamins such as biotin, choline, pantothenic acid, riboflavin, B6 and B12 may lead to some form of lameness in pigs. Although limited evidence is available, previous studies suggested a possible association of biotin deficiency and lameness in gilts (Misir et al., 1986; de Jong et al., 2011). Recent studies in rats (Huot et al., 2013) and humans (Huot et al., 2013) did not observe changes in bone content of newborns when folate was supplemented to the dam. However, when folate was supplemented to the dam at 10 fold the requirement, femoral bone strength of the newborn rats was decreased (Huot et al., 2013).

2.3.5 Analytical tools to monitor bone health

2.3.5.1 Biochemical markers of bone turnover

Non-invasive techniques that are available to monitor bone health in humans have also been used in livestock. Bone markers provide a dynamic measure of bone turnover providing quantitative data on bone activity and are an alternative to histomorphometric examination of bone biopsies. Unlike bone biopsies which provide information on site specific metabolism, serum and urinary assays of bone markers reflect total bone metabolism (Allen, 2003). Repeated samplings do not interfere with bone activity (Frost, 1983). Markers of bone turnover currently in use, include peptides derived from bone cells or matrix, and are classified based on the metabolic activity of osteoblasts (cells that form bone) and osteoclasts (cells that resorb bone) (Seibel, 2005). Collagen-degradation products such as PYD, DPD, CTX and NTX reflect a majority of bone resorption markers while molecules that reflect bone formation are mostly by-products of collagen synthesis or osteoblast-related proteins such as OC and AP (Allen, 2003; Seibel, 2005).

In livestock, bone marker assays have been used to study bone turnover in dairy cattle (Liesegang et al., 2000; Liesegang et al., 2007; Esser et al., 2009; Moreira et al., 2009; Taylor et al., 2009), beef cattle (Erickson et al., 2002), sheep and goats (Liesegang and Risteli, 2004), horses (Price et al., 1995; Hoekstra et al., 1999; Hiney et al., 2000) and pigs (Carter et al., 1996;

Nicodemo et al., 1998; Eklou-Kalonji et al., 1999; Larsen et al., 2000; Liesegang et al., 2005; Shaw et al., 2006a; Lauridsen et al., 2010; Varley et al., 2011b; Witschi et al., 2011). Studies in horses (Price et al., 1995; Hiney et al., 2000) and dogs (Puustjarvi et al., 1995) showed similar trends with high concentrations of both bone markers in young animals indicating a high bone turnover rate, which decreased as the animal reached maturity. In piglets, concentrations of bone absorption (OC) and resorption (CTX) markers gradually increased from d 21 post-farrowing to approximately 77 d of age (Witschi et al., 2011). However, when piglets were fed low dietary vitamin D, a plateau for both markers occurred around weaning suggesting the involvement of vitamin D in regulating Ca and P homeostasis by reducing bone mineralization (Witschi et al., 2011).

Studies in different species using bone markers to study the effects of dietary Ca and P on bone turnover have met with contradictory results. Studies in growing and finishing pigs (Carter et al., 1996; Eklou-Kalonji et al., 1999; Shaw et al., 2006a), rats (Lian et al., 1987; Tanimoto et al., 1991; Hämäläinen, 1994) and adult humans (Åkesson et al., 1998) have shown that serum OC was inversely correlated with dietary Ca and P but no significant differences were seen in studies in neonatal (Hillman et al., 1993) or growing/finishing (Nicodemo et al., 1998) pigs. In another, more recent study in growing pigs, different levels of dietary Ca resulted in only slight changes in bone marker concentrations in serum of pigs fed the high Ca diet (Larsen et al., 2000).

In sows, serum concentrations of bone formation markers, such as AP and OC decrease while concentration of the bone resorption marker, CTX increased at the onset of lactation compared to late gestation (Liesegang et al., 2005; Lauridsen et al., 2010). Similarly, in cows, goats and sheep, decreased concentrations of bone formation markers and increased concentration of bone resorption markers were seen around parturition, presumably an indication of the acceleration of bone resorption to spare Ca for the acute demands of Ca for milk production (Liesegang et al., 2000; 2004; 2007). Studies in humans and monkeys however, showed both formation and absorption markers increased during late gestation and at the onset of lactation (Cross et al., 1995b; Lees et al., 1998). In these studies, bone formation markers decreased close to baseline levels at the start of pregnancy and gradually increased towards late gestation. Although it is unclear why the bone markers results differ between species, it has been

suggested that this may be due to the type of markers used and the uniqueness of each regarding the mechanisms regulating the synthesis and metabolic clearance (Cross et al., 1995b).

As opposed to human studies, none of the few currently available studies in sows demonstrated a response to dietary Ca or P on bone marker status during gestation or lactation (Nimmo et al., 1981; Liesegang et al., 2005; Sipos et al., 2011). One study however, reported that sows fed low dietary Ca and P had lower ash values indicating lower bone mineral content, suggesting that bone resorption leading to bone mineral loss, occurred in these sows to meet the demands of fetal development (Mahan and Fetter, 1982). The discrepancy between the studies can be due to several factors including the differences in dietary Ca and P concentration, sow reproductive performance or the lack of validation between bone markers and bone cell activity in vivo. However, results from this limited number of studies indicate the need for more research examining the regulation of bone metabolism during gestation and lactation in sows.

2.3.5.2 Non-invasive imaging techniques for assessing bone status

Dynamic, but indirect measures of bone health can be obtained with bone marker assays, but direct, static measures can be obtained from invasive techniques such as histomorphometry of bone biopsies or non-invasive imaging techniques such as dual-energy x-ray absorptiometry (DXA), radiography absorptiometry (RA) or quantitative computed tomography (QCT) (Orth, 2003). These techniques are considered static as they provide information on the appearance of the bone at one specific moment and not changes in bone cells activity. The most common method, DXA, has its limitations as it measures areal bone mineral density in g/cm2 (aBMD) by projecting a three-dimensional object to a two-dimensional area assessment and thus, the size of the bone is a confounding factor due to the missing depth value for the calculation of volume (Gasser, 1995). In contrast, peripheral quantitative computed tomography (pQCT) a newer, three-dimensional imaging technique, provides measurement of bone geometry related to bone strength as it provides the actual volumetric bone mineral density in g/cm^3 (BMD) and has the ability to distinguish the cortical and trabecular compartments (Pollock et al., 2007). Studies in rats using pQCT, demonstrated better estimation of long bone strength compared to DXA (Ferretti, 1995; Gasser, 1995; Ferretti et al., 1996; Jämsä et al., 1998) or a three-point bending test (Jämsä et al., 1998). The ability of pQCT to provide bone strength indices based on the bone

material properties such as bone mass and bone density, and bone structural properties such as cross-sectional area, may therefore serve as a more reliable method to predict long bone strength (Siu et al., 2003; Willnecker, 2003; Kontulainen et al., 2008).

Animal models have been widely used for bone research to address bone disorders including osteoporosis and effects of drugs on bone metabolism in humans. This however, has shown that differences in bone composition and density of different species are not always reflected in human bones. A study by Aerssens et al. (1998) showed marked differences between species in terms of bone composition, density, and mechanical properties. However, it was suggested that bone composition of canine best-resembled humans whereas rats have very different bone composition. Dogs and pigs are similar and comparable to humans with regards to higher fracture incidences with lower bone density values. In recent years, studies have been conducted on infants (Koo, 2000), children (Nelson and Koo, 1998; Binkley and Specker, 2000), and developing animals (Abrams et al., 1988; Mitchell et al., 1998; Koo et al., 2001) to examine the body composition and bone strength using non-invasive measurements. The comparison of these measurements however, must be based on the same bone (Koo et al., 2001). Nevertheless, the ability of pQCT to assess bone strength noninvasively allows for an accurate and precise measure of the mechanical properties of the given skeletal region, particularly in smaller bones such as those of the newborn piglets.

2.4 Sow Housing

2.4.1 Housing options for gestating gilts and sows

In today's modern pork production, gestating and lactating sows are kept in individual stalls to allow separate feeding to minimize weight gain during gestation and allow proper management. Although crates, the most common sow housing system, allow individual control of feed intake; social interaction (McGlone et al., 2004) and freedom of movement (Gonyou, 2001) are limited, thus welfare is negatively impacted. Recently, there has been pressure on the swine industry to move from individual stall housing to group housing for sows. The revised Canadian Code of Practice for the Care and Handling of Pigs (National Farm Animal Care Council, 2014) which was released in March 2014, called for the implementation of group housing for sows to occur over the next 10 yr.

The housing system must meet the requirements of the animals and the producer. Required criteria include positive effects on welfare and health, maintenance of productivity, labor and management requirements cost, and potential effects on the environment (den Hartog et al., 1993). It is natural behavior for sows to establish a social hierarchy by showing dominance through aggression, which can lead to injury (Morris et al., 1993; Gjein and Larssen, 1995; Borell et al., 1997). Individual stalls reduce aggression however, the potential to cause stress increases because of the limited social interactions (Broom et al., 1995). Main factors to consider for group housing includes control of individual sow feed intake, number of sows in the group, and reducing aggression associated with feeding, and mixing (Gonyou, 2001; Spoolder et al., 2009; Gonyou and Rioja-Lang, 2012; Edwards, 2013; Schau et al., 2013). There are various criteria that must be considered for group housing. Edwards and Gonyou (2001) described the main housing systems based on feeding methods; pens with floor feeding or individual feeders, free-access stalls, short stall feeders with trickle feeding systems, and pens with electronic sow feeders.

To evaluate the different type of housing system for sows, particularly group housing and individual stalls, researchers have focused on behavior, physiology, performance and health of the sows. Despite the intense public debate on welfare of sow housed in groups versus stalls, scientific reviews have concluded that there were no effects of housing systems on reproductive performance (McGlone et al., 2004; Rhodes et al., 2005; Curtis et al., 2009).

2.4.2 Mobility of sows in gestation stalls and lameness

Individual stalls are currently the primary housing system for gestating sows. The practice of confining sows in stalls results in a restriction in space and a reduced ability to exercise. Using animal models such avian, Lanyon (1984, 1987) stated that exercise is required to maintain bone composition and strength. A decrease in physical loading will eventually lead to Ca mobilization from the bone, subsequently affecting the cortical bone mass (Lanyon, 1993).

Earlier studies showed that confinement housing altered lying and standing behavior. A study by Krohn and Munksgaard (1993) demonstrated that the duration of lying was generally longer in dairy cows that were housed in stalls compared to cows in loose housing. In layer hens, studies have been done quite extensively on exercise and its effect on bone strength, as

osteoporosis has become a widespread problem in layer flocks (Jendral et al., 2008). Studies in layers have shown that physical activity enhanced bone strength while weaker bones were seen in confined layer hens (Knowles and Broom, 1990; Whitehead, 1996; Newman and Leeson, 1997).

Compared to group housing, posture and movement restrictions were seen in individually confined sows which may be due to the availability of space per animal (McGlone et al., 2004). Results from an earlier study by Marchant and Broom (1996a) indicated that sows that were confined in stalls had reduced muscular weight and bone strength due to lack of mobility. High demands for Ca and P, particularly in late gestation and lactation, may cause a severe depletion of these minerals in maternal bones if there is dietary inadequacy. This may eventually lead to increased susceptibility to fracture and bones may be further weakened due to other factors such as the lack of exercise. Paradoxically however, findings from a recent study demonstrated that the occurrence of lameness was higher in sows housed in groups compared to those housed in gestation stalls (Calderón Diaz et al., 2014). The study also showed that sows in groups required a shorter time to lie down due to the fact that they had more space or perhaps because they were lame. Although there are different causal factors for lameness, studies in sows have shown that lame animals have more difficulty lying down (Bonde et al., 2004) and they spend more time lying down compared to non-lame sows (KilBride et al., 2009). Similar results have been found in broilers where a negative correlation was seen between the time spent standing and lameness score (Berg and Sanotra, 2003). Overall, this suggests that postural analysis may be a useful tool for lameness assessment (Ringgenberg et al., 2010). In summary, although currently there is no conclusive evidence that indicates confinement as a cause of lameness, results from studies suggest that reduced mobility has the potential to cause lameness.

2.4.3 Posture and stepping behavior of sows using acceleration data

Postural behavior is often used to study the influence of housing and feeding system on sow behavior (Rhodes et al., 2005). Postures are usually measured using observations by trained observers or video observations. While these are the more common methods, they are time-consuming in both the recording and interpretation of results. The observations can also be subjective, dependent on the criteria and may lead to errors due to perception, or observer bias

(Bateson and Martin, 2007; Brown et al., 2013). In recent years, the used of accelerometers have been shown to be a promising tool for postural assessments. Apart from being a more objective method, this device is relatively cheap, effective, and provides standardized data (Ringgenberg et al., 2010).

While accelerometers have been used extensively in studies to measure postural and moving behavior in livestock species such as dairy cattle (Endres and Barberg, 2007; Ito et al., 2009; Trenel et al., 2009; de Passille et al., 2010; Nielsen et al., 2010; de Mol et al., 2011; Mattachini et al., 2013) and goats (Moreau et al., 2009), limited studies with sows are available (Cornou and Lundbye-Christensen, 2008). Pain and lameness assessments studies using acceleration data have been recorded in cattle, horses and sows (Ashley et al., 2005; Rushen et al., 2007; Leach et al., 2009; Grégoire et al., 2013). Validation studies demonstrated that these devices were reliable methods to measure posture and steps in cows and sows (Müller and Schrader, 2003; McGowan et al., 2007; Nielsen et al., 2010; Ringgenberg et al., 2010). A study by Ringgenberg et al. (2010) showed accelerometers successfully measured posture and steps in sows while a more recent study by Grégoire et al. (2013) found a possible relationship between these measures and lameness. In the same study by Grégoire et al. (2013), lame sows spent less time standing during feeding over a 24 h period. The correlation between the amount of time spent standing after feeding and time spent standing in 24 h was suggested as a possible indicator of lameness. Results were in agreement with those seen in lame dairy cattle that spent more time lying, possibly as an attempt to reduce pain (Blackie et al., 2011).

Sows spend most of their time lying and, studies have used lying time as a measure of comfort to compare different housing systems (Li and Gonyou, 2007; Tuyttens et al., 2008). These studies used either live or video observations as a measure of lying or standing behavior. Older studies (Vestergaard, 1981; Jensen, 1988) showing that sows in individual stalls spent more time lying, agrees with more recent research showing that sows spent less time standing in narrower floor spaces (Li and Gonyou, 2007; Salak-Johnson et al., 2012). The restricted space also decreased the frequency of postural changes between standing and lying (Marchant and Broom, 1996b; Anil et al., 2002). Another video-observation study demonstrated that gilts spent less time standing as gestation advanced, however, there were no differences in the percentage of time spent standing, lying or sitting due to housing, groups or stalls (Harris et al., 2006). Overall, accelerometers provide an effective and standardized method to measure posture and stepping

behavior, and are therefore a potential tool to assess the behavior of sows in different housing and feeding systems.

2.5 Summary

Among all the mineral elements, Ca and P are the most abundant found in the body, with a majority of these minerals being deposited in skeletal tissue. These minerals are continually deposited and resorbed, and their accumulation in skeletal tissue is interdependent. The dietary Ca and P requirement for sows depends on age, weight, litter size and physiological status. Gestation and lactation place significant demands on the sows to provide sufficient minerals, and especially Ca and P, for fetal skeletal mineralization during late gestation and milk production during lactation. Based on studies in human and rats, maternal Ca metabolism adapts to the increased Ca demand by the fetuses via adaptive mechanisms, which includes mobilization of Ca from skeletal reserves, intestinal Ca absorption and reduced renal Ca excretion. The extent of these adaptations however, in response to increased Ca demand during gestation and lactation is not clearly understood in sows. Additionally, sows of the modern genetics has improved litter size, however, a negative result of these larger litters is a decreased average birth weight and increased within-litter variation in birth weight. This is of concern because the variation is often associated with reduced litter growth, preweaning mortality, and variation in weaning weight. Sow nutrition, including minerals such as Ca and P, has important role in supporting the needs of both sow and fetuses, particularly with these larger litters. Incorrect maternal nutrition may lead to fetal growth retardation and subsequently poor piglet performance such as decreased piglet birth weight. As most of the work that was carried out to establish the dietary Ca and P requirements are based on older studies with less prolific sows, a re-evaluation of the Ca and P requirements for sows of modern genetics with larger litters is required.

In the wake of increasing public pressure to move from gestation stalls to group housing for sows, the revised Canadian Code of Practice for the Care and Handling of Pigs which was released in March 2014, called for the implementation of group housing for sows to occur over the next 10 yr. However, current recommendations for dietary Ca and P levels for gestating sows are primarily based on sows housed in gestation stalls. More studies are therefore required to determine if the current recommended feeding levels of these minerals are adequate for sows housed in groups, who have a potential for increased mobility. Behavior and movement of sows

can be monitored using accelerometers, which provide less subjective and standardized results than manual observation.

Currently, there are new biological tools and non-invasive techniques to monitor bone health in humans and animals. Dynamic measurements such as bone markers in serum or urine provide information on the current status of bone metabolism. On the other hand, static measurement of bone health, which includes DXA and pQCT, provides data on specific bone mineral content and density. Additionally, the pQCT has the ability to measure volumetric bone density of smaller subjects such as a newborn piglet.

3 THE EFFECTS OF DIETARY CALCIUM AND PHOSPHORUS LEVELS ON REPRODUCTIVE PERFORMANCE AND BONE TURNOVER OF SOWS HOUSED IN GROUPS OR STALLS

3.1 Introduction

Current issues related to welfare of reproducing sows, which includes gestation housing and lameness, are an important concern for the pork industry. A recent survey of a large Canadian sow barn showed that 60 % of the sows in the herd were lame on at least 1 limb (Seddon and Brown, 2014). In Ireland, based on a survey of 68 pig farms, Boyle and Lawlor (2013) reported that the prevalence of lameness was 48 % in gestating sows. Genetic improvements in sow productivity raises the issue of bone health of reproducing sows (Cromwell et al., 1970; Orth, 2003; Ryan et al., 2010).

A majority of the Ca and P in the body is located in the bone and these minerals play a major role in the growth, development and maintenance of the skeletal system (Maxson, 1985; Giesemann et al., 1998; Crenshaw, 2001; Favus et al., 2006). Authors have agreed that the Ca and P requirement for bone mineralization and strength is higher than the requirement for growth and reproductive performance (Crenshaw et al., 1981; Combs et al., 1991; Giesemann et al., 1998; NRC, 2012). As the current nutrient requirements for reproducing sows are based on older and limited research (Ball et al., 2008), the National Research Council (NRC) Nutrient Requirements of Sows 2012 concluded that sow nutrition should be a current focus for research as there was inadequate data to update the requirements (NRC 2012).

Previous studies investigating the role of Ca and P in reproducing sows yielded contradictory results. No improvement in reproductive performance or longevity was observed when sows were fed increased levels of Ca and P (Kornegay et al., 1985; Maxson and Mahan, 1986) however, other studies have shown a higher incidence of lameness in sows with low Ca and P intake (Nimmo et al., 1981; Giesemann et al., 1998). Additionally, a study by Marchant and Broom (1996a) indicated that confinement of sows in stalls reduced bone strength, perhaps increasing the risk of lameness.

Sows are commonly kept in individual stalls to control feed intake and protect them from aggression during feeding. This however, limits movement and mobility. In recent years,

consumers have been advocating for group housing (den Hartog et al., 1993; Curtis et al., 2009; Edwards, 2013). The new, revised Canadian Code of Practice for the Care and Handling of Pigs (National Farm Animal Care Council, 2014) which was released in March 2014, called for an implementation for group housing and a transition from individual crates will occur over the next 10 yr.

The older requirements were developed for stall-housed sows and we hypothesized that, in contrast to sows housed in stalls, sows housed in groups will respond to increased dietary Ca and P with an increase bone turnover. The objective of this study therefore, was to determine if the recommended levels of dietary Ca and P are adequate for sows housed in groups who have a potential for increased mobility. The specific objectives were to 1) determine the effect of dietary Ca and P level on bone turnover in gestating and lactating sows, 2) compare bone turnover of sows housed in individual stalls vs. those housed in groups, and 3) determine if there is an interaction between dietary Ca and P intake and housing on sow reproductive performance and bone metabolism.

3.2 Materials and methods

The study was carried out at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada). Sows were cared for according to the Prairie Swine Centre Inc. standard operating procedures and the experiment was approved by University of Saskatchewan's Committee on Animal Care and Supply (UCACS, protocol 20120067) for compliance with the Canadian Council on Animal Care guidelines.

3.2.1 Treatments

The experiment was designed using a randomized complete block design (RCBD) with a 3 × 2 factorial arrangement of treatments consisting of 3 levels of dietary Ca and P and 2 housing systems. A total of 180 sows were randomly assigned to 1 of the 6 treatments. Sows were blocked by breeding group to ensure batches of sows entering the gestation room from the breeding room did not confound the response. The trial started when sows were moved from

breeding to the gestation room (approximately d 28 of gestation) and ended when the piglets were weaned (average d 26 post-farrowing).

3.2.2 Animals and housing

3.2.2.1 Gestation

All sows utilized in this experiment were PIC Camborough Plus (commercial synthetic sow lines) (PIC Canada Ltd. Winnipeg, Canada). Sow reproductive history was obtained from a breeding log and identified to ensure each treatment group had a balance of parities. Each wk, 16 sows were mated with pooled semen and remained in the breeding stalls for 4 or 5 wk. Sows were moved to the gestation room every 2 wk, thus 4 or 5 wk post-breeding. Sows were housed in gestation pens at an ambient temperature (16.5 to 20°C). The gestation pens consisted of an alley (3.0 m × 10.7 m) with slatted flooring running between 2 lanes of 16 free access stalls (1.65 m × 0.67 m) on each side and a solid floor loafing area at one end (3.8 m × 7.1 m). Each stall had a single space feeder located at the front. Water was provided ad libitum through nipple drinkers located beside the feeders. A nipple drinker was also available in the loafing area.

3.2.2.2 Lactation

Sows were moved to the farrowing room 1 wk prior to anticipated farrowing. Sows were maintained in farrowing crates (overall size 1.83 m × 2.41 m) with a room temperature of 22.5°C at the start of farrowing which was gradually reduced to approximately 18.5°C prior to weaning. Cross-fostering of piglets was allowed within the first 24 h but only within dietary treatment.

3.2.3 Diets and feeding

Three dietary Ca and P levels were evaluated throughout gestation and lactation (Table 3.1). The control level for the gestation phase was based on NRC (1998) and National Swine Nutrition Guide (2010). These levels also met the requirements of NRC (2012). The control level for the lactation phase was based on the lactating sow model from NRC (2012). The other treatment diets were formulated to achieve Ca and P levels 15 % lower or higher than the

control. The ratio of Ca and P was maintained across diets within each phase. The barley and wheat-based diets were formulated to meet or exceed NRC (1998) requirements for all nutrients, with the exception of Ca and P (Table 3.2). An indigestible marker, Celite 545 (Celite Corporation, Lompoc CA, USA) was included into the diets as a source of acid insoluble ash for digestibility calculations.

Gestation feeding began when the sows were moved to the gestation room from breeding (approximately d 28 of gestation). Sows were fed 2.3 kg/d until 2 wk prior to farrowing, when this was increased to 3.0 kg/d. Sows were manually fed individually in their stalls. However, depending on housing treatment they were either i) forced out of the individual stalls to the loafing area 1 hr after feeding until feeding the following d (group) or ii) locked-in their individual stalls throughout the trial period (stalls). Sows were provided ad libitum access to their treatment diets throughout lactation except for the first 3 d; when allotments were gradually increased, starting with approximately 0.5 kg on d 1.

Table 3.1. Dietary Ca and P levels used in gestation and lactation phases

Feed intake and nutrients	Treatment 1 (-15% Ca:P)	Treatment 2 Control	Treatment 3 (+15% Ca:P)
Gestation			
Daily feed intake[1], kg/d	2.30	2.30	2.30
Ca, %	0.60	0.70	0.81
Total P, %	0.47	0.55	0.63
Lactation			
Daily feed intake[1], kg/d	6.65	6.65	6.65
Ca, %	0.61	0.71	0.82
Total P, %	0.53	0.62	0.71

[1] Daily feed intake estimation was based on National Swine Nutrition Guide 2010 for gestation and a previous sow trial conducted at Prairie Swine Centre Inc, Saskatoon, SK, Canada for lactation.

Table 3.2. Ingredient and nutrient composition of the three experimental diets used in gestation and lactation (as-fed basis)

Item	Gestation			Lactation		
	-15% Ca:P	Control	+15% Ca:P	-15% Ca:P	Control	+15% Ca:P
Ingredient, % as fed						
Barley	67.59	67.60	67.46	15.00	15.00	15.00
Wheat	21.04	20.49	20.00	60.92	60.02	59.12
Soybean meal	7.00	7.00	7.00	20.40	20.40	20.40
Canola oil	1.20	1.33	1.53	0.53	0.87	1.20
Limestone	0.97	1.13	1.30	0.97	1.07	1.13
Monocalcium P (21.1 % P)	0.87	1.10	1.37	0.77	1.23	1.73
Salt	0.60	0.60	0.60	0.60	0.60	0.60
Choline chloride	0.06	0.06	0.06	0.06	0.06	0.06
Lysine HCl	0.013	0.013	0.017	0.090	0.090	0.090
Minerals[1]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamins[2]	0.17	0.17	0.17	0.17	0.17	0.17
Celite[3]	0.40	0.40	0.40	0.40	0.40	0.40
Formulated nutrient content, % as fed						
DM, %	88.76	88.08	88.87	89.00	89.08	89.16
DE, Mcal/kg	3.19	3.19	3.18	3.35	3.35	3.35
Crude protein, %	16.0	16.0	16.0	21.0	21.0	21.0
SID Lys[4],	0.45	0.45	0.45	0.80	0.80	0.80
Ca, %	0.60	0.70	0.80	0.61	0.71	0.82
Total P, %	0.47	0.55	0.63	0.53	0.62	0.71
Available P, %	0.27	0.32	0.37	0.30	0.36	0.42

[1] Provided (per kg of diet): Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; Se, 0.10 mg as sodium selenite.

[2] Provided (per kg of diet): Vitamin A, 8250 IU; Vitamin D_3, 825 IU; Vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; Vitamin B_{12}, 25 ug.

[3] Celite 545, Celite Corporation, Lompoc CA, USA

[4] Standardized ileal digestible lysine

3.2.4 Data collection

Body weights of the sows were determined approximately 1.5 h after feeding at 3 time points; prior to entry into the gestation room (d 28 gestation), upon entry to farrowing (d 107 gestation), and prior to weaning (approximately d 26 post-farrowing). During the lactation phase, individual feed intake was recorded for the first 3 d and weekly thereafter until weaning. The numbers of piglets born alive, stillbirths or mummies were recorded at birth. Individual piglet weights were also obtained at birth and 1 d prior to weaning.

3.2.4.1 Behavioural data

Skin lesion scores, video recordings and accelerometer data were collected to aid the interpretation of effects of housing on aggression or movement. Lesion scores were obtained during wk 1 of the experiment (initial entry into the gestation room) and wk 5, by the same individual. Sows were scored 0 to 3 according to severity of the lesion. The number of scratches on all regions of the body was counted and scored as: 0 (no scratches), 1 (less than 5 scratches), 2 (5 to 10 scratches) and 3 (more than 10 scratches) (Brown et al., 2009). Only fresh scratches such as red scratches from vascular exposure or inflammation during healing were recorded (Brown et al., 2009). Each group of sows was video recorded using 3 to 5 video cameras per pen placed at different angles for a maximum of 13 h on wk 1 and wk 5 of the experiment. Sows were marked with spray paint to differentiate the treatment groups. One individual observed all videos and scan sampling was done every 5 min. Data was recorded and frequency of posture; sitting, standing and lying recorded. The group housing space was divided into different zones to prevent repeated observations on the same sow This was done by first identifying the overlapping section of the pen in the video recordings and then using an obvious landmark in the pen such as the columns on floor grates to separate the observations in the videos.

On wk 5, a 1-time behavioural measurement was recorded by using accelerometers (HOBO Pendant G Acceleration Data Logger, Onset Computer Corporation, Pocasset, MA, USA) which were placed on the right hind limb of a subset of sows for recording posture (9 sows per treatment for a 24 h period) or for the evaluation of stepping (8 sows per treatment for 1 h period) (Fig. 3.1). The standing posture and steps procedure, set-up and analyses were done

according to previously described studies (Ringgenberg et al., 2010; Grégoire et al., 2013). Programming and data conversion to degrees of tilt were done using HOBOware Pro software (Ringgenberg et al., 2010). Macros were used to convert acceleration data to detect steps and standing posture. The hobo device was placed inside a pouch attached with Velcro straps (Velcro USA Inc, Manchester, US) and wrapped with Vetrap (3M Vetrap, 3M Corporate Headquarters, St. Paul, MN, US). For data on posture (to complement video observations), the device recorded the acceleration on the x-axis (interval of 5 s) for 24 h. The device was placed with the x-axis parallel to the limb, oriented down. For data on stepping, the device recorded the acceleration only on the x-axis (10 Hz = 0.1 s), for 1 h during feeding time to measure the number of hind limb steps. Data loggers were programmed to sample acceleration signals 10 times/s for 1 h. When the x-axis was equal to or more than 0.59 *g* (where *g* is the static acceleration of gravity, providing information on the angle the device is tilted at with respect to earth), a sow was recorded as standing; otherwise sow posture was classified as other. A step was considered underway if the x-axis acceleration was below 0.6 *g* or above 1.4 *g*.

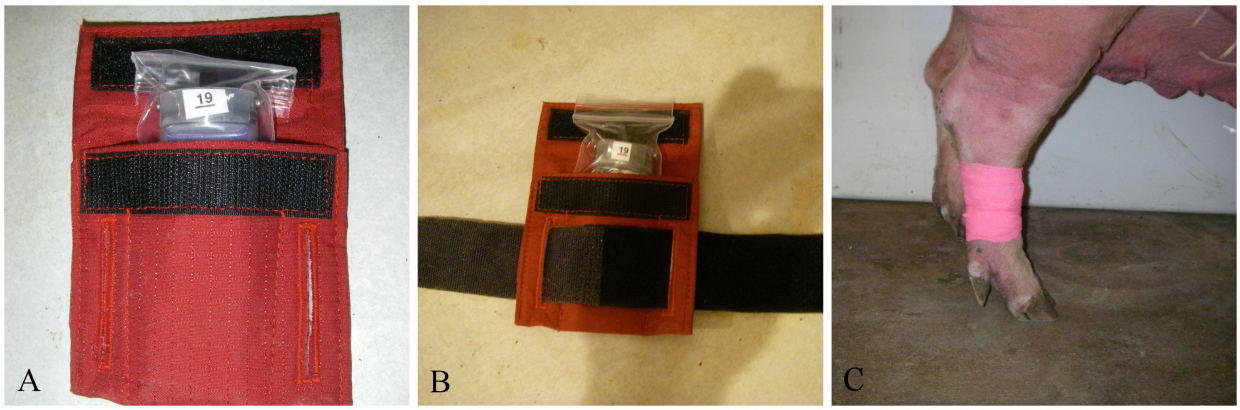


Figure 3.1. Accelerometer for the evaluation of posture and steps in sows. (A) Accelerometer secured in a pouch, (B) Velcro strap attached to pouch, (C) Pouch secured with Vetrap and placed on hind limb of sows.

3.2.4.2 Sample collection

Feed samples for each dietary treatment were collected from the 3 batches of feed produced, analyzed and an average was reported. Fecal samples were collected by grab sampling directly from a subset of sows (10 sows per treatment) on d 60 of gestation and d 14 of lactation for 3 consecutive days. Fecal samples were then pooled, and a subset sample was taken for fecal Ca and P analysis. Blood and milk samples were collected at a few time points as previously outlined by Maxson et al., 1986 and Giesemann et al., 1998. Approximately 8 mL of blood was collected via cranial vena cava venipuncture on gestation d 28 (entry to gestation room), gestation d 100 (2 wk prior to farrowing), lactation d 14 ± 2 (mid-lactation), and lactation d 26 ± 2 (prior to weaning). Blood sampling was done in the morning approximately 1 h after feeding (postprandial) using 10-mL blood collection tubes (red top, no additives, BD Vacutainer; Becton, Dickinson and Company, New Jersey, US) and 18-gauge × 38-mm needles (BD PrecisionGlide; Becton, Dickinson and Company, New Jersey, US). Samples were centrifuged at 830 × *g* for 15 min (Beckman TJ-6 Centrifuge, Beckman Coulter, Mississauga, Ontario, Canada) and serum transferred to microcentrifuge tubes. A total of 20 mL of milk per time period was collected from all functional teats on lactation d 12 ± 2 (mid-lactation) and d 25 ± 2 (prior to weaning). Sows were injected with 20 to 30 IU of oxytocin (Oxyto-Sure 20 IU/mL, 1 mL; Vetoquinol, Lavaltrie, Quebec, Canada) intravulvally to stimulate milk letdown. All samples were stored frozen at -20˚C until analysis.

3.2.5 Sample analysis

3.2.5.1 Chemical analysis

All laboratory analyses were conducted at the Department of Animal and Poultry Science, University of Saskatchewan, unless otherwise stated. All samples were analyzed in duplicates. Feed and fecal samples were freeze-dried with shelf and condenser temperatures of 15°C and -50°C respectively (VirTis Genesis 25, SP Industries, Gardiner, NY, US) and then ground through a 1 mm mesh screen (Retsch Mill ZM1, Newtown, PA) prior to analysis. Feed and fecal samples were analyzed for DM, ash, and AIA. GE was determined by bomb calorimetry (1281 Oxygen Bomb Calorimeter, Parr Instrument Company, Moline, Illinois, US).

Feed and fecal samples were also analyzed for Ca and P using a modification of AOAC 968.08 and 935.13A method (Central Testing Laboratory Ltd., Manitoba, Canada). A pooled sample for each dietary treatment was analyzed for CP using AOAC 990.03 method (Central Testing Laboratory Ltd., Manitoba, Canada). Water samples were collected from the nipple drinkers provided to the sows for Ca analysis (ALS Canada Ltd., Saskatoon, SK, Canada). Serum samples were analyzed for Ca and P using a COBAS Chemistry Analyzer (Prairie Diagnostic Services Inc., Saskatoon, Canada). Serum samples were also analyzed for markers of bone formation, serum osteocalcin (OC, intra-assay CV, 8.8 %; interassay CV, 9.4 %) and bone resorption, serum pyridinoline (PYD, intra-assay CV, 7.4 %; interassay CV, 9.8 %; MicroVue; Quidel Corporation, San Diego, CA) (Shaw et al., 2006a). Milk samples were dried at 80°C for 24 h. After the dry weight was obtained, samples were ashed at 550°C for 24 h and ash weight was recorded. The ash from each sample was solubilized in 5 mL of 2 *N* HCL for 16 to 24 h. Ashed samples were analyzed for Ca using atomic absorption spectrophotometry (Thermo Scientific iCE 3000 Series) (AOAC 1990; method 968.08; Lauridsen et al., 2010) and P using colorimetry (ThermoElectron Helios Delta Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, US) (AOAC 1990; method 965.17).

3.2.5.2 Calculations

Apparent total tract digestibility values for energy, Ca and P were calculated using the acid-insoluble ash as an indigestible marker, with the following equation:

$$AD = \{1 - [(IA_d/IA_f)/(N_d/N_f)]\} \times 100 \quad (3.1)$$

where AD is the apparent digestibility, IA_d and IA_f are the insoluble ash levels in the diet and feces respectively, and N_d and N_f are the nutrient levels (energy, Ca and P) in the diets and feces (Adeola, 2001).

Milk output was estimated based on the equation:

$$\text{Milk output, g/piglet}\cdot d^{-1} = 2.50 \times ADG + 80.2 \times BWi + 7 \quad (3.2)$$

where ADG is the average daily gain of piglets nursed from birth to weaning and BWi is the average birth weight of piglets per litter (Noblet and Etienne, 1989).

3.2.6 Statistical analysis

The experiment was designed as a randomized complete block with breeding group as the block and sow as the experimental unit. Treatments were arranged as a 3 × 2 factorial with 3 dietary Ca and P levels and 2 housing systems The effects of dietary Ca and P and housing were analyzed by analysis of variance (ANOVA) using the MIXED procedure of SAS 9.3 (2011 SAS Inst. Inc., Cary, NC) with dietary Ca and P and housing as fixed effects and block (8 gestation groups) as a random effect. The statistical model for all analysis included the main effects of dietary Ca and P levels, housing, block and the interaction between dietary treatments and housing. All data were checked for normality using the Proc UNIVARIATE procedure of SAS. Non-normally distributed data (Shapiro-Wilk test; P-value < 0.05) were analyzed using the Proc GLIMMIX procedure of SAS. Each ANOVA analysis that was significantly different was followed by a multiple comparison procedure using the Tukey-Kramer method. Main effects only are presented unless significant interactions were found. Variables with values of more than one time point were also analyzed as deviations from the initial value at the start of the trial. Parity was included in a second model as the third factor with 2 parity groups; gilts and parity 1, and above parity 1. The effects of dietary Ca and P, housing and parity were analyzed by analysis of variance (ANOVA) using the MIXED procedure of SAS. There were no three-way interactions; hence only the main effects are reported. As diet, housing and diet by housing interaction effects were similar to the first model; results are reported based on the first model.

A repeated measures analysis (MIXED procedure of SAS) was used to determine the effects of dietary treatments, housing and parity over time on serum Ca and P and bone markers. Data were analyzed by using the best-fit covariance structure model assessed by model-fit criteria, Akaike Information Criterion (AIC) within SAS. Means that were significantly different were followed by a multiple comparison procedure using the Tukey-Kramer method.

The Pearson correlations between selected variables (serum and milk Ca and P, bone markers and milk output) and dietary Ca and P intake were estimated using the CORR function of SAS. The behavioural data (lesions and posture) was tested by Fisher's exact test using Proc FREQ for proportions. The majority of the accelerometer data had a skewed distribution; hence, depending on the skewness, a square root or log transformation was applied. Transformed data that failed to normalize were analyzed using the non-parametric Kruskal-Wallis test. As results

were comparable with those from parametric tests, results from the GLIMMIX procedure were reported.

A *P*-value of ≤ 0.05 was considered significant and a *P*-value of > 0.05 but ≤ 0.10 was considered a trend. Treatment means were analyzed using the LSMEANS statement with all values reported as LSMEANS ± standard error mean (SEM).

3.3 Results

3.3.1 General

A total of 18 sows were removed during the course of the trial; 7 during gestation and 11 during lactation. Fisher's exact test showed that the number of sows removed differed by parity and the reasons for removal ($P < 0.01$; Fig. 3.2). Most of the young sows (gilts and parity 1) removed during early lactation were because of apparent poor milking ability. Older sows (above parity 1) were removed mainly due to sudden hind limb lameness and poor body condition score. There was no effect of dietary treatment or housing on the number of sows removed in the different categories ($P > 0.10$).

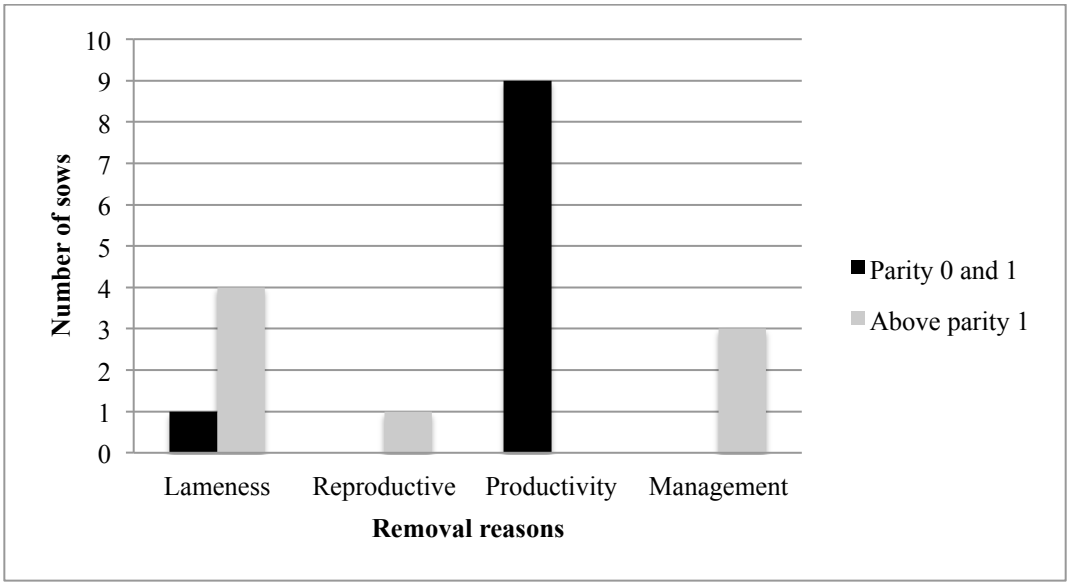


Figure 3.2. Reasons for sow removal by parity. Data presented as number of sows removed from the total 180 sows on trial. *P*-value obtained by Fisher's exact test. The proportion of removed sows by removal reasons and parity of sows was different ($P < 0.01$). Young sows were removed mainly due to poor milking ability (productivity) whereas older sows were removed due to lameness and poor body condition score (management).

3.3.2 Apparent digestibility of nutrients

Analysis of Ca and P % in the gestation and lactation diets is presented in Table 3.3. Except for the gestation diet with high Ca (+15 %), the analyzed Ca and P % of all diets was between 0 to 0.08 % units higher than formulated values. The Ca % of gestation diet (+15 %) was 0.05 % units lower than formulated. However, the 15 % variance between the treatments and the ratio of Ca and P were maintained. The apparent total tract digestibility of energy, DM, Ca and P calculated (Eq. 3.1) are shown in Table 3.4. There were no effects of diet or housing on the AD of these nutrients during gestation ($P > 0.10$). The AD of energy ($P = 0.04$) and dry matter ($P < 0.01$) decreased linearly with increasing levels of dietary Ca and P during lactation. Treatment had no effect on the apparent digestibility of Ca and P during lactation ($P > 0.10$).

Table 3.3. Formulated and analyzed chemical composition of experimental diets used in the gestation and lactation phases (% as fed)[1]

Nutrient	Gestation			Lactation		
	-15%	Control	+15%	-15%	Control	+15%
Formulated						
Ca, %	0.60	0.70	0.81	0.61	0.71	0.82
Total P, %	0.47	0.55	0.63	0.53	0.62	0.71
Analyzed						
Ca, %	0.67	0.70	0.76	0.64	0.78	0.89
Total P, %	0.54	0.59	0.63	0.58	0.67	0.79
Moisture, %	11.8	11.8	11.7	12.0	11.9	11.9
CP, %	13.5	13.2	13.1	19.7	19.8	19.9
GE[2], Mcal/kg	3.8	3.8	3.8	3.8	3.8	3.8

[1] Feed samples collected from 3 batches of feed produced for both gestation and lactation. Each batch of was analyzed for Ca and P for each dietary treatment and a pooled sample was used to analyze CP (Central Testing Laboratory Ltd., Manitoba, Canada).
[2] GE (% of DM) of each diet was analyzed at the Department of Animal and Poultry Science, University of Saskatchewan.

Table 3.4. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) and housing on apparent digestibility (%) of nutrients

Nutrient	Diet			SEM	Housing		SEM	*P*-value		
	-15%	Control	+15%		Stall	Group		Diet	House	Diet × House
Gestation										
Energy	85.0	83.9	84.9	0.37	84.8	84.4	0.30	0.08	0.34	0.70
Ca	19.4	14.9	17.4	3.14	15.1	19.3	2.56	0.59	0.25	0.57
P	27.5	25.6	24.3	2.89	24.8	26.8	2.36	0.73	0.55	0.55
DM	84.6	83.4	84.0	0.36	84.1	83.9	0.29	0.05	0.64	0.68
Lactation										
Energy	88.0	88.6	86.9	0.47	87.7	88.0	0.39	0.04	0.51	0.09
Ca	14.6	28.8	20.7	4.75	17.2	25.5	3.92	0.16	0.15	0.81
P	26.7	37.7	37.3	4.96	30.3	37.5	4.10	0.18	0.23	0.97
DM	87.4	87.9	85.7	0.45	86.8	87.2	0.37	<0.01	0.52	0.16

3.3.3 Sow body weight and feed intake

The effects of dietary Ca and P and housing on sow BW and ADFI during lactation are shown in Table 3.5. An effect of housing was seen on sow BW at d 107 of gestation ($P = 0.01$) with sows housed in groups recording higher average BW compared to sows housed in stalls. The change in BW, however, from d 28 of gestation (start of trial) to d 107 of gestation was similar among the treatment groups ($P > 0.10$). No effect of dietary Ca and P or housing was observed on the ADFI of sows during lactation ($P > 0.10$).

Differences in sow BW were seen when parity was included as a factor with higher BW recorded for older sows ($P < 0.01$; Table 3.6). Younger sows however, gained more weight from the start of the trial to day 107 of gestation ($P < 0.01$) and from trial start to weaning ($P = 0.04$). Older sows consumed 1 kg/d more feed during lactation ($P < 0.01$). The change in BW of younger sows from the start of the trial to d 107 of gestation ($P < 0.01$) and weaning ($P < 0.05$) however was larger than older sows.

Table 3.5. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) and housing on sow body weight during gestation and lactation and lactation feed intake[1]

Item	Diet			SEM	Housing		SEM	*P*-value		
	-15%	Control	+15%		Stall	Group		Diet	House	Diet × House
Sows, *n*	51	54	57	-	81	81	-	-	-	-
Sow BW, kg										
d 28[2]	231.7	234.2	234.9	5.3	229.6	237.6	4.5	0.88	0.15	0.72
d 107[2]	257.9	265.7	259.9	3.9	255.5	266.9	3.2	0.32	0.01	0.95
Weaning[3]	240.2	249.8	239.2	5.0	239.5	246.7	4.1	0.25	0.21	0.79
Change, d 28 to d 107[4]	26.7	33.1	28.4	3.8	27.3	31.5	3.6	0.12	0.12	0.55
Change, d 28 to weaning[4]	9.2	15.1	9.1	3.1	11.5	10.8	2.8	0.17	0.83	0.65
ADFI[5], kg	7.0	6.8	6.9	0.2	6.9	6.9	0.2	0.53	0.69	0.24

[1] A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments. Sows were blocked by breeding group. A total of 18 sows were removed during the course of the trial.
[2] Gestation d 28 (start of trial) and d 107.
[3] Lactation d 26 ± 2 d.
[4] Difference between values at weaning and gestation d 28 (start of trial).
[5] Average daily feed intake for total number of lactation days.

Table 3.6. Effect of parity on sow body weight changes during gestation and lactation and lactation feed intake[1]

Item	Gilts & parity 1	Above parity 1	SEM	*P*-value
				Parity
Sows, *n*	80	82	-	-
Sow BW, kg				
d 28[2]	201.0	261.3	2.4	< 0.01
d 107[2]	241.4	280.1	2.5	< 0.01
Wean[3]	217.9	266.9	3.1	< 0.01
Change, d 28 to d 107[4]	38.7	20.3	3.1	< 0.01
Change, d 28 to weaning[4]	14.1	8.0	2.7	0.04
ADFI[5], kg	6.4	7.4	0.1	< 0.01

[1] A total of 180 sows were used; 18 sows were removed during the course of the trial.
[2] Gestation d 28 (start of trial) and d 107.
[3] Lactation d 26 ± 2 d.
[4] Difference between values at gestation d 107 or weaning and gestation d 28 (start of trial).
[5] ADFI for entire lactation period. Sows were provided ad libitum access to their treatment diets throughout lactation except for the first 3 d; when allotments were gradually increased, starting with 0.5 kg on d 1.

3.3.4 Sow reproductive performance

There were no effects of dietary Ca and P or housing observed for litter size, piglet weaning weight or ADG ($P > 0.10$; Table 3.7). Group-housed sows produced 1 more live born piglet than sows housed in stalls ($P = 0.03$) and these piglets were heavier ($P = 0.04$). Overall, there was no interaction of diet and housing observed for any sow reproductive parameters ($P > 0.10$). The number of piglets born alive, litter size and average birth weight was similar among sows of different parities ($P > 0.10$; Table 3.8). Older sows however, produced piglets with higher ADG and higher average weaning weight ($P = 0.01$).

Table 3.7. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) and housing on sow reproductive performance[1]

	Diet			SEM	Housing		SEM	*P*-value		
	-15%	Control	+15%		Stall	Group		Diet	House	Diet × House
Sows, *n*	51	54	57	-	81	81	-	-	-	-
Liveborn[2], *n*	14.2	14.3	14.7	0.43	13.9	14.9	0.36	0.60	0.03	0.54
Litter[3], *n*	15.2	15.6	16.1	0.55	15.2	16.1	0.38	0.38	0.07	0.46
Birth wt[4], kg	1.56	1.51	1.49	0.03	1.49	1.55	0.03	0.26	0.04	0.87
Weaning wt[5], kg	6.67	6.58	6.72	0.14	6.59	6.73	0.12	0.76	0.39	0.86
ADG[6], kg/piglet·d^{-1}	0.23	0.23	0.23	0.01	0.23	0.23	0.01	0.99	0.41	0.21

[1] A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments. Sows were blocked by breeding group. A total of 18 sows were removed during the course of the trial.
[2] Number of piglets born alive.
[3] Total number of piglets born / litter (Live + stillborn + mummies).
[4] Piglet average birth weight.
[5] Piglet average weaning weight (average d 26 lactation).
[6] Average daily gain during lactation.

Table 3.8. The effect of parity on sow reproductive performance[1]

Item	Gilts & parity 1	Above parity 1	SEM	*P*-value
				Parity
Sows, *n*	80	82	-	-
Liveborn[2], *n*	14.4	14.5	0.36	0.74
Litter[3], *n*	15.6	15.7	0.38	0.83
Birth wt[4], kg	1.52	1.52	0.03	0.98
Weaning wt[5], kg	6.45	6.87	0.12	0.01
ADG[6], kg/piglet·d^{-1}	0.22	0.24	0.01	< 0.01

[1] A total of 180 sows were used; 18 sows were removed during the course of the trial.
[2] Number of piglets born alive.
[3] Total number of piglets born / litter (Live + stillborn + mummies).
[4] Piglet average birth weight.
[5] Piglet average weaning weight (average d 26 lactation).
[6] Average daily gain for the total number of farrowing days.

3.3.5 Sow serum constituents: Total Ca and P and bone markers in sow serum

There were no dietary or housing effects on serum Ca concentration postprandially at d 28 of gestation (start of trial), at mid-lactation or weaning ($P > 0.10$). A diet by housing interaction was observed for serum total Ca concentration at d 100 of gestation ($P = 0.02$; Table 3.9). The lowest serum total Ca concentration was seen in group-housed sows that received the low Ca diet (Fig. 3.3). The initial levels of serum P were lower for the group housed treatment group ($P < 0.10$), and therefore values for d 100 gestation, mid-lactation and weaning were also analyzed as deviations from d 28 of gestation. A diet by housing interaction was observed for the change of serum P concentration from d 28 to d 100 gestation ($P = 0.04$). Sows housed in stalls fed the high level of dietary Ca and P had the smallest change in serum P levels while the biggest decline in serum P levels were group-housed sows that were fed low dietary Ca and P (Fig. 3.4).

Postprandial Serum total Ca and P concentration were different between the younger sows (gilts and parity 1) and older sows (above parity 1) at the start of the trial ($P < 0.01$; Table 3.10) and on d 100 gestation ($P < 0.01$). The reduction in serum total Ca and P concentration within the 72-d period during gestation did not differ between the parities ($P > 0.10$). Changes in serum total Ca concentration from the initial value at d 28 gestation to mid-lactation and weaning were similar between parities ($P > 0.10$) but a higher reduction in serum P was observed in younger sows compared to older sows within these 2 periods ($P < 0.01$).

Repeated measures were used to determine the effect of parity over time on the serum constituents. Younger sows generally had higher serum total Ca and P concentrations at all time points ($P < 0.01$; Table 3.11). Serum concentrations of both minerals however, were reduced by d 100, regardless of parity ($P < 0.01$). Serum total Ca concentration increased by mid-lactation and dropped slightly at weaning in the older, but not the younger, sows (parity × time; $P < 0.01$; Fig. 3.7 A.). A different trend was observed for serum P concentrations. In the young sows, serum P levels decreased at mid-lactation compared to late gestation but increased by weaning, whereas serum P levels in older sows increased during the initial stage of lactation and dropped slightly by the time the piglets were weaned (parity × time; $P < 0.01$; Fig. 3.7 B.).

Table 3.9. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) and housing on postprandial response of total Ca and P in sow serum[1]

Item	Diet			SEM	Housing		SEM	*P*-value		
	-15%	Control	+15%		Stall	Group		Diet	House	Diet × House
Sows, *n*	51	54	57	-	81	81	-	-	-	-
Serum Ca										
d 28[2]	2.43	2.43	2.42	0.04	2.45	2.41	0.04	0.88	0.11	0.68
d 100[2]	2.26	2.29	2.34	0.03	2.32	2.27	0.03	0.05	0.06	0.02[3]
Mid-lactation[4]	2.45	2.44	2.47	0.02	2.45	2.45	0.22	0.34	0.62	0.58
Weaning[5]	2.41	2.39	2.40	0.03	2.41	2.39	0.02	0.72	0.36	0.70
Change, d 28 to d 100[6]	-0.18	-0.14	-0.09	0.05	-0.13	-0.14	0.05	0.05	0.77	0.34
Change, d 28 to mid-lactation[6]	0.01	-0.01	0.03	0.05	0.00	0.02	0.05	0.54	0.36	0.60
Change, d 28 to weaning[6]	-0.03	-0.05	-0.04	0.05	-0.05	-0.03	0.04	0.88	0.40	0.50
Serum P										
d 28[2]	2.21	2.20	2.18	0.05	2.24	2.16	0.05	0.65	0.03	0.39
d 100[2]	2.10	2.14	2.16	0.03	2.17	2.10	0.03	0.32	0.02	0.38
Mid-lactation[4]	2.09	2.15	2.18	0.05	2.15	2.13	0.05	0.21	0.65	0.80
Weaning[5]	2.13	2.16	2.19	0.04	2.19	2.13	0.04	0.48	0.16	0.21
Change, d 28 to d 100[6]	-0.13	-0.08	-0.03	0.05	-0.08	-0.08	0.05	0.03	0.92	0.04[3]
Change, d 28 to mid-lactation[6]	-0.15	-0.05	-0.02	0.07	-0.10	-0.05	0.07	0.09	0.24	0.96
Change, d 28 to weaning[6]	-0.12	-0.06	-0.01	0.06	-0.08	-0.05	0.05	0.17	0.51	0.11

[1] A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments. Sows were blocked by breeding group. A total of 18 sows were removed during the course of the trial.
[2] Gestation d 28 (start of trial) and d 100.
[3] Significant diet × housing interactions presented in Fig. 3.2 and 3.3 ($P < 0.05$).
[4] Lactation d 14 ± 2 d.
[5] Lactation d 26 ± 2 d.
[6] Difference between values at gestation d 100 or mid-lactation or weaning and gestation d 28 (start of trial).

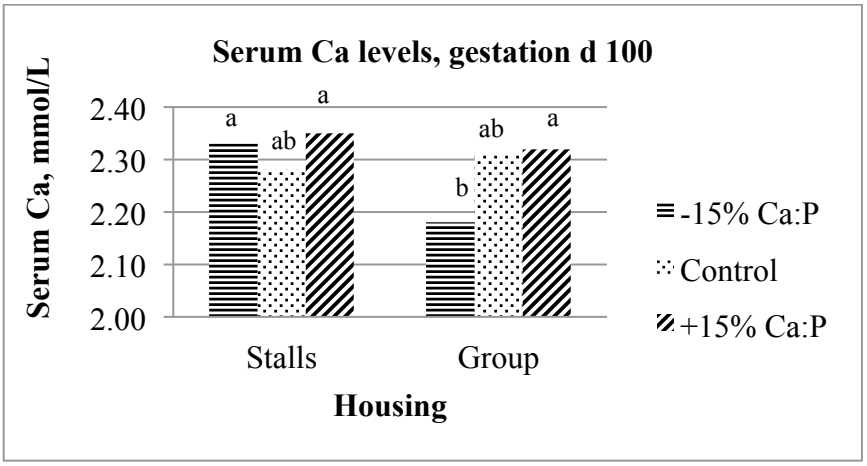


Figure 3.3. The interaction of diet and housing on sow serum total Ca (postprandial) at d 100 of gestation (diet × housing $P = 0.02$; SEM = 0.04). Means with the same letter are not significantly different ($P > 0.10$). A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments; 18 sows were removed during the course of the trial (stalls, $n = 81$; group, $n = 81$).

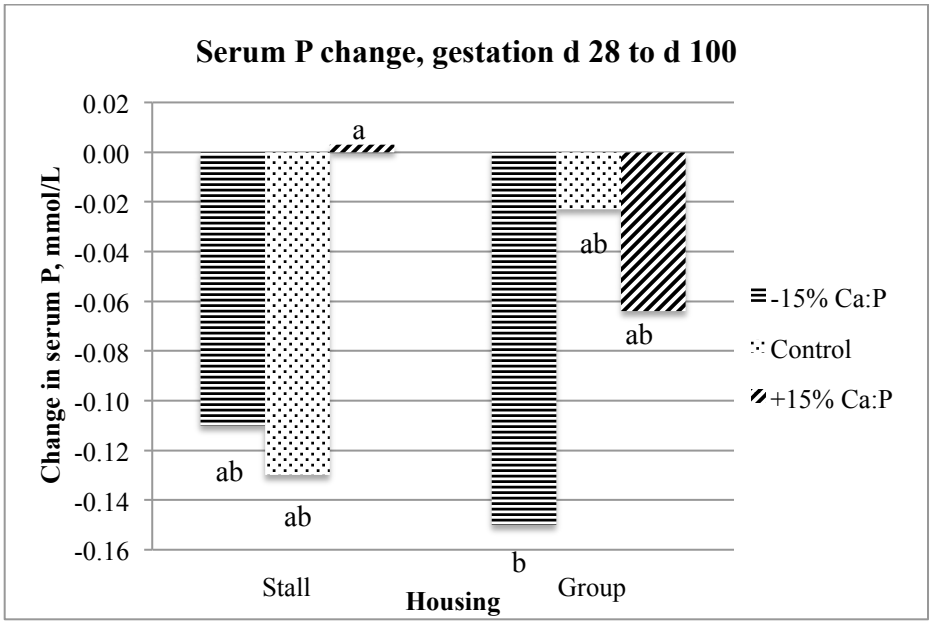


Figure 3.4. The interaction of diet and housing on the change in sow serum P (postprandial) from d 28 to d 100 of gestation; Diet × housing $P = 0.04$; SEM = 0.06. Means with same letter are not significantly different ($P > 0.10$). A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments; 18 sows were removed during the course of the trial (stalls, $n = 81$; group, $n = 81$).

Table 3.10. The effect of parity on postprandial response of total Ca and P (mmol/L) in sow serum[1]

Item	Gilts & parity 1	Above parity 1	SEM	*P*-value Parity
Sows, *n*	80	82	-	-
Serum Ca				
d 28[2]	2.49	2.37	0.04	< 0.01
d 100[2]	2.33	2.26	0.03	< 0.01
Mid-lactation[3]	2.47	2.43	0.02	0.02
Weaning[4]	2.44	2.36	0.02	< 0.01
Change, d 28 to d 100[5]	-0.16	-0.12	0.05	0.28
Change, d 28 to mid-lactation[5]	-0.02	0.04	0.05	0.07
Change, d 28 to weaning[5]	-0.05	-0.03	0.04	0.55
Serum P				
d 28[2]	2.37	2.05	0.03	< 0.01
d 100[2]	2.26	2.01	0.03	< 0.01
Mid-lactation[3]	2.17	2.12	0.05	0.21
Weaning[4]	2.23	2.09	0.04	< 0.01
Change, d 28 to d 100[5]	-0.10	-0.06	0.05	0.20
Change, d 28 to mid-lactation[5]	-0.19	0.03	0.06	< 0.01
Change, d 28 to weaning[5]	-0.14	0.01	0.05	< 0.01

[1] A total of 180 sows were used; 18 sows were removed during the course of the trial.
[2] Gestation d 28 (start of trial) and d 100.
[3] Lactation d 14 ± 2 d.
[4] Lactation d 26 ± 2 d.
[5] Difference between values at gestation d 100 or mid-lactation or weaning and gestation d 28 (start of trial).

There were no effects of dietary Ca and P or housing on the bone formation marker, serum osteocalcin (OC), or serum pyridinoline (PYD), a marker of bone resorption, regardless of sampling time ($P > 0.10$). However, there was an effect of time on both markers ($P < 0.01$; Fig. 3.5). Serum OC was reduced slightly at d 100 of gestation (Fig. 3.5 A. and B.). A further decline was seen during mid-lactation before rising again at weaning but with a level lower than the initial value. A similar trend was seen for serum PYD during gestation but the values increased to above 8.0 mmol/L during mid-lactation and were reduced at weaning to a level that was comparable to the initial value on d 28 of gestation (Fig. 3.5 C. and D.). When parity was included as a factor, a parity by time interaction was observed ($P < 0.05$; Table 3.11). Generally, the younger sows had higher values of serum OC compared to the older sows at all time points (Fig. 3.6 A.). Multiple comparison procedure (Tukey-Kramer method) showed that the serum OC levels were similar at the start of the experiment (d 28 gestation) and d 100 gestation ($P > 0.05$) for all sows before decreasing at mid-lactation ($P < 0.05$). The level increased just prior to weaning in the older sows, with the level similar to the start of the experiment. In the younger sows, however, the serum OC level at prior to weaning did not return to the initial levels at d 28 gestation ($P < 0.05$). The serum PYD levels in both young and old sows were similar at most time points ($P > 0.05$; Fig. 3.6 B.) except for the levels during mid-lactation. The levels reached its peak during this time ($P < 0.05$).

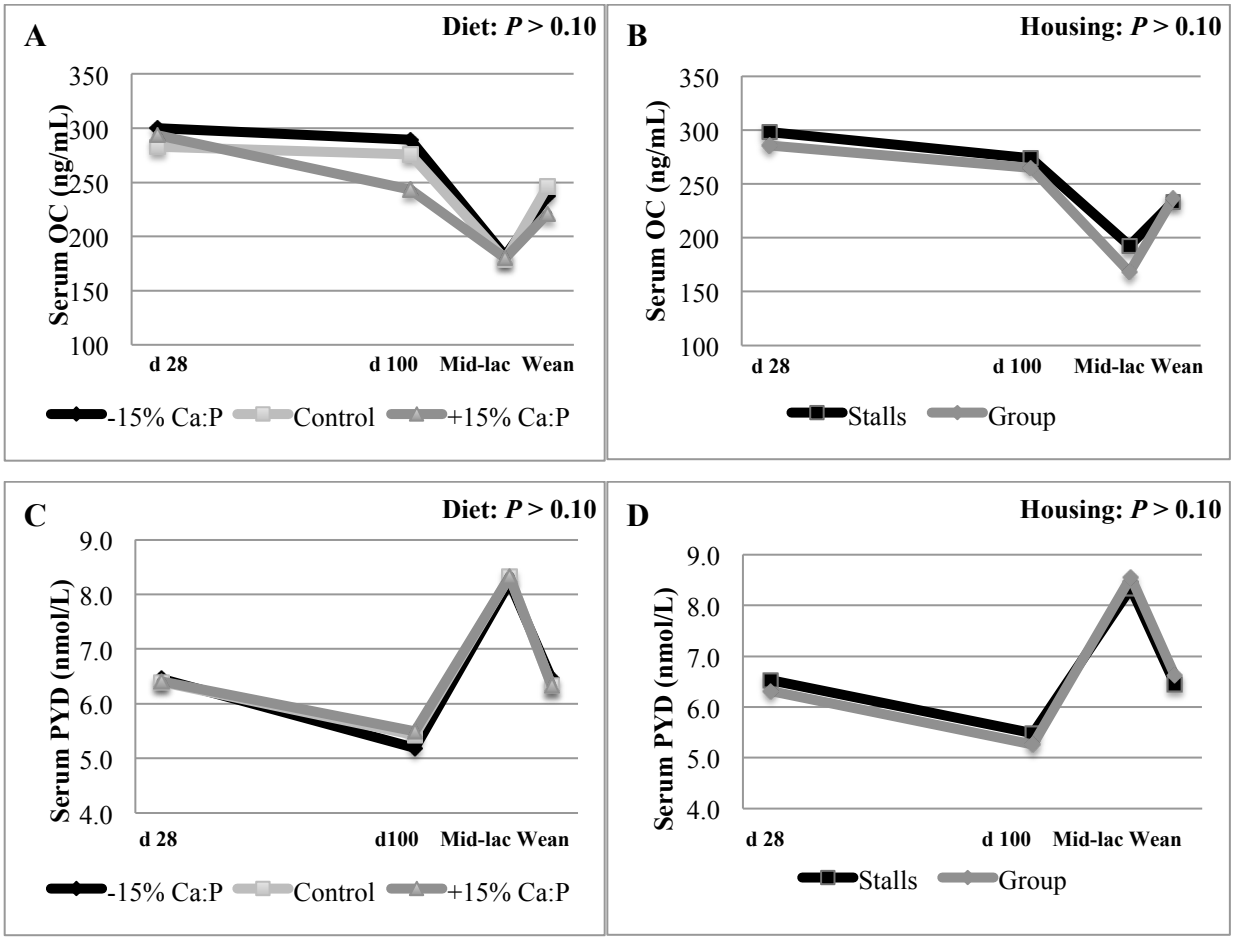


Figure 3.5. Main effects of diet and housing on serum osteocalcin (OC) (A and B) and serum pyridinoline (PYD) (C and D). Data were analyzed using repeated measures ANOVA. Diet × housing × time interactions; $P > 0.10$; (SEM A = 19.77; B = 13.69; C = 0.28; D = 0.24). A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments; 18 sows were removed during the course of the trial.

Table 3.11. Parity by time interaction for serum Ca and P (postprandial) and bone marker concentration in sows[1]

Serum constituents	Gilts & parity 1				Above parity 1				SEM	*P*-value
	d 28[2]	d 100[2]	Mid-Lac[3]	Wean[4]	d 28[2]	d 100[2]	Mid-Lac[3]	Wean[4]		Parity × time[5]
Sows, *n*	80	80	80	80	82	82	82	82	-	-
Osteocalcin, ng/mL	362.6^{a}	338.9^{a}	212.7^{b}	277.1^{c}	221.5^{bc}	199.9^{b}	147.8^{d}	192.7^{b}	13.7	< 0.01
Pyridinoline, nmol/L	6.04^{ab}	5.39^{a}	8.59^{c}	6.49^{b}	6.79^{b}	5.34^{a}	8.31^{c}	6.57^{b}	0.24	0.04
Ca, mmol/L	2.49^{a}	2.32^{bc}	2.47^{a}	2.44^{a}	2.36^{b}	2.27^{c}	2.43^{a}	2.36^{b}	0.02	< 0.01
P, mmol/L	2.39^{a}	2.26^{b}	2.17^{bcd}	2.23^{bc}	2.04^{ef}	2.01^{f}	2.12^{cde}	2.08^{def}	0.02	< 0.01

[a-f] Means within the same row with the same letter are not significantly different ($P > 0.05$).
[1] A total of 180 sows were used; 18 sows were removed during the course of the trial.
[2] Gestation d 28 and d 100.
[3] Lactation d 14 ± 2.
[4] Lactation d 26 ± 2.
[5] Data analyzed using repeated measures ANOVA. Significant parity × time interactions presented in Fig. 3.6 and 3.7 ($P < 0.05$).

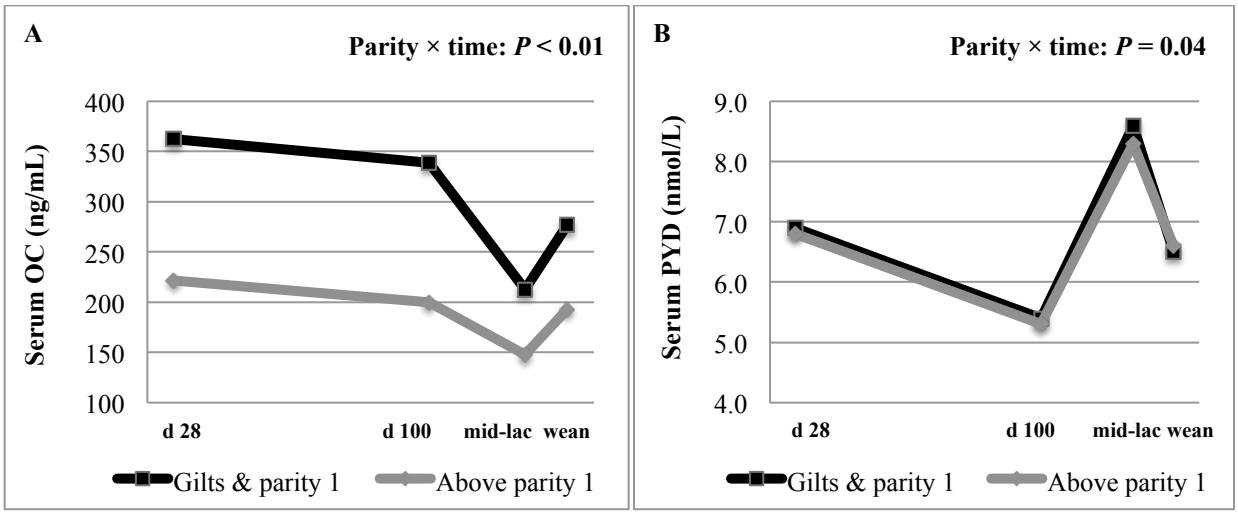


Figure 3.6. The effects of parity and time on serum osteocalcin (OC) (A; Parity × time interactions; $P < 0.01$; SEM = 13.7) and serum pyridinoline (PYD) (B; Parity × time interactions; $P = 0.04$; SEM = 0.24). Data were analyzed using repeated measures ANOVA. A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments; 18 sows were removed during the course of the trial (gilts and parity 1, $n = 80$; above parity 1, $n = 82$).

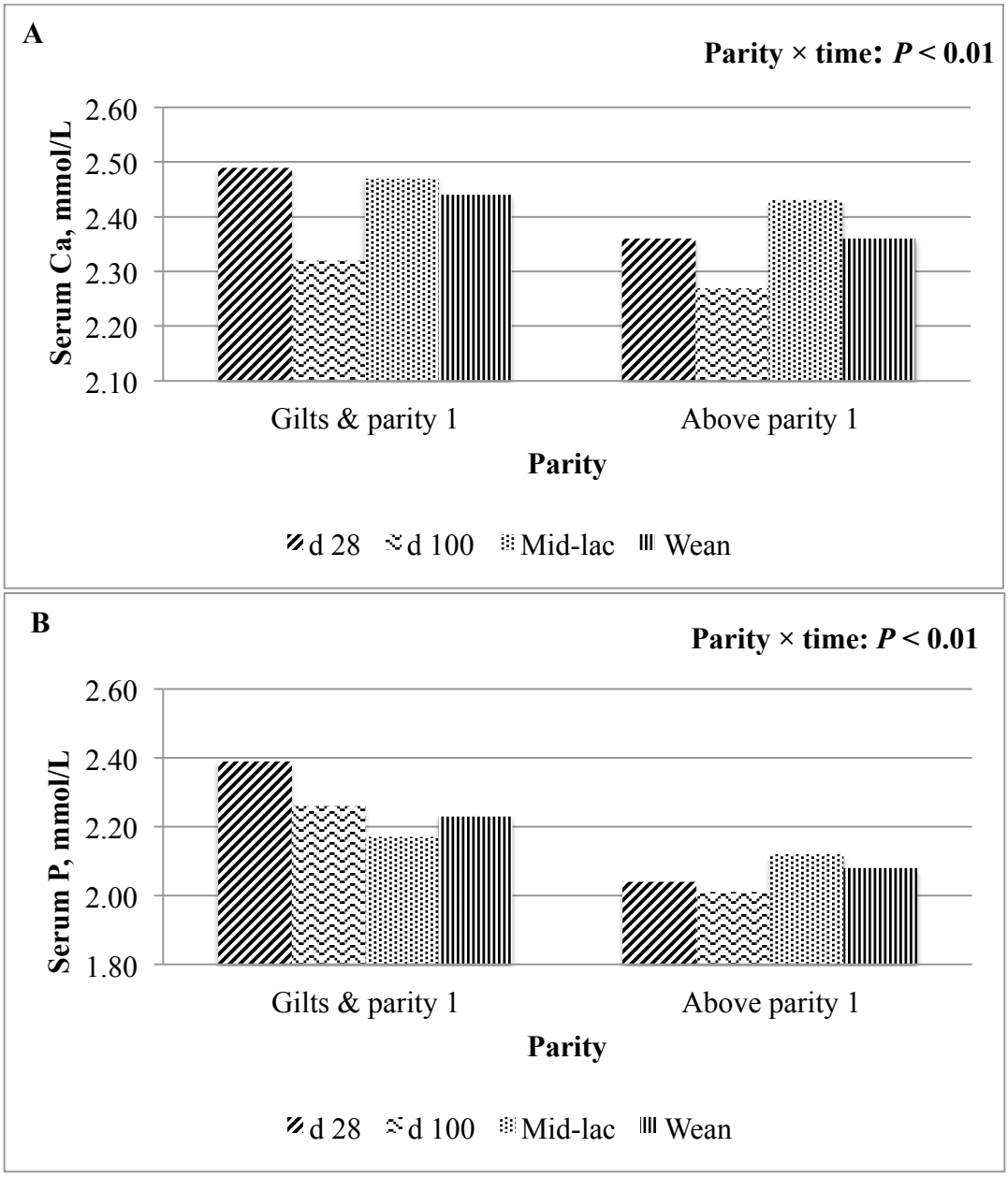


Figure 3.7. The effects of parity and time on serum Ca postprandial (A; Parity × time interactions; $P < 0.01$; SEM = 0.02) and serum P (B; Parity × time interactions; $P < 0.01$; SEM = 0.02). Data were analyzed using repeated measures ANOVA. A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments; 18 sows were removed during the course of the trial (gilts and parity 1, $n = 80$; above parity 1, $n = 82$).

3.3.6 Sow milk Ca and P and milk output

A diet by housing interaction for milk Ca % was observed at weaning with group-housed sows fed the high Ca diet producing milk with the highest amount of Ca ($P = 0.03$, Fig. 3.8). There were no effects of diet on milk Ca % at mid-lactation ($P > 0.10$, Table 3.12), but a higher milk Ca % was observed in group, compared to stall-housed sows at this time ($P = 0.01$). Neither diet, nor housing, had an effect on the amount of P in milk at mid-lactation ($P > 0.10$) but group-housed sows produced milk with a higher P concentration at weaning ($P = 0.01$).

A parity by housing interaction for milk Ca % was observed at mid-lactation with younger sows housed in stalls producing milk with the lowest Ca % whereas milk produced by the other treatment groups had similar Ca % ($P = 0.01$; Table 3.13). Older sows produced milk with higher P % compared to young sows ($P = 0.01$). Estimation of milk output (Eq. 3.2) for the first 21 d of lactation showed older sows produced more milk compared to their younger counterparts ($P < 0.01$; Table 3.14). There was no effect of diet or housing on milk output at this time period ($P > 0.10$).

There was a weak positive correlation between dietary Ca % during gestation and serum Ca levels at d 100 of gestation (r = 0.17; $P < 0.05$; Table 3.15) whereas a tendency for a positive correlation was observed between Ca intake during gestation and milk Ca % only at weaning (r = 0.15; $P < 0.10$). Average daily Ca intake during lactation was positively correlated with milk Ca % during mid-lactation (r = 0.31; $P < 0.05$) and at weaning (r = 0.20; $P < 0.05$). As expected from the experimental design, P intake was highly correlated with Ca intake (r = 1.0, data not shown). A similar weak positive correlation was observed for milk P % during mid-lactation (r = 0.20; $P < 0.05$) and a tendency to be correlated at weaning ($P < 0.10$). A positive correlation was also seen between Ca and P intake during lactation and milk output level (r = 0.56; $P < 0.05$).

Table 3.12. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) and housing on milk Ca and P[1]

Item	Diet			SEM	Housing		SEM	*P*-value		
	-15%	Control	+15%		Stall	Group		Diet	House	Diet × House
Sows, *n*	51	54	57	-	51	51	-	-	-	-
Milk Ca, %										
Mid-lactation[2]	0.159	0.158	0.163	0.004	0.155	0.165	0.004	0.42	0.01	0.83
Weaning[3]	0.188	0.185	0.198	0.004	0.184	0.197	0.003	0.01	<0.01	0.03[4]
Milk P, %										
Mid-lactation[2]	0.139	0.134	0.139	0.002	0.137	0.138	0.002	0.15	0.45	0.47
Weaning[3]	0.150	0.146	0.151	0.002	0.146	0.152	0.002	0.15	0.01	0.56
Milk output[2], g/piglet·d^{-1}										
	711.0	704.0	705.1	23.8	697.5	716.0	22.5	0.93	0.25	0.27

[1] A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments. Sows were blocked by breeding group. A total of 18 sows were removed during the course of the trial.
[2] Milk samples collected on d 12 ± 2 of lactation.
[3] Milk samples collected prior to weaning and 25 ± 2 of lactation.
[4] Significant diet x housing interaction presented in Fig. 3.5 ($P < 0.05$).

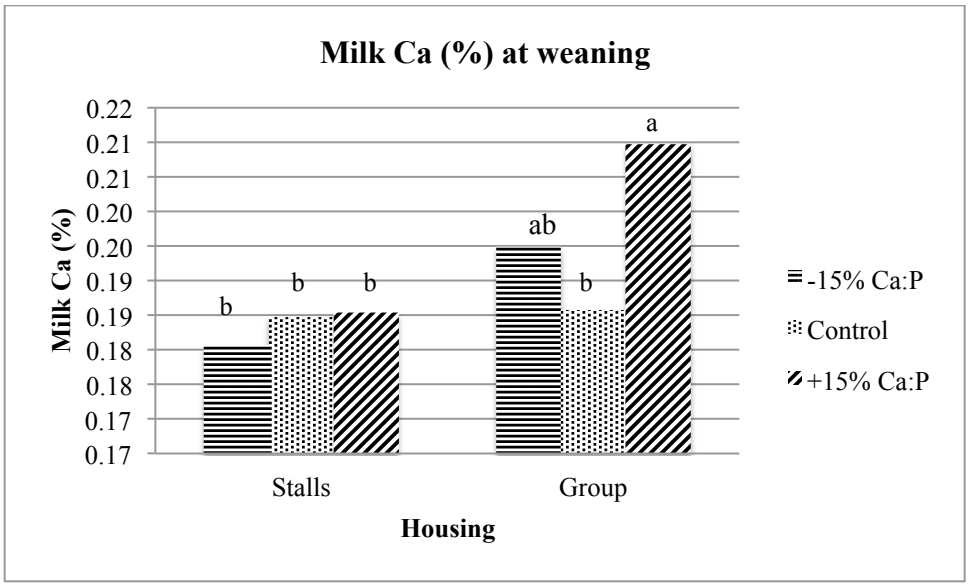


Figure 3.8. The interaction of diet and housing on concentration of Ca in milk of sows at weaning (diet × housing $P < 0.05$; SEM = 0.005). Means with same letter are not significantly different ($P > 0.10$). A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments; 18 sows were removed during the course of the trial (stalls, $n = 81$; group, $n = 81$).

Table 3.13. Effects of parity and housing on milk Ca and P of sows[1]

Item	Stalls		Group		SEM	*P*-value	
	Gilts & parity 1	Above parity 1	Gilts & parity 1	Above parity 1		Parity	Parity × House
Sows, *n*	39	42	41	40	-	-	-
Milk Ca, %							
Mid-lactation[2]	0.146	0.163	0.165	0.164	0.005	0.03	0.01
Weaning[3]	0.181	0.185	0.201	0.193	0.004	0.60	0.11
Milk P, %							
Mid-lactation[2]	0.131	0.137	0.141	0.139	0.003	0.01	0.10
Weaning[3]	0.144	0.147	0.153	0.152	0.003	0.72	0.50

[1] A total of 180 sows were used in a 3 × 2 randomized complete block design; 18 sows were removed during the course of the trial.
[2] Milk samples collected on d 12 ± 2 of lactation.
[3] Milk samples collected prior to weaning on d 25 ± 2 of lactation.

Table 3.14. The effect of parity on average milk output of sow[1]

Item	Parity		SEM	*P*-value
	Gilts & parity 1	Above parity 1		Parity
Sow, *n*	81	81	-	-
Milk output[2], g/piglet·d^{-1}	680.04	732.79	21.66	<0.01

[1] A total of 180 sows were used; 18 sows were removed during the course of the trial.
[2] Estimation of milk output (d 1 to d 21 lactation) calculated using equation, M = 2.50 × ADG + 80.2 × BWi + 7 (Noblet and Etienne 1989); BWi = average birth weight of piglets within a litter.

Table 3.15. Pearson correlation coefficients (r) for serum constituents and milk Ca and P[1]

Item	Sow Ca intake, g/d[4]	
	Gestation	Lactation
Serum Ca, mmol/L		
d 28[2]	-0.04	-
d 100[2]	0.17**	-
Mid-lactation[3]	0.09	0.11
Wean[4]	-0.06	0.06
Serum P, mmol/L		
d 28[2]	-0.06	-
d 100[2]	0.11	-
Mid-lactation[3]	0.14	0.07
Wean[4]	0.11	-0.01
Serum osteocalcin (ng/mL)		
d 28[2]	-0.03	-
d 100[2]	-0.11	-
Mid-lactation[3]	-0.01	0.04
Wean[4]	-0.05	0.14*
Serum pyridinoline (nmol/L)		
d 28[2]	-0.01	-
d 100[2]	0.07	-
Mid-lactation[3]	0.04	0.01
Wean[4]	-0.10	-0.12
Milk Ca (%)		
Mid-lactation[3]	0.07	0.31**
Wean[4]	0.15*	0.20**
Milk P (%)		
Mid-lactation[3]	0.01	0.20**
Wean[4]	0.02	0.14*
Milk output (g/piglets.d^{-1})[6]	-0.05	0.56**

[1] A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments. Sows were blocked by breeding group. A total of 18 sows were removed during the course of the trial. Blood samples were collected postprandially.
[2] Gestation d 28 (start of trial) and d 100.
[3] Lactation d 12 ± 2 (milk); d 14 ± 2 (serum).
[4] Lactation d 25 ± 2 (milk); d 26 ± 2 (serum).
[5] Dietary Ca % x ADFI of individual sows.
[6] Estimation of milk output (d 1 to d 21 lactation), M = 2.50 × ADG + 80.2 × BWi + 7 (Noblet and Etienne 1989); BWi=average birth weight of piglets within litter.
* $P < 0.10$; ** $P < 0.05$

3.3.7 Calcium balance of sows

Ca balance during gestation and lactation was unaffected by either dietary Ca and P or housing ($P > 0.10$; Table 3.16). An effect of diet was observed for fecal Ca excretion during both stages of reproduction as the amount of Ca excreted in the feces increased as the levels of dietary Ca increased ($P < 0.05$). A lower calculated Ca retention was generally observed in sows, regardless of housing during lactation compared to gestation. During mid-lactation, there was a tendency for reduced Ca retention as dietary Ca decreased ($P = 0.09$). Although group-housed sows produced milk with higher Ca % at weaning, there was no effect of diet or housing on the amount of Ca retained ($P > 0.10$).

Table 3.16. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) and housing on Ca balance of sows[1]

Item	Diet			SEM	Housing		SEM	*P*-value		
	-15%	Control	15%		Stall	Group		Diet	House	Diet × House
Gestation										
Intake[2], g/d	17.3	18.2	19.7	-	18.4	18.4	-	-	-	-
Digestibility[3], %	19.4	14.9	17.4	3.1	15.1	19.3	2.6	0.59	0.25	0.57
Excreted[4], g/d	13.9	15.5	17.3	0.7	16.0	15.2	0.6	<0.01	0.33	0.68
Balance[5], g/d	3.3	2.7	2.4	0.7	2.4	3.2	0.6	0.60	0.33	0.68
Lactation										
Intake[2], g/d	44.8	53.6	61.0	3.6	52.8	53.5	3.0	0.01	0.86	0.08
Digestibility[3], %	14.6	28.8	20.7	4.8	17.2	25.5	3.9	0.16	0.15	0.81
Excreted[4], g/d	37.1	38.6	48.3	3.4	42.9	39.8	2.8	0.04	0.44	0.08
Balance[5], g/d	7.7	15.0	12.7	2.4	10.6	13.0	2.0	0.10	0.42	0.95
Milk output[6], g/piglet.d^{-1}	691.0	713.0	688.5	23.9	697.5	716.0	22.5	0.93	0.25	0.27
Mid-Lactation Milk Ca output[6], g/d	11.6	11.7	10.9	1.5	10.4	12.3	1.2	0.91	0.28	0.93
Ca retained[8], g/d	-3.9	3.3	1.9	2.4	0.2	0.6	2.0	0.09	0.89	0.87
At weaning Milk Ca output[7], g/d	14.0	14.7	13.0	1.5	12.0	15.8	1.3	0.75	0.04	0.94
Ca retained[8],g/d	-6.3	0.3	-0.3	2.6	-1.3	-2.8	2.1	0.12	0.62	0.97

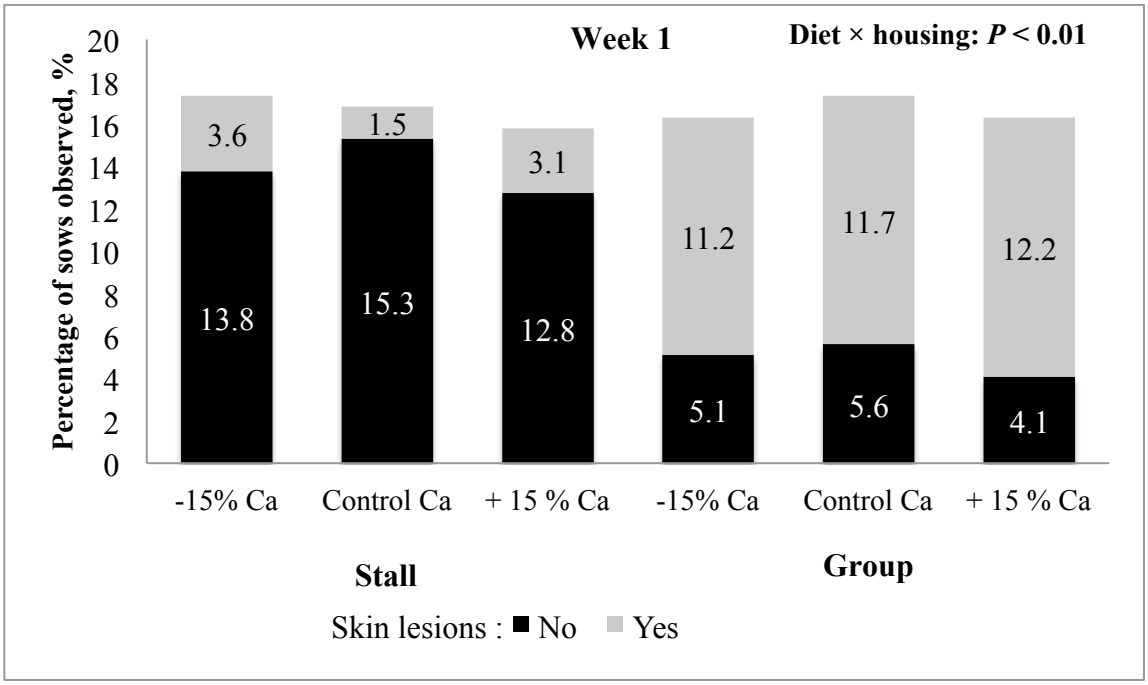
[1]A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments. Sows were blocked by breeding group. A total of 18 sows were removed during the course of the trial. All balance data are based on 10 sows/treatment (gestation) and 5 sows/treatment (lactation). Apparent digestibility calculated based on a 3-d grab sampling of feces from these sows.
[2] DM % × feed intake of sows.
[3]All digestibility values are apparent.
[4] Fecal loss.
[5] Intake – fecal loss.
[6] Estimation of milk output (d 1 to d 21 lactation) calculated using equation, M = 2.50 × ADG + 80.2 × BWi + 7 (Noblet and Etienne 1989); BWi = average birth weight of litter.
[7] Milk Ca % (Table 3.7) × milk output × No. of piglets nursed.
[8] Ca balance – milk Ca output for lactation

3.3.8 Sow behavioural analysis

Based on Fisher's exact test the number of sows with or without skin lesions in each treatment group at wk 1 and wk 5 of the trial was different ($P < 0.01$; Fig. 3.9). The number of sows skin with lesions at wk 1 was proportionately larger in the sows housed in groups compared to sows in stalls with approximately 70 % of group-housed sows having one or more skin lesions. Although the number of sows with lesions was reduced at wk 5 with sows in stalls recording almost no lesions, the majority of sows with skin lesions were from those housed in groups.

Video observation of sows recorded over 13 h showed that the proportion of sows in each posture category differed between treatment groups at wk 1 ($P < 0.05$; Fig. 3.10a.). During the 13 h observation period, approximately 57 % of the sows housed in stalls were observed to be lying down whereas an even higher percentage of sows in group housing (approximately 90 % of sows) were exhibiting this posture. However, by wk 5, 75 % of all sows were lying down during the observation period, regardless of treatment ($P > 0.10$; Fig. 3.10 b.). The lack of a treatment effect during wk 5 is consistent with results from the accelerometers, which recorded the sow posture over 24 h during the same time period (Table 3.17). There were no effects of dietary Ca and P or housing on the number of steps, standing time or the duration that each sow spent on different postures at wk 5 ($P > 0.05$). Sows spent approximately 20 % of their time standing; the rest of the time they were lying down or sitting.

a)



b)

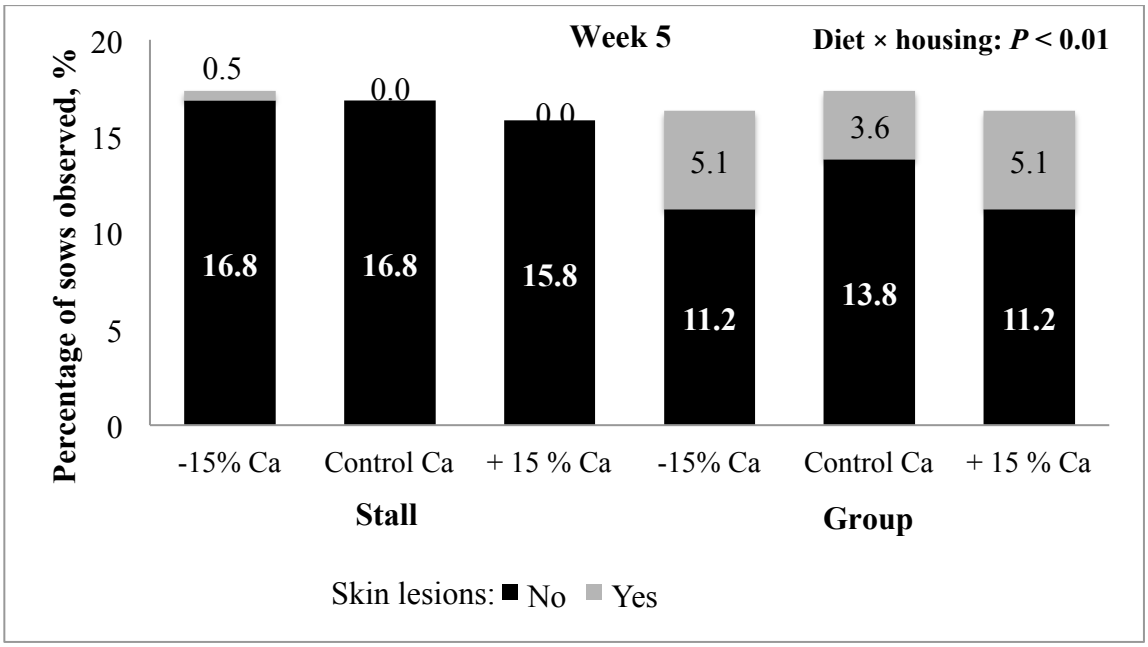
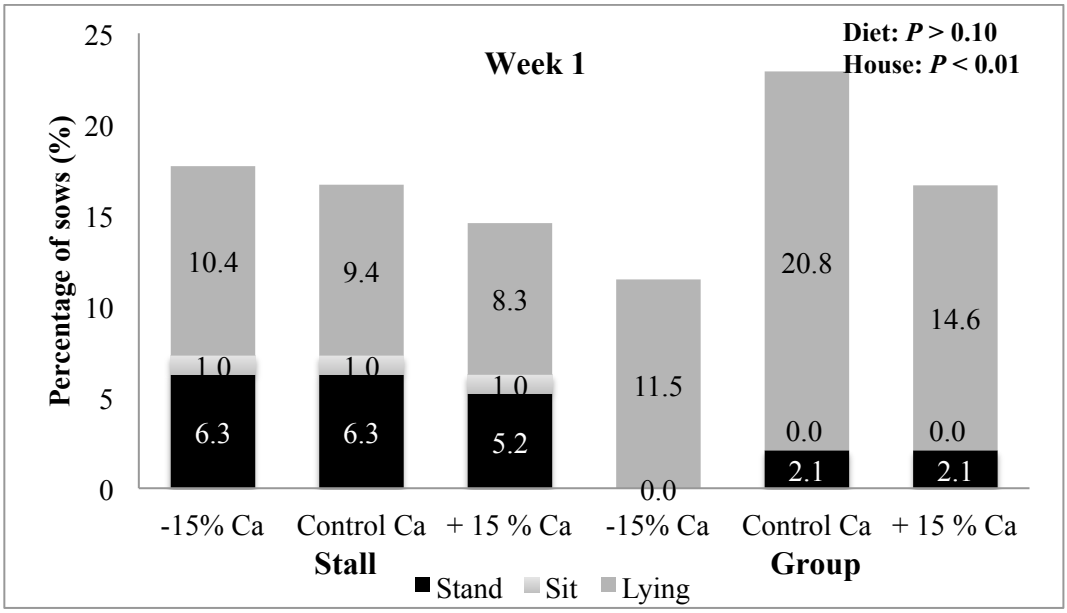


Figure 3.9. The distribution of sows by diet and housing interaction and presence (Yes) or absence (No) of skin lesions on (a) wk 1 and (b) wk 5 of the trial. Data presented as number of sows per total of number of the sows observed. *P*-values obtained using Fisher's exact test. The presence or absence of skin lesions in sows was associated with a diet by housing interaction at wk 1 and wk 5; $P < 0.01$. A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments; 18 sows were removed during the course of the trial (stalls, $n = 81$; group, $n = 81$).

a)



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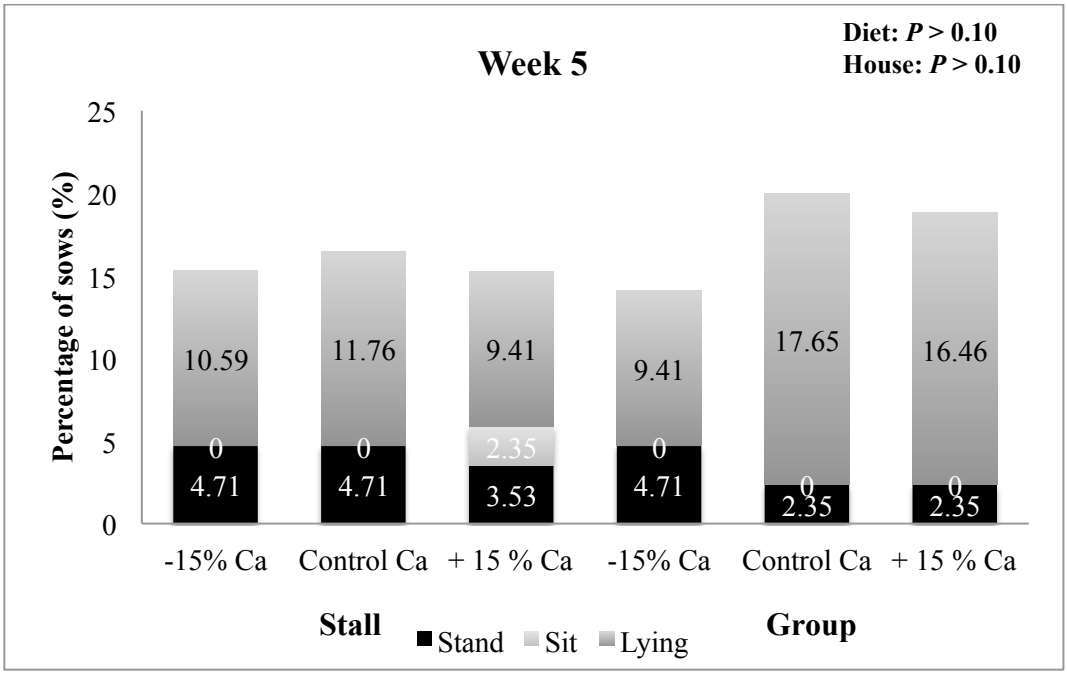

Figure 3.10. Video observation of sows, % by treatment and posture on (a) wk 1 and (b) wk 5 of trial over 13 h (light period). Data presented as number of sows per total number of sows observed. *P*-values obtained using Fisher's exact test. The proportion of sows in each posture category differed between treatment groups; $P < 0.05$ at wk 1 but no difference was observed at wk 5; $P > 0.10$. A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments; 18 sows were removed during the course of the trial (stalls, $n = 81$; group, $n = 81$).

Table 3.17. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) and housing on steps and posture of sows[1] on wk 5 of the trial using accelerometers

Item	Diet			SEM	Housing		SEM	*P*-value		
	-15%	Control	+15%		Stall	Group		Diet	House	Diet × House
Steps[2]										
No. of steps	258	273	254	34.1	250	273	28.8	0.90	0.55	0.66
Standing time, min	33.9	45.1	40.6	5.2	39.6	40.2	4.4	0.29	0.92	0.80
Steps/min standing[3]	7.3	7.2	7.6	1.1	7.2	7.5	0.9	0.96	0.82	0.69
Posture[4]										
Duration, %										
Standing	17.1	11.4	16.6	1.2	14.3	15.3	1.1	0.11	0.87	0.34
Other	80.6	85.5	79.9	3.2	83.0	81.0	2.8	0.29	0.50	0.83

[1] A total of 180 sows were used in a randomized complete block design with a 3 × 2 factorial arrangement of treatments. Sows were blocked by breeding group. A total of 18 sows were removed during the course of the trial. Accelerometers were attached to one hind limb of a subset of sows; steps (n = 8 sows per treatment) and posture (n = 9 sows per treatment).

[2] Number of steps taken by sows were recorded for 1 h starting approximately 30 min after feeding time.

[3] Number of steps taken by sows in 1 min while standing.

[4] Sow posture was recorded over a 24 h period starting 1 h after feeding. Posture recorded as standing if acceleration along x-axis was ≥ 0.59 g (static acceleration of gravity); otherwise, the posture is other.

3.4 Discussion

The objective of this study was to determine if the recommended levels of dietary Ca and P are adequate for sows housed in groups, who have a potential for increased mobility.

Culling rate during the experiment was approximately 5 %, which was lower than the herd average (12 to 14 %). Removal due to lameness however, was approximately 28 % compared to the herd average of less than 1%. The type and severity of lameness, the timing of treatment (Craig, 2001) and the subjective detection of lameness (Grégoire, et al., 2013) may be the reasons in the difference observed between sows on study and those in herd.

Consistent with results from previous, older studies (Kornegay et al., 1973; Harmon et al., 1974; Mahan and Fetter, 1982; Maxson and Mahan, 1986), dietary Ca and P did not affect sow BW or ADFI during gestation. Although sows housed in stalls had a slightly lower BW compared to group-housed sows at d 100 of gestation, the change of BW from the start of the trial to late gestation and weaning were not different between sows from either housing system. Previous studies have shown that stall housing decreased muscle weight of certain locomotor muscles and bone strength compared to group-housed sows, thus assuming that movement or exercise is required for bone and muscle development (Marchant and Broom, 1996a). The same study however, showed that although muscle weight was strongly correlated with BW, there was no correlation between BW and bone strength among sows in the same housing system. In our current study, the lack of differences in the movement of sows (number of steps and standing posture) probably explains the lack of change in BW between sows in group or stall housing. The lower BW of gilts and parity 1 compared to older sows is expected. Moreover, the larger increase in BW for young sows at late gestation and at weaning as compared to their initial weight was also expected as these sows would still be growing while meeting the demands for reproductions.

Previous studies have reported that altering the levels of dietary Ca and P during gestation had no effect on sow reproductive performance (Harmon et al., 1974; Nimmo et al., 1981; Mahan and Fetter, 1982; Kornegay et al., 1985; Maxson and Mahan, 1986; Everts et al., 1998; Liesegang et al., 2005). Similar results were obtained in the current study. A housing effect however, was also observed. Group-housed sows had a higher number of liveborns and piglets with heavier birth weight compared to stall-housed sows. The reverse was reported in a

study by Marchant and Broom (1996a) where sows kept in stalls had a tendency to produce more live piglets. A compilation of studies on sow housing also concluded that sows in stalls had greater or equal reproductive performance as group-housed sows (McGlone et al., 2004). These results however may be influenced by various factors in each individual study such as the number of reproductive cycles involved in each study and particularly the type of group housing and feeding systems used. Individual feeding systems are a common practice to control individual feed intake and aggression, however, movement is limited (Gonyou, 2001; Lund, 2002; Salak-Johnson et al., 2012). On the other hand, feed may be gained through aggression in a competitive feeding system within group housing systems such as floor feeding or non-gated stalls (Gonyou and Rioja-Lang, 2012). The current study employed a form of group housing and non-competitive feeding system (sows were individually fed), hence, results may not be reflected if different feeding systems were used. Additional research is still required on feeding systems in group housing to lessen the potential for aggression, injury and subsequent decrease in reproductive efficiency.

Mahan and Fetter (1982) reported an effect of parity on reproduction, where second or third parity sows generally produced higher numbers of liveborn, and these piglets were heavier than those produced from the first parity sows. Conversely, in the current study, piglet birth weights were similar across parities but piglets from older sows had higher weaning weights. Maxson and Mahan (1986) suggested that individual piglet gains were similar across parity, a result that may be confounded by the differences in sows' feed intake between small and large litters. Conversely, our results indicate that while litter size was similar across parities, the higher ADG of piglets from older sows may be attributed to the larger ADFI and higher milk output per piglet estimated for the older sows.

Findings from older studies showed that serum Ca and P concentrations were not affected when dietary Ca and P treatments were fed to sows for 3 to 5 parities (Kornegay et al., 1973; Mahan and Fetter, 1982; Kornegay and Kite, 1983; Maxson and Mahan, 1986). In the current study, the diet by housing interaction observed for serum total Ca on d 100 of gestation showed that group-housed sows that were fed low Ca diets had the lowest level of serum total Ca. Their level of serum Ca, 2.18 mmol/L, was not different from sows fed the control diets while the serum Ca value in sows fed the low Ca diet was below the normal reference range of gilts or sows in late gestation or during lactation (Lauridsen et al., 2010; Weber et al., 2014). Lanyon

(1984) proposed that decreased dynamic loading results in Ca mobilization from the bone. This theory was supported by Marchant and Broom (1996a) where confined sows with restricted movement had considerably lighter muscle weight and a reduction in bone strength. Decreased serum Ca triggers parathyroid hormone (PTH) to induce changes in target organs such as the bone, kidney or intestines which subsequently increases the serum Ca to a homeostatic level (Crenshaw, 2001). If the Lanyon (1984) theory is utilized for the current study, the group-housed sows that have more potential for movement and increased dynamic loading would have a higher Ca requirement for bone formation. This may explain the lower levels of serum Ca observed in the group-housed sows fed the low Ca diet.

Serum P levels differed between housing groups at the start of the trial, and therefore, the values for d 100 gestation and lactation were measured as deviations from the initial value. This allowed us to evaluate how the extracellular levels of P adapted to changes in dietary and housing treatments. The diet by housing interaction observed at d 100 of gestation suggests that group-housed sows fed low P levels had the largest decline in serum P levels. This result complements the diet by housing interaction for serum Ca at d 100 of gestation. The lack of a response of diet or housing on serum Ca and P during lactation may be due to the variable feed intake of sows and circulating Ca and P regulation via hosmeostatic mechanisms (Maxson and Mahan, 1986)

Contradictory results have been reported for an effect of parity on serum Ca and P concentrations in sows. Several studies have reported higher levels of serum Ca and P in first parity sows (Mahan and Fetter, 1982; Giesemann et al., 1998; Liesegang et al., 2005; Weber et al., 2014) which is consistent with the current study, suggesting that lactation intensity may be responsible for the decreased mineral levels in the serum of the older sows. On the contrary, (Maxson and Mahan, 1986) reported that while dietary Ca and P did not affect serum mineral levels, the increased serum Ca and P levels in second compared to first parity sows, is the result of the higher feed intake and higher skeletal reserves of the older sows.

Serum total Ca and P levels differed during the course of the reproductive cycle. In the current study, regardless of parity, serum total Ca and P declined towards late gestation. This is when these minerals are required for rapid fetal development (Mahan and Fetter, 1982; Liesegang et al., 2005; Weber et al., 2014). A fluctuation in serum mineral levels was observed during lactation where serum total Ca rose at mid-lactation before declining again towards

weaning. Adaptive mechanisms such as maternal skeletal resorption and decreased renal excretion (Kovacs and Kronenberg, 1997) and the gradual increase in feed intake may explain the rise in serum Ca at mid-lactation. The slight decline in serum Ca levels prior to weaning (d 26 of lactation) observed in this study could conceivably be due to the continuous Ca demand for milk production which reaches its peak between the second and third wk of lactation (Hansen et al., 2012; Lien et al., 2014). Conversely, serum P of the young sows decreased at late gestation and continued to decline towards mid-lactation before rising at weaning. In older sows however, serum P displayed a trend similar to serum Ca. Although significant differences were present for serum Ca and P between the reproductive stages, the levels were always within the normal reference range reported by others (Friendship et al., 1984; Liesegang et al., 2005; Lauridsen et al., 2010; Weber et al., 2014), providing further evidence on the importance of maternal homeostatic mechanisms in maintaining serum Ca and P concentrations (Harmon et al., 1974; Weber et al., 2014). In the current study, the effect of dietary Ca and P of gestating sows on other serum minerals such as Mg, Zn, Cu and Se, was not monitored. The gestation and lactation diets were formulated to meet or exceed the mineral requirements of the sows as recommended by NRC (2012). Moreover, there is only limited evidence of Ca interactions with other minerals in human and laboratory animals. Further, in the one study conducted with lactating sows, there was no effect of dietary Ca and P on serum or milk Mg (Maxson and Mahan, 1986). Similarly, there are other nutrients that may be predisposing factors for changes in bone health of sows, such as minerals (Zn, Mg, Cu, Se and Mn) and vitamins (vitamin B and D). However, apart from vitamin D, limited evidence is available in sows and their role in Ca homeostasis are yet to be established. For this reason, in the current study, the mineral and vitamin premixes were added into the diets to meet or exceed the requirements as suggested by NRC (2012).

In the current study, serum bone marker concentrations were influenced by parity and physiological state but not by dietary Ca and P levels, findings which were consistent with previous findings on reproducing sows (Liesegang et al., 2005; Lauridsen et al., 2010) and cows (Taylor et al., 2009). On the other hand, several studies using growing pigs have shown significant effects of dietary Ca and P on serum bone markers. Diets deficient in Ca and P increased serum pyridinoline (PYD), a marker of bone resorption, with no effect on serum osteocalcin (OC), a marker of bone formation (Nicodemo et al., 1998). However, separate studies reported increased levels of serum OC in growing pigs (Carter et al., 1996) and rats (Lian

et al., 1987; Tanimoto et al., 1991) receiving Ca-deficient diets. These studies suggested a possible involvement of 1,25(OH)D_3 in the elevation of serum OC. All diets used in our study contained 825 IU vitamin D/kg, a level which exceeds the requirement of vitamin D for sows based on NRC (1998) and NRC (2012).

To our knowledge, few studies have been performed examining the effects of different housing systems on bone turnover of sows. Studies in other species were primarily conducted in growing animals. In young horses, confinement in stalls resulted in reduced bone formation and higher bone resorption marker concentrations (Hoekstra et al., 1999). The lack of movement in horses (Hiney et al., 2000) and calves (Hiney et al., 2004) was also correlated with a decreased rate of bone formation. In the current study, housing did not affect serum bone markers concentrations which may be due to the lack of effect of housing system on movement of the sows. Since the levels of serum Ca and P were mostly within the normal reference range (Friendship et al., 1984), this may reflect the involvement of other maternal Ca adaptation mechanisms.

Gilts and first parity sows generally had greater levels of bone markers compared to the older sows, which may be due to the growing bones of the younger sows (Verheyen et al., 2007; Weber et al., 2014). Similar results were also seen in bone markers of growing horses and dogs (Allen, 2003). The decrease in both serum bone markers in late gestation suggests an uncoupling of bone formation from resorption. As the highest requirement for Ca and P is known to be during late gestation when rapid fetal accretion would require maternal skeletal mobilization, it would be expected that the marker of bone formation, serum OC would decline while the bone resorption marker, serum PYD, would increase (reviewed by Kovacs and Kronenberg (1997). The current study however, showed a slight decline in serum PYD rather than the expected increase during late gestation. According to Kovacs and Kronenberg (1997), a 1,25(OH)D_3 mediated increase in intestinal Ca absorption appeared to be the major maternal adaptive mechanism used in humans (Gertner et al., 1986; Cross et al., 1995b) and rats (Quan-Sheng and Miller, 1989; Baylis, 1994) to meet the rapid fetal Ca accretion observed in late gestation. Unfortunately, serum 1,25(OH)D_3 was not measured in the current study. Moreover, blood samples were obtained around the time when the daily feed allotment was increased from 2.3 to 3.0 kg/d, which might have also contributed to increased Ca absorption. Conversely, an older study in rats (Rasmussen 1977, cited by Miller et al. (1982) also suggested that Ca and P are

supplied to the developing fetus without mobilizing these minerals from the maternal skeleton, provided that intake is adequate (Rasmussen, 1977). Consistent with other studies, the levels of serum PYD at mid-lactation was higher than during gestation as opposed to serum OC, which is attributable to the acute, increased demand for Ca at the onset of lactation, leading to a subsequent rise in mobilization of the skeletal Ca stores (Liesegang et al., 2005; Lauridsen et al., 2010). This supports the review by Kovacs and Kronenberg (1997) stating that skeletal resorption is probably the main maternal adaptation used during lactation. In response to the immediate need for Ca, Ca flux from bone will result in increased serum Ca levels, and while the mechanism is not fully understood, it has been suggested that this flux occurs at a rate faster than changes in bone formation and resorption (Crenshaw, 2001). Although the levels of bone formation marker, OC, eventually increased at prior to weaning with levels comparable to early gestation in the older sows, the serum OC level in the younger sows were lower than the initial level at early gestation. Apart from the need for Ca for their own growth, the lower level of feed intake observed in this young sows may have contributed to the lower levels of serum OC.

Milk Ca and P concentrations increased from mid-lactation (approximately d 12 lactation) to just prior to weaning (approximately d 25 lactation). This is consistent with previous studies in sows. However, the average concentrations in the current study are slightly lower than formerly reported for milk Ca and P levels at either mid-lactation (within d 7 to d 14 lactation) or weaning (within d 21 to d 28 lactation) (Harmon et al., 1974; Harmon et al., 1975; Allen, 1982; Mahan and Fetter, 1982; Maxson and Mahan, 1986; Miller et al., 1994). The majority of these studies did not see an effect of dietary Ca and P on in milk Ca and P (Mahan and Fetter, 1982; Maxson and Mahan, 1986; Mahan and Vallet, 1997; Liesegang et al., 2005). Additionally, Maxson and Mahan (1986) reported that the concentration of these minerals in milk increased from d 7 to d 21 of lactation. Results from this older study are consistent with results obtained in the current study, and as suggested by Maxson and Mahan (1986), this is probably due to the increased feed and thus mineral intake from d 3 onwards of lactation as recorded in this study. In contrast, a study by Miller et al. (1994) showed a tendency for increased milk Ca when sows were fed a high Ca diet, a result that is consistent with the weak positive correlation between Ca intake and milk Ca % in the present study. Moreover, a diet by housing interaction for milk Ca at weaning showed group-housed sows fed the high dietary Ca and P produced milk with the highest Ca composition. The difference between the lowest and highest milk Ca levels however,

was only 0.03 %, and all concentrations were close to the normal level of 0.21 %, reported in the literature (Pond and Maner, 1974 as cited by Miller et al. 1994). As serum Ca levels at weaning were similar regardless of dietary Ca and P or housing, this again suggests the importance of the homeostatic mechanism in maintaining these levels, particularly when the diet is not severely deficient in Ca or P (Maxson and Mahan, 1986). Statistical analysis showed a significant effect of housing on milk P levels at weaning (1.46 g/L for sows in stalls versus 1.52 g/L for group housed sows)

As opposed to data reported by Giesemann et al. (1998), milk Ca and P % differed between young and mature sows at mid-lactation. In the current study, mature sows had higher levels of milk Ca and P % compared to the younger sows although the differences were only 0.01 %. According to King'ori (2012), age, stage of lactation and especially parity affect milk yield. In this study, olders sows had an overall higher estimated milk output compared to the younger sows. The estimation of milk output, based on the equation by Noblet and Etienne (1989), takes into consideration the average initial birth weight of piglets and ADG of piglets nursed. As the birth weight and litter size was similar across parities, the higher weaning weight and ADG of piglets by the older sows resulted in a calculated higher milk production value. The greater milk yield for the older sows may be attributed to the greater lactation feed and thus energy intake as suggested by others (Noblet and Etienne, 1986; Neill and Williams, 2010; King'ori, 2012). Although the younger sows had lower ADFI, the estimated milk output per kg feed for these sows was higher compared to the older sows. In general, young sows have a higher requirement for lysine for lean tissue growth and an inadequate supply of this amino acid from their daily ration may lead to mobilization of body protein and subsequent body weight loss (Jang et al., 2014). This may then eventually lead to lowered milk production and litter weight gain (Neill and Williams, 2010). Similarly, the younger sows in this study also had a higher loss of BW by the end of lactation, a lower milk output per piglet and piglet ADG compared to the mature sows.

In this study, the larger milk yield in older sows, but with a similar milk Ca and P composition as the younger sows observed would suggest a possible higher mineral from the bones in older sows as reported in other studies (Mahan and Fetter, 1982; Maxson and Mahan, 1986). However, the older sows had approximately 1 kg more of feed daily, hence, a higher Ca and P intake compared to the young sows. The higher Ca and P intake may have compensated

for the larger amount of Ca and P required for milk production. Moreover, results from this study also showed that bone resorption marker levels were quite similar across parities, although the levels of bone formation markers were lower in the older parities as compared to the younger sows. Observation from this study over 1 parity would therefore suggest that in the older sows, mobilization of Ca from the skeleton was minimal as the higher requirement for Ca and P for milk production was compensated by higher feed intake.

The estimation of Ca balance in sows during gestation and lactation was calculated using the AD of Ca, which does not take into consideration the endogenous or urinary losses (Gonzalez-Vega and Stein, 2014) or the contribution of Ca from the drinking water. Analysis of the water used in this experiment showed that it contained approximately 28 mg/L Ca. The average water consumption in other studies varied between 17 to 30 L per sow per d (Peng et al., 2007; Oliviero et al., 2009; Kruse et al., 2011). If sows in the current study consumed this amount of water, the contribution of Ca from water intake would be approximately 2 to 3 % of dietary Ca during gestation and 0.8 to 1 % of dietary Ca during lactation. The AD of Ca obtained from this study is comparable with values reported by Jongbloed et al. (2004); Shaw et al. (2006b), but lower than the average AD of Ca of approximately 32 % in sows reported by Everts et al. (1998). The higher Ca digestibility obtained during lactation compared to gestation is consistent with most previous studies (Harmon et al., 1974; Harmon et al., 1975; Nimmo et al., 1981; Kornegay and Kite, 1983; Kemme et al., 1997a; Giesemann et al., 1998). The increased demand of Ca for milk production may have triggered the release of $25(OH)D_3$ resulting in increased Ca absorption (Kemme et al., 1997a). The intake of Ca influenced fecal Ca excretion, with increased fecal Ca excretion as Ca intake increased, consistent with other studies in pigs and even cows (Everts et al., 1998; Liu et al., 1998; Crenshaw, 2001; Taylor et al., 2009). In our study, despite the larger feed intake and fecal Ca excreted during lactation as compared to gestation, a greater percentage of Ca was also absorbed and retained, suggesting an adaptation to absorb and retain Ca during lactation (Giesemann et al., 1998). The amount retained was not affected by dietary treatments which is in accordance with Everts et al. (1998). Fats are commonly added to sows diet during gestation and lactation to improve energy density and evidence suggest that addition of fat can improve milk yield, fat content in milk and survival of pigs (NRC, 2012). Ca, which forms insoluble calcium fatty acid soaps are often added to diets of ruminants to improve energy digestiblity (Block et al., 2005; Lorenzen 2007). In this study, fats

(canola oil) was added at approximately 0.5 to 1.5 % in all diets. Results showed that both gestation and lactation diets had slight differences in AD of energy among the treatment groups. As the fat content in the feces was not measured in this study, it remains unknown if the formation of Ca soap would have affected Ca or energy digestibility to some degree. However, as there was no difference in Ca digestibility between the dietary treatments, it can be safely assumed that there was very little or no effect of Ca soap formation on Ca availability.

To our knowledge, there have been no studies examining the effect of gestation housing on Ca digestibility. In growing pigs, Kemme et al. (1997b) reported a higher AD of Ca in pigs housed in pens compared to those in metabolism crates whereas pigs in metabolic crates that were supplemented with feces in their diet also had numerical increase in AD of Ca compared to those without the intake of feces. These results indicate that increased movement (pens or metabolic crates) and coprophagy (feces supplementation in the diet) by the pigs housed in pens are possible factors for the improved digestibility. However, in the current study, there was no significant difference in the AD of Ca and amount of Ca retained between stall or group-housed sows, suggesting that sows are able to adapt to intestinal Ca absorption regardless of housing. Apart from that, the partly slatted flooring which limits the access to feces and similarity in Ca retention between both housing would imply that coprophagy was probably not a major concern in the group-housed sows. The current study however, employed a non-competitive feeding system with each sow receiving the same amount of feed,regardless of housing. Results may have been different if the group housing used a competitive feeding system. Urinary Ca excretion was not taken into account in this study as urine collection requires sows to be either kept in metabolism crates or undergo bladder catheterization which would not allow our housing treatments to be maintained. Furthermore, some studies on pigs (Fernández, 1995) and cows (Taylor et al., 2009) reported that urinary Ca excretion is not affected by dietary Ca unless the animals are severely deficient. This suggests that intestinal absorption plays a larger role than renal reabsorption in regulation of Ca balance. Conversely, several studies have reported that urinary P excretion increases as P intake increases (Vipperman Jr. et al., 1974; Nimmo et al., 1981; Fernández, 1995; Varley et al., 2011a), implying that renal regulation of P is important. For this reason, estimation of P balance was not considered in the current study

Behavioural measurements of the sows in this study were required to determine if the activity of sows housed in stalls or groups might explain differences in bone metabolism. Unlike

some studies on housing for gestating sows (Salak-Johnson et al., 2012; Schau et al., 2013), the expected results of increased activity as space increased was not seen in this study. In fact, sows in stalls stood or moved as much as sows that had access to a loafing area. The increased space allowance would have allowed sows to lie down with increased comfort as indicated by the larger proportion of group-housed sows lying down as seen in the video observation. This probably explains the lack of a significant effect of housing on the majority of behavioural variables measured. The greater presence of skin lesions, however, indicates increased aggression in the group-housed sows. In our study, the video observation, accelerometers and aggression data was only collected once during the 1st and 5th wk of the trial; conclusions may have differed if sows were monitored more frequently. Additionally, the potential for increased movement and aggression may have been even more prominent between the 2 housing systems if a competitive feeding system, such as group floor feeding had been used (Gonyou and Rioja-Lang, 2012).

In conclusion, results from the bone markers suggest that bone turnover of sows in this study was not affected by diet or housing. In fact, group-housed sows showed improved reproductive efficiency with increased birth weight and number of piglets born alive. There was also no interaction between dietary Ca and P intake and housing on sow reproductive performance and bone turnover. Results from this study suggest that the recommended level of dietary Ca and P as prescribed by NRC (1998), and NRC (2012) is adequate for high-producing sows of modern genetics, whether housed in stalls or groups.

4 EFFECTS OF DIETARY CALCIUM AND PHOSPHORUS IN GESTATING SOWS ON BONE DEVELOPMENT IN PIGLETS

4.1 Introduction

Adequate nutrition, including minerals, is important for gestating sows, particularly gilts, as growth and development of their piglets needs to be supported while their own growth is maintained (Noblet et al., 1985). Nutrient requirements obtained from older studies may not be applicable to the modern, highly prolific sow as they were based on sows farrowing smaller litters (Ball et al., 2008). Analysis of data collected from 1994 to 2004 showed that genetic improvements resulted in approximately 1 to 2 piglets more per litter in commercial sows (Quesnel et al., 2008). As cited by Kim, 2013, the National Agriculture Statistic Service US reported that litter size has increased by 3 pigs in the past 40 years (NASS, 2011). The increased prolificacy of sows is associated with higher within-litter weight variation which increases with litter size and parity (Quesnel et al., 2008). Although there are several factors which influence within-litter weight variation, insufficient maternal nutrition may be related to fetal growth retardation, which leads to negative effects on piglet birth weight and within-litter birth weight uniformity (Campos et al., 2012). The larger litter size of the modern sow hence, suggests a need for increased mineral content in their diets (Mahan et al., 2009).

The effects of maternal Ca intake on bone development of the fetus have not been clearly defined (Hacker et al., 2012). In humans, Ca supplementation for pregnant women with adequate Ca intake did not improve bone development of the newborn (Koo et al., 1999; Chang et al., 2003; Prentice, 2003a). Conversely, other studies suggest that a low or high maternal Ca intake may affect fetal skeletal development (Koo et al., 1999; Chang et al., 2003). Similarly, an effect of maternal Ca on fetal skeletal tissue was reported for ewes (Corbellini et al., 1991; Lima et al., 1993) and rats (Shackelford et al., 1993), but limited information is available for pigs. A study in the 1960s suggested that sow Ca intake influenced the transfer of Ca to the fetuses, and low Ca intake in sows affected fetal skeletal development (Itoh et al., 1967). The largest fetal Ca accretion occurred in late gestation (Hansard et al., 1966; Itoh et al., 1967) and dietary mineral inadequacy requires sows to mobilize its body reserves to meet the needs of the developing fetuses (Mahan et al., 2009).

We hypothesized that the concentration of Ca and P in the diet of gestating sows will 1) affect bone metabolism and skeletal development of the fetuses and thus of the newborn piglets, and 2) the smaller birth-weight piglets will be the most affected. The objective of this study was to determine the influence of Ca and P intake by young, gestating sows on the growth and skeletal development of their piglets and if smaller birth-weight piglets are at a greater risk of mineral deficiency.

4.2 Materials and methods

The study was carried out at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada). Sows were cared for according to the Prairie Swine Centre Inc. standard operating procedures and the experiment was approved by the University of Saskatchewan's Committee on Animal Care and Supply (UCACS protocol 20130056) for compliance with the Canadian Council on Animal Care guidelines.

4.2.1 Treatments

A total of 30 sows (gilts and parity 1) were randomly assigned to 3 treatment groups consisting of 3 dietary Ca and P levels (Table 4.1). The control treatment for the gestation phase was based on guidelines in the National Research Council (NRC) Nutrient Requirements of Swine 1998 (NRC, 1998; Table 3.1) and the National Swine Nutrition Guide (2010). Retrospective analysis confirmed that these levels also met the requirements of NRC (2012). The other treatment diets were formulated with Ca and P levels 15 % lower and 15 % higher than the control. The ratio of Ca and P was maintained across diets within each phase.

4.2.2 Animals and housing

All sows utilized in this experiment were PIC Camborough Plus (commercial synthetic sow lines) (PIC Canada Ltd. Winnipeg, Canada) and were housed in the gestation room at ambient temperature (16.5 to 20°C). Gilts (parity 0) and parity 1 sows were identified from the breeding log to ensure that each treatment was represented equally across parities. Body weight

of sows ranged from 220 to 230 kg at trial initiation. Within the parity group, sows were randomly assigned to 1 of the 3 treatment groups. All sows were housed in individual stalls (1.65 m × 0.67 m) during gestation. The stalls contained partially slatted concrete flooring. Sows were moved to the farrowing room 1 wk prior to the anticipated farrowing date and maintained in farrowing crates (1.83 m × 2.41 m). The room temperature was set at 22.5°C at the start of farrowing and was gradually reduced to approximately 18.5°C prior to weaning. The crates contained fully slatted metal flooring with a rubber mat in the piglet area. The piglet area (90 cm × 90 cm) had a hood with a heat lamp hanged from the top of the hood. Piglets were cross-fostered within the first 24 h of birth but only within the dietary treatment assigned during gestation. Each gestation stall or farrowing crate had a single space feeder located at the front. Water was provided ad libitum through nipple drinkers located beside the feeders.

4.2.3 Diets and feeding

The trial started when the sows were moved from the breeding to the gestation room (approximately d 28 of gestation) and ended when the piglets were weaned (average 26 d post-farrowing). Gestation phase feeding began when sows were moved to the gestation room from breeding (approximately d 28 of gestation). Sows were fed 2.3 kg/d until 2 wk prior to farrowing, when this allotment was increased to 3.0 kg/d. A standard lactation diet was available ad libitum to all sows on study during lactation (Appendix A-1). With the exception of Ca and P, the barley and wheat-based diets were formulated to meet or exceed NRC 1998 requirements for all nutrients (Table 4.2).

Table 4.1. Feed allotments and dietary Ca and P levels used in gestation

Nutrients	Treatment 1 (-15 % Ca:P)	Treatment 2 Control	Treatment 3 (+15 % Ca:P)
Gestation			
Daily feed intake, kg/d	2.30	2.30	2.30
Ca, %	0.60	0.70	0.81
Total P, %	0.47	0.55	0.63
Lactation[1]			
Daily feed intake[2], kg/d	6.65	6.65	6.65
Ca, %	0.90	0.90	0.90
Total P, %	0.53	0.53	0.53

[1] A standard lactation diet used at Prairie Swine Centre Inc., Saskatoon, SK, Canada was provided ad libitum to all sows during lactation period.
[2] Daily feed intake estimation was based on a previous sow trial conducted at Prairie Swine Centre Inc, Saskatoon, SK, Canada.

Table 4.2. Ingredient and nutrient composition of the three experimental diets used in gestation (% as fed)

Ingredient	Gestation, %		
	-15% Ca:P	Control	+15% Ca:P
Barley	67.59	67.60	67.46
Wheat	21.04	20.49	20.00
Soybean meal	7.00	7.00	7.00
Canola oil	1.20	1.33	1.53
Limestone	0.97	1.13	1.30
Monocalcium P (21.1 % P)	0.87	1.10	1.37
Salt	0.60	0.60	0.60
Choline chloride	0.06	0.06	0.06
Lysine HCl	0.013	0.013	0.017
Minerals[1]	0.10	0.10	0.10
Vitamins[2]	0.17	0.17	0.17
Celite[3]	0.40	0.40	0.40
Nutrients, (formulated)			
DM, %	88.76	88.08	88.87
DE, Mcal/kg	3.19	3.19	3.18
Crude protein, %	16.0	16.0	16.0
SID Lys, %	0.45	0.45	0.45
Ca, %	0.60	0.70	0.81
Total P, %	0.47	0.55	0.63
Available P, %	0.27	0.32	0.37

[1] Provided (per kg of diet): Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; Se, 0.10 mg as sodium selenite.

[2] Provided (per kg of diet): Vitamin A, 8250 IU; Vitamin D_3, 825 IU; Vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; Vitamin B_{12}, 25 ug.

[3] Celite 545, Celite Corporation, Lompoc CA, USA

4.2.4 Data collection

Only sows farrowing 12 or more piglets remained on the experiment. The number of live born, still born, mummies and litter size were recorded. Piglets were weighed on d 3 post-farrowing and prior to weaning. In each litter, the smallest or second smallest piglet (SMALL) and a piglet representing the heaviest 20 % of the piglets (NORM) were selected and birth weight recorded.

4.2.5 Sample collection

Gestation feed samples for each dietary treatment were collected from the 2 batches of feed produced, analyzed and an average reported (Table 4.2). Approximately 8 mL of blood was collected from sows via cranial vena cava venipuncture 2 wk prior to entry to the farrowing room and 1 d prior to weaning. Blood sampling was done in the morning, 2 to 3 h post feeding, using 10-mL blood collection tubes (no additives, BD Vacutainer; Becton, Dickinson and Company, New Jersey, US) and needles (18-gauge × 38-mm; BD PrecisionGlide; Becton, Dickinson and Company, New Jersey, US). Two piglets per litter were selected from each sow. The selected piglets, (SMALL and NORM) were euthanized using a non-penetrating captive bolt (Zephyr-E, Bock Industries, Pennsylvania). Blood was immediately collected by cardiac puncture using two 4-mL blood collection tubes (no additives) to reduce the damage to red blood cells and probability of aggregation due to the higher suction pressure in larger tubes and a 21-gauge needle. Samples were centrifuged at 830 × *g* for 15 min (Beckman TJ-6 Centrifuge, Beckman Coulter, Mississauga, Ontario, Canada) and serum transferred to microcentrifuge tubes. All samples were stored frozen at -20˚C until analysis.

The left hind limb was dissected and removed at the femoral joint. Soft tissues surrounding the femur were removed and the bone length measured. The femur midshaft was marked for the bone scan. The dissected limb with the exposed femur bone was then wrapped with saline-soaked gauze and finally with plastic wrap.

4.2.6 Sample analysis

4.2.6.1 Chemical analysis

All laboratory analyses were conducted at the Department of Animal and Poultry Science, University of Saskatchewan, unless otherwise stated. Feed samples were analyzed for Ca and P using a modification of AOAC 968.08 and 935.13A (Central Testing Laboratory Ltd., Manitoba, Canada). All samples were analyzed in duplicates. Serum samples were analyzed for Ca and P using a COBAS Chemistry Analyzer (Prairie Diagnostic Services Inc., Saskatoon, Canada). Serum samples were also analyzed for the bone markers osteocalcin (OC; intra-assay CV, 2.2 %; interassay CV, 4.9 %) and serum pyridinoline (PYD; intra-assay CV, 1.3 %; interassay CV, 1.6 %) using ELISA kits, which were developed for humans but have been validated for some animal species including pigs (MicroVue Osteocalcin EIA and Serum PYD EIA; Quidel Corporation, San Diego, CA) (Shaw et al., 2006a). The femurs were dried at 100°C for 24 h to obtain a dry weight. Samples were then ashed at 550°C for 24 h and the ash weight was recorded. The ash from each sample was solubilized in 5 mL of 2 *N* HCL for 16 to 24 h. Samples were then analyzed for Ca by atomic absorption spectrophotometry using lanthanum reagent to absorb light at a wavelength of 422.7 nm (Thermo Scientific iCE 3000 Series; Thermo Fisher Scientific, Inc., Waltham, MA) (AOAC 1990, method 968.08; Lauridsen et al., 2010). Phosphorus was determined by a colorimetric method using ammonium vanadate-ammonium molybdate reagent and read on a spectrophotometer at 400 nm (ThermoElectron Helios Delta Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, US) (Stuffins, 1967; AOAC 1990, method 965.17).

4.2.6.2 Peripheral quantitative computed tomography (pQCT) scanning

Prior to ashing, the frozen femur bones (together with the intact left hind limb) were thawed to room temperature. The thawed limbs were then transferred in a cooler box to the College of Kinesiology, University of Saskatchewan for scanning using a Norland/Stratec pQCT (XCT 2000, Stratec Medizintechnik GmbH, Pforzheim, Germany) (Ferretti et al., 1996;

Liesegang et al., 2005, Kontulainen et al., 2008; Buhler et al., 2010; Witschi et al., 2011). Limbs were maintained at room temperature throughout the scanning procedure.

A phantom scan was performed at the beginning of scan day to ensure the instrument was properly calibrated. Each limb was positioned into the pQCT gantry in the exact same position with the lateral side of the limb facing up. The limb was positioned perpendicular to the laser of the gantry (Fig. 4.2). The specimen was aligned so that the laser was focused on the mid shaft of the femur, which had been previously marked. A 2.4 mm slice was scanned using 0.2 mm voxel size. Of the 60 specimens, 30 specimens were randomly selected for repeated measurements. Reproducibility was then measured and calculated as root mean square averages of the standard deviations (SD) of repeated measurements (Glüer et al., 1995):

$$\mathrm{SD} = \sqrt{\sum_{j=1}^{m} \mathrm{SD}_j^2 / m} \quad \text{..... (4.1)}$$

where j= 1….m are the individual subjects. Precision error was calculated on a % basis as coefficient of variation (CV):

$$\mathrm{CV}_{SD} = \left(\mathrm{SD} / \sum_{j=1}^{m} \bar{x}_j / m\right) \times 100\ \% \quad \text{.....(4.2)}$$

Norland/Stratec XCT 5.50 software (Stratec Medizintechnik GmbH, Pforzheim, Germany) was used for the data analysis. At the measured site, the periosteal (outer) boundary was defined with a threshold of 200 mg/cm^3 using contour mode 1. Peel mode 2 with a threshold of 280 mg/cm^3 (inner threshold) was used to separate trabecular from the subcortical compartment. The cortical compartment was defined by using separation mode 4 with an outer threshold of 200 mg/cm^3 and an inner threshold of 280 mg/cm^3. The cortical bone mineral density was determined as the average density of the voxels used to define the cortical compartment. Based on the parameters as defined by the software, the measurements obtained included ToA and CoA (cross-sectional area, mm^2), CRT_CNT (cortical mineral content, mg^2/mm), CRT_DEN (apparent cortical bone mineral density, mg/cm^3) and RP_CM_W (SSI=strength strain index, mm^3), an index for bone strength used in pQCT.

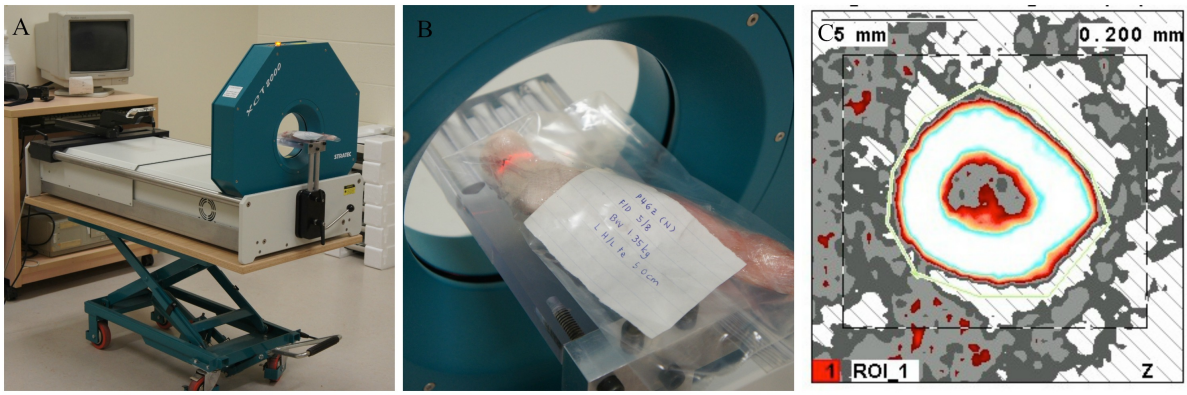

Figure 4.1. Peripheral quantitative computed tomography (pQCT) scan in the midshaft of the femur in a newborn piglet left hind limb; (A) Norland/Stratec pQCT, (B) Limb positioned perpendicular to the laser of the gantry, (C) Scanned region of interest (ROI) at femur midshaft.

4.2.7 Statistical analysis

The effects of dietary Ca and P in the sows' diet during gestation were analyzed by ANOVA using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC) with sow as the experimental unit. The piglet data was analyzed by ANOVA using a split-plot design with diet assigned to the whole plot (sow) and piglet size in the subplot (piglet). All data were checked for normality using the PROC Univariate procedure of SAS. Non-normally distributed data (Shapiro-Wilk test; P-value < 0.05) were analyzed using the Proc GLIMMIX procedure of SAS. P-values less than 0.05 were considered to be significant and a P-value of > 0.05 but ≤ 0.10 was considered a trend. Treatment means were analyzed using the LSMEANS statement with all values reported as LSMEANS. Each ANOVA analysis that revealed significant differences was followed by a multiple comparison procedure using the Tukey-Kramer method. Only main effects are presented if the interactions were not significant.

Correlations between selected variables were analyzed using the CORR function of SAS. A regression model (REG function) was fitted using all possible predictors of bone parameters that were significant and statistics that were diagnostic for collinearity were requested. The variance inflation factor (VIF) was used to check for multicollinearity. VIF values that were more than 10 were of concern. In order (starting with the highest VIF), variables were removed and repeated until all VIF were below 10. To determine which variable contributed the prediction of cortical density, the same variables used in the final regression model (after VIF selection) were entered into a stepwise regression. Variables with $P > 0.15$ were excluded from the final model.

4.3 Results

All 30 sows farrowed 12 piglets or more, therefore remained on the trial. There were no health, reproductive or lameness issues. Analysis of the Ca and P levels in the gestation diet are presented in Table 4.3. The Ca and P % (as fed values) for all dietary treatments were approximately 0.04 to 0.08 % units higher than formulated. The variance between dietary treatments was maintained at about 15 % except for P in the low treatment diet. The Ca:P ratio however, were maintained at a range of 1.1 to 1.3 for all diets.

4.3.1 Sow reproductive performance

Table 4.4 shows the effects of dietary Ca and P on reproductive performance. There were no treatment effects on the number of live born, litter size, d 3 BW, weaning weight or ADG of the piglets ($P > 0.10$). The expected differences in birth weight of the SMALL and NORM piglets were seen with BW's averaging 0.86 and 1.36 kg for the SMALL and NORM piglets, respectively ($P < 0.01$; Table 4.5).

Table 4.3. Formulated and analyzed dietary Ca and P levels in gestation diets[1] (% as fed)

Nutrients	Diet		
	- 15 %	Control	+15 %
Formulated			
Ca, %	0.60	0.70	0.81
Total P, %	0.47	0.55	0.63
Analyzed			
Ca, %	0.64	0.75	0.89
Total P, %	0.54	0.59	0.67
Moisture, %	10.8	11.5	11.2

[1] Feed Ca and P analysis was performed by Central Testing Laboratory Ltd. (Manitoba, Canada) and values represent an average of 2 batches of samples per diet).

Table 4.4. Main effects of dietary Ca and P (15 % variance from 1998 NRC recommendations) in the diet of gestating sows on reproductive performance[1]

Item	Diet			SEM	*P*-value
	-15%	Control	+15%		
Liveborn[2], *n*	15.5	15.4	14.4	0.8	0.53
Total[3], *n*	16.3	15.8	15.3	0.7	0.58
d 3 wt[4], kg	1.49	1.53	1.59	0.06	0.58
CV_{d3wt}[5], %	14.67	19.21	17.25	1.50	0.12
Weaning wt[6], kg	6.93	7.59	6.84	0.35	0.27
ADG[7], kg/piglet·d^{-1}	0.22	0.23	0.21	0.01	0.18

[1] A total of 30 sows (gilts and parity 1) were randomly assigned to 3 dietary Ca and P treatments.
[2] Number of piglets born alive per litter.
[3] Total number of piglets born/litter (Live + stillborn + mummies).
[4] Piglet average BW at d 3.
[5] Coefficient of variation of piglets d 3 BW.
[6] Piglet average weaning weight (average lactation d 26).
[7] Average daily gain of piglets throughout lactation.

Table 4.5. The effects of dietary Ca and P and piglet size on birth weight[1]

Item	Diet			SEM	Size of piglet		SEM	*P*-value		
	-15%	Control	+15%		SMALL	NORM		Diet	Size	Diet × Size
Birth wt, kg	1.16	1.04	1.12	0.05	0.86	1.36	0.04	0.30	<0.01	0.33

[1] Two piglets per litter (*n* = 30 sows) were selected; the smallest or second smallest piglet in each litter (SMALL) and the heaviest 20 % of the piglets in each litter (NORM).

4.3.2 Sow and piglet serum constituents

An effect of dietary Ca and P on serum total Ca of sows was observed at weaning ($P < 0.01$; Table 4.6). Sows that were fed lower Ca during the gestation period had the highest level of serum Ca prior to weaning while sows fed the high Ca:P treatment had serum Ca levels comparable to the control. There was no effect of dietary Ca on serum Ca at d 100 of gestation ($P > 0.10$). Dietary Ca and P had no effect on the concentration of serum P, serum OC or serum PYD at late gestation or prior to weaning ($P > 0.10$).

A diet by piglet size interaction was observed for serum total Ca of piglets at birth ($P = 0.04$; Fig. 4.2). The SMALL piglets from sows fed the high levels of Ca during gestation had the highest level of serum Ca at birth while those from control sows had the lowest level of serum Ca. In contrast, no differences were observed in the serum Ca concentrations of NORM piglets. Values at weaning calculated as deviations from d 0 showed a diet by size interaction where the SMALL piglets produced by sows fed the high Ca diet had the smallest increase in serum Ca prior to weaning ($P = 0.02$). Neither gestational diet Ca and P concentration nor piglet size affected serum P levels of the piglets at birth, but NORM piglets had higher concentrations of serum P prior to weaning ($P = 0.01$; Table 4.7). NORM piglets had a greater increase in serum P from d 0 to weaning ($P = 0.05$) compared to SMALL piglets ($P = 0.05$). Comparable to that seen with the sows, dietary Ca and P did not affect the levels of serum OC and PYD of piglets either at birth or at weaning ($P > 0.10$; Table 4.7).

Table 4.6. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) on serum Ca and P (postprandial) and bone markers, osteocalcin and pyridinoline, in sows[1]

Serum constituent	Diet			SEM	*P*-value
	-15%	Control	+15%		
Serum Ca, mmol/L					
d 100 Gestation	2.51	2.45	2.47	0.03	0.30
Wean[2]	2.66[a]	2.52[b]	2.52[b]	0.03	<0.01
Serum P, mmol/L					
d 100 Gestation	2.33	2.23	2.27	0.05	0.37
Wean[2]	2.21	2.17	2.15	0.07	0.83
Serum osteocalcin, ng/mL					
d 100 gestation	375.2	397.0	327.2	35.9	0.39
Wean[2]	181.2	184.8	139.9	18.9	0.19
Serum pyridinoline, nmol/L					
d 100 gestation	6.81	7.42	6.98	0.31	0.37
Wean[2]	7.36	6.86	7.20	0.52	0.79

[1] A total of 30 sows (gilts and parity 1) were randomly assigned to 3 dietary Ca and P treatments.
[2] Serum samples collected at lactation d 26 ± 2 from all 30 sows..
[ab] Within a row, means without a common superscript differ ($P < 0.05$).

Table 4.7. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) for gestating sows and piglet size on Ca and P and bone markers, osteocalcin and pyridinoline, in piglet serum[1]

Serum constituent	Diet			SEM	Size[2]		SEM	*P*-value		
	-15%	Control	+15%		SMALL	NORM		Diet	Size	Diet × Size
Serum Ca, mmol/L										
d 0 (birth)	2.45	2.23	2.48	0.09	2.45	2.32	0.07	0.13	0.21	0.04[3]
Weaning[4]	2.84	2.76	2.80	0.03	2.77	2.83	0.03	0.26	0.10	0.13
Change, d 0 to weaning[5]	0.41	0.58	0.35	0.09	0.33	0.56	0.07	0.21	0.03	0.02[3]
Serum P, mmol/L										
d 0 (birth)	2.06	1.91	1.98	0.10	1.97	1.99	0.08	0.55	0.85	0.30
Weaning[4]	3.11	3.11	3.09	0.04	3.04	3.16	0.03	0.96	0.01	0.11
Change, d 0 to weaning[5]	1.06	1.30	1.16	0.09	1.07	1.28	0.07	0.15	0.05	0.31
Serum osteocalcin, ng/mL										
d 0 (birth)	77.7	77.7	68.2	10.0	65.6	83.4	8.2	0.73	0.13	0.31
Weaning[4]	401.0	400.0	357.5	29.9	374.6	397.7	24.4	0.51	0.51	0.80
Change, d 0 to weaning[5]	325.1	325.8	294.8	32.0	311.3	319.2	26.2	0.74	0.83	0.68
Serum pyridinoline, nmol/L										
d 0 (birth)	88.9	111.0	74.1	29.9	89.2	93.4	24.4	0.69	0.90	0.65
Weaning[4]	6.3	5.8	5.9	0.4	5.8	6.3	0.4	0.65	0.31	0.60
Change, d 0 to weaning[5]	-92.6	-116.4	-78.2	31.9	-90.8	-94.0	26.0	0.66	0.93	0.50

[1] A total of 30 sows (gilts and parity 1) were randomly assigned to 3 dietary Ca and P treatments. Serum samples were collected from 2 piglets per litter; the smallest or the second smallest piglet and an average-sized piglet.
[2] SMALL = smallest or second smallest piglet in each litter; NORM = heaviest 20 % of the piglets in each litter.
[3] Diet by size interactions presented in Fig. 4.3 ($P < 0.05$).
[4] Serum samples collected at lactation d 26 ± 2.
[5] Difference between values at weaning and d0 (within 24 h of birth).

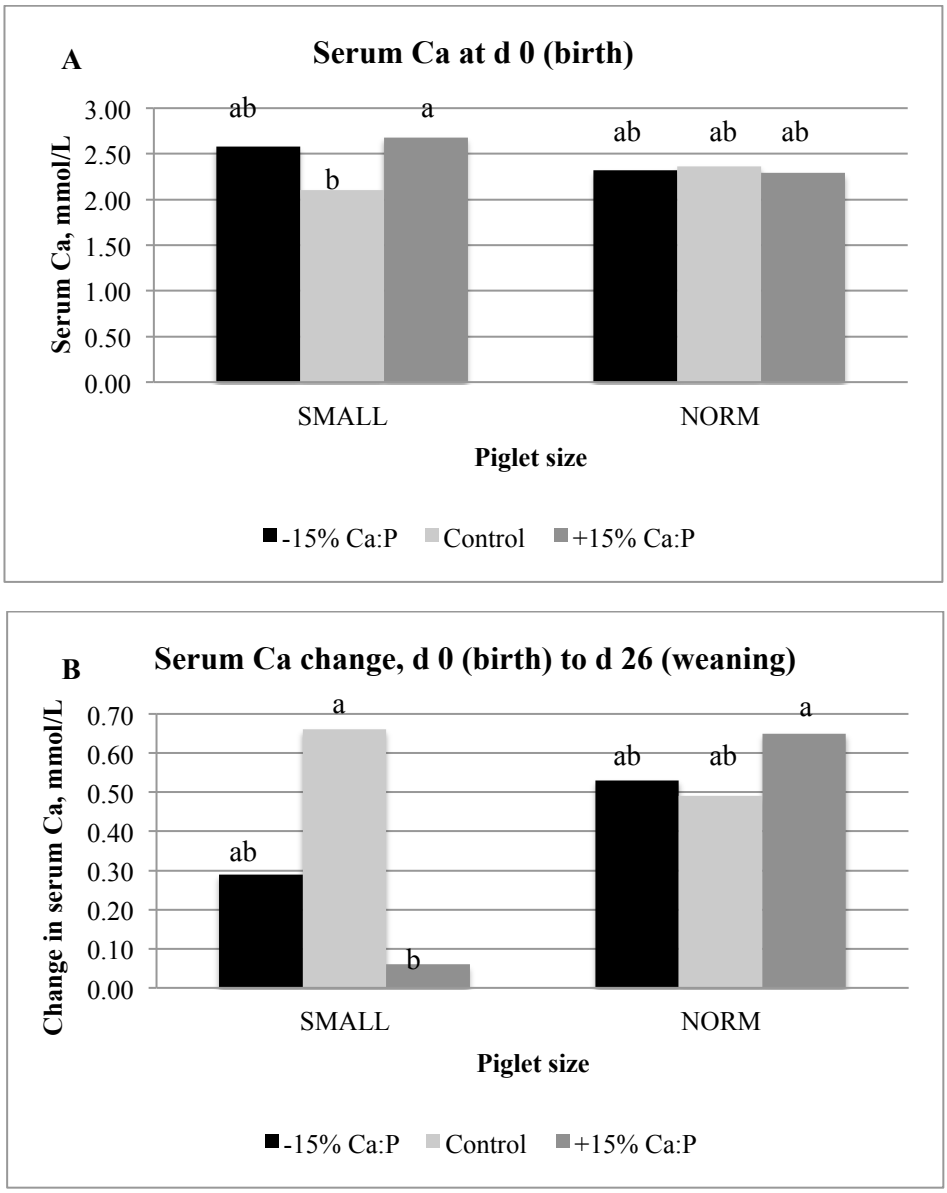

Figure 4.2. The effects of dietary Ca and P (15 % variance from 1998 NRC recommendations) for gestating sows and piglet size on serum Ca concentration of piglets (SMALL = smallest or second smallest piglet in each litter; NORM= heaviest 20 % of the piglets in each litter) on serum Ca of piglets *(P* < 0.05); (A) d 0; SEM = 0.13, (B) Change, d 0 (birth) to weaning (d 26); SEM = 0.12. Means with the same letter are not significantly different (*P* > 0.05).

4.3.3 Piglets' bone parameters

The expected differences in the fresh weight, dry weight and ash weight of femoral bones were observed between the SMALL and NORM piglets at birth; femoral bones from NORM piglets were heavier (fresh, dry or ash weights) ($P < 0.01$; Table 4.8). Except that SMALL piglets had a tendency for higher ash % compared to the NORM piglets ($P = 0.07$), the fresh weight, dry weight and ash weight, when calculated as percentages of DM showed no differences due to dietary treatment or piglet size ($P > 0.10$). Femoral bone ash Ca and P were also not affected by sows' dietary Ca and P or piglet size ($P > 0.10$).

Femoral bone scans using pQCT revealed significant differences in bone parameters between the SMALL and NORM piglets. Based on the scans, SMALL piglets had smaller total area, cortical bone area, cortical bone content and SSI ($P < 0.01$; Table 4.9). Femoral bones of piglets from sows fed the low Ca and P diet during gestation had the highest cortical density while the lowest cortical density was seen in piglets produced from sows fed the control diet ($P = 0.03$). Piglet size did not influence the cortical density of the femoral bone ($P > 0.10$).

Further analysis showed that cortical density was positively correlated with piglet birth weight (r = 0.31; $P = 0.02$), cortical content (r = 0.46; $P < 0.01$), bone strength (r = 0.36; $P < 0.01$) and bone ash (r = 0.42; $P < 0.01$; Table 4.10). The strong positive correlation between SSI and birth weight (r = 0.90) and cortical content (r = 0.94) indicated possible collinearity between the variables. Apart from a positive correlation with cortical density, bone ash was also positively correlated with the d 3 litter weights (r = 0.34), ash Ca % (r = 0.43) and ash P % (r = 0.41) whereas a tendency for a positive correlation was reported with litter size (r = 0.23; $P = 0.08$). Table 4.11 shows the final regression model after VIF selection with cortical density as dependent variables whereas Table 4.11 shows the regression equations for the effects of birth weight, bone ash composition and bone scan parameters on cortical density. Bone ash % explained only 19 % of the variations in cortical density ($R^2 = 0.19$; $P < 0.01$). Inclusion of birth weight in the equation increased the R^2 ($R^2 = 0.31$; $P < 0.01$) and it was further improved when cortical area was also included ($R^2 = 0.41$; $P < 0.01$).

Table 4.8. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) and piglet size on ash, Ca and P content of femoral bone[1]

Bone constituent	Diet			SEM	Size[2]		SEM	*P*-value		
	-15%	Control	+15%		SMALL	NORM		Diet	Size	Diet × Size
Weight, g	6.26	5.87	6.15	0.27	4.69	7.50	0.22	0.58	<0.01	0.33
DM, g	1.88	1.72	1.85	0.08	1.39	2.24	0.07	0.34	<0.01	0.30
Ash, g	0.80	0.71	0.78	0.04	0.60	0.93	0.03	0.21	<0.01	0.29
DM, %	30.16	29.25	30.05	0.51	29.66	29.98	0.42	0.40	0.59	0.43
Ash[3], %	42.63	41.68	42.31	0.69	42.94	41.48	0.57	0.61	0.07	0.85
Ca[4], %	23.53	22.57	21.72	1.20	23.41	21.80	0.98	0.57	0.25	0.92
P[4], %	16.40	16.12	16.05	0.13	16.22	16.16	0.10	0.11	0.70	0.57
Ca:P	1.44	1.42	1.37	0.08	1.48	1.34	0.06	0.81	0.10	0.61

[1] A total of 30 sows were randomly assigned to 3 dietary Ca and P treatments. The left femoral bone was obtained from 2 piglets per litter.
[2] SMALL = smallest or second smallest piglet in each litter; NORM = heaviest 20 % of the piglets in each litter.
[3] Bone ash reported as % of dry matter (DM).
[4] Ca and P reported as % of bone ash content.

Table 4.9. Main effects of dietary Ca and P (15% variance from 1998 NRC recommendations) and piglet size on geometric and densitometric parameters of femoral bone[1]

Bone scan parameter	Diet			SEM	Size[2]		SEM	CV[3]	*P*-value		
	-15%	Control	15%		SMALL	NORM			Diet	Size	Diet × Size
Total area, mm^2	35.0	32.8	34.2	1.0	29.5	38.5	0.9	5.33	0.32	<0.01	0.34
Cortical											
Area, mm^2	27.7	27.1	27.7	1.1	22.9	32.0	0.9	7.47	0.89	<0.01	0.41
Density, mg/cm^3	712.1	660.0	691.2	13.9	675.7	699.8	11.3	4.04	0.03	0.14	0.72
Content, mg/mm	19.7	18.0	19.2	0.8	15.6	22.4	0.7	2.15	0.35	<0.01	0.46
SSI[4], mm^3	27.2	24.1	26.5	1.2	20.1	31.7	1.0	5.66	0.15	<0.01	0.19

[1] A total of 30 sows were randomly assigned to 3 dietary Ca and P treatment. The left femoral bone was obtained from 2 piglets per litter; the smallest or second smallest piglet and an average-sized piglet in each litter.
[2] SMALL = smallest or second smallest piglet in each litter; NORM = heaviest 20% of the piglets in each litter.
[3] Root-mean-square average of precision errors (CV in %) for 2-point repeated measurements of 30 samples with repositioning.
[4] Strength strain index (SSI).

Table 4.10. Pearson correlation coefficient (r) and significance level (*P*-value) of all variables evaluated

	Litter, n	Dietary Ca, %	Dietary P, %	Birth wt, kg	Total d 3 wt, kg/litter	Total area, mm^2	Cortical			SSI, mm^3	Ash Ca, %	Ash P, %
							Density, mg/cm^3	Content, mg/mm	Area, mm^2			
Dietary Ca, %	-0.09											
Dietary P, %	-0.09	1.00***										
Birth wt, kg	-0.22	-0.06	-0.06									
Total d 3 wt, kg/litter	0.43***	0.02	0.02	0.01								
Total area, mm^2	-0.15	-0.05	-0.05	0.85***	0.09							
Cort density, mg/cm^3	0.03	-0.12	-0.13	0.31*	0.04	0.05						
Cort content, mg/mm	-0.12	-0.03	-0.04	0.83***	0.15	0.82***	0.46***					
Cort area, mm^2	-0.16	<0.01	<0.01	0.81***	0.14	0.90***	0.13	0.93***				
SSI, mm^3	-0.18	-0.03	-0.03	0.90***	0.10	0.92***	0.36**	0.94***	0.91***			
Ash Ca, %	0.39**	-0.14	-0.14	-0.25	0.27*	-0.21	0.09	-0.06	-0.07	-0.18		
Ash P, %	0.39**	-0.27*	-0.27*	0.10	0.09	0.19	0.17	0.08	0.03	0.13	0.09	
Bone ash, %	0.23[1]	-0.04	-0.04	-0.13	0.34**	-0.05	0.42***	0.16	0.01	0.04	0.43***	0.41***

[1] $0.05 < P < 0.10$

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Table 4.11. Final regression model after VIF selection with cortical density as the dependent variable

Variables	Parameter estimate	SE	*P*-value	VIF[1]	Model probability > F	Adj R^2
Birth weight, kg	229.60	64.91	<0.01	8.88	<0.01	0.36
Bone ash, %	12.70	2.97	<0.01	1.71		
Cortical area, mm^2	-4.91	1.99	0.02	3.32		
Ash P, %	-22.99	16.58	0.17	1.75		
Dietary Ca, %	-104.89	86.57	0.23	1.12		
Total d 3 wt, kg/litter	-3.71	3.16	0.25	1.44		
Piglets/litter, n	2.86	4.55	0.53	1.97		
Ash Ca, %	0.37	1.67	0.82	1.60		

[1] All variables in the model have been tested for multicollinearity by using variance inflation factors (VIFs). The highest values of VIF were removed stepwise until all VIFs were below 10. Dietary P was removed from the model due to autocorrelation with dietary Ca.

Table 4.12. Prediction equations of cortical density, mg/cm^3 from various independent variables using stepwise regression[1]

Eq. No.	Equation	R^2	*P*-value
1	Cortical density = 187.58 + 157.7 × Birth[2] - 5.38 × Cortical area[3] + 11.12 × Bone ash[4]	0.41	0.005
2	Cortical density = 190.81 + 70.28 × Birth + 9.86 × Bone ash	0.31	0.004
3	Cortical density = 299.51 + 9.14 × Bone ash	0.19	0.001

[1] Variables tested were variables from the final regression model after VIF selection. All variables left in the model are significant at $P = 0.15$.
[2] Piglet wt within 24 h after birth.
[3] Cortical area, mm^2.
[4] Femur bone ash as % of DM.

4.4 Discussion

Several studies have shown that the majority of fetal Ca accretion occurs during late gestation with humans and rats accruing about 80 % and 95 %, respectively, of the required Ca (Kovacs and Kronenberg, 1997). The rapid demand for Ca by the developing fetus is required for skeletal mineralization and growth. Apart from using the placenta, the fetus utilizes the kidneys, bones and intestines, which are comparable to the adult human to maintain serum mineral concentrations and simultaneously, providing sufficient Ca (among other minerals) to enable mineralization of the fetal skeleton (Kovacs, 2006). In pigs, past studies have reported a similar pattern where the rate of Ca deposition in the fetuses increased as gestation advanced, particularly during the final trimester (Hansard et al., 1966). The increased demand for Ca by the fetus will be met from ingestion of feed, maternal Ca stores and deposits of nutrients in the placenta (Itoh et al., 1967).

Consistent with results from Exp. 1 (Table 3.7), Ca and P content of the diet fed to the gestating sows did not influence reproductive performance. The young sows in this study were highly prolific, averaging 16 piglets per litter. The smallest piglets had an average birth weight of 0.86 kg while the normal-sized piglets averaged 1.36 kg. Within-litter variation in piglet birth weight may be affected by several factors that influences fetal growth and development such as genetics, distribution of blood flow and maternal nutrition (Campos et al., 2012). Quiniou et al. (2002) showed that as litter size increased from 11 to 16 piglets, within-litter birth weight variation increased from 16 to 24 % The mean within-litter CV for d 3 BW in the current study was approximately 17 % with sows having an average litter size of 16 piglets, comparable to the data reported by Milligan et al. (2001) but lower than that obtained by Quiniou et al. (2002) for birth weight. A study by Milligan et al. (2001) showed that although BW CV of piglets was similar at d 0 (birth) and d 3 of lactation, the BW CV of piglets was slightly lower at d 21 of lactation compared to d 3, suggesting that this may be due to the increasing mean BW over time. Based on the current study, as BW measurement was recorded on d 3, the BW CV would not have differed much from the variation in BW at birth. Additionally, Quesnel et al. (2008) reported that young sows (parity 1 and 2) had less heterogeneous litters compared to older parity sows thus, a lower birth weight CV (20 %) compared to older sows (25 %). Further, a lower CV observed in the current study can also be attributed to the improvement and uniformity of sow genetics for uterine capacity compared to those used in studies reported in the previous years. A negative correlation was seen between uterine blood flow and litter size in an earlier study (Père and Etienne, 2000),

suggesting that a reduced blood flow per fetus in a large litter will affect nutrient delivery and fetal growth. According to Wu et al. (2006), fetal growth is a complex process influenced by genetic and environmental factors, and maternal maturity. These factors can affect the utero-placental blood flow and transfer of nutrients from the sow to the fetus, which ultimately leads to intrauterine growth retardation. In our study, sows produced between 15 to 16 piglets, regardless of treatment thus, the lower fresh weight, DM weight and ash weight of the femoral bone of the SMALL piglets suggests a possible limitation of fetal growth and development during gestation. Older studies have reported that maternal undernutrition during gestation reduced fetal growth and subsequently caused a reduction in piglet birth weight (Pond et al., 1969; Buitrago et al., 1974). Unfortunately recent data using the modern, prolific sow is lacking. Furthermore, our current study did not show an effect of maternal dietary Ca and P levels on the birth weight of piglets, suggesting that there are other non-nutritional factors involved as previously mentioned.

Maternal serum Ca and P were unaffected by dietary Ca and P levels at d 100 of gestation. These concentrations were slightly higher than the piglets' serum total Ca and P at birth. This may be due to an abrupt loss of Ca infusion from the placenta after birth following the separation of the piglet from the umbilical cord (Kovacs, 2006). Studies in the past have shown that the concentration of serum Ca is usually maintained at a higher level in fetus than in maternal serum (Itoh et al., 1967). According to this study (Itoh et al., 1967), when dietary Ca was adequate, absorbed Ca was transferred to the fetus and less was deposited into maternal bones, indicating that maternal intake influences the concentration of Ca in fetuses. As described by Kovacs (2006), in humans, this suggests that the maternal skeleton, being a source of Ca for the fetus, can be compromised should a deficiency in mineral intake occur. At weaning, sows fed the low level of Ca during gestation had the highest level of serum total Ca and levels were similar between sows fed the control and high Ca diet despite the same lactation diet being fed to all sows. In fact, serum Ca levels of sows fed the low level of Ca, were higher than what is considered to be within the normal reference range as suggested by Weber et al. (2014). Lactation ADFI was not recorded in his study however; since sows were all gilts or parity 1, and balanced across treatment, we assumed that the feed intake level would be comparable across treatments. If this is true, it indicates that maternal adaptive mechanisms such as intestinal Ca absorption, as described by Kovacs and Kronenberg (1997); Prentice (2000); Kovacs (2005); and Abrams (2007), might have played a role in maintaining constant serum mineral concentrations during the periods of high demand even though one of the diets was lower in Ca. Following a review of the literature (Kovacs and

Kronenberg, 1997), it was suggested that intestinal Ca absorption is probably the major maternal adaptation to meet fetal demands for Ca during pregnancy and increases in fasting urinary Ca can be attributed to the increased intestinal Ca absorption. This is also supported by the lack of an effect of the dietary Ca and P levels on bone formation and resorption markers in the current study. Serum P concentrations followed a similar trend as serum total Ca but the levels were not affected by dietary P levels during gestation at either time point.

Normal-sized piglets had similar levels of serum Ca at birth regardless of the Ca and P in the diet of their sows during gestation, while the serum Ca of the small piglets was affected by the Ca intake of their sows. These results are in contrast with previous studies which showed that the concentration of serum Ca in piglets was unaffected by sows receiving inadequate levels of Ca in their diets (Mahan and Fetter, 1982; Mahan and Vallet, 1997), but suggested to be due to bone resorption in the sow (Liesegang et al., 2005). In our study, the sows' serum Ca and bone markers were unaffected at late gestation by dietary Ca or P, indicating that the rate of bone metabolism in the sow was similar. A newborn loses its Ca source abruptly when separated from the placenta, and is required to rapidly adjust its mineral homeostasis regulation via adaptive mechanisms such as intestinal Ca absorption, mobilization of Ca from the bones or increased renal Ca reabsorption to maintain the extracellular Ca levels while at the same time, sustaining Ca accretion for skeletal growth (Kovacs, 2006). In a review of the literature by Kovacs and Kronenberg (1997), studies in human (Schauberger and Pitkin, 1979) and rats (Garel and Barlet, 1976) showed that serum total Ca decreases within the first few hours of birth before increasing gradually in succeeding days and weeks. In the current study, blood samples from newborn piglets were collected within 2 to 24 h after birth; hence, this may have allowed the newborn to begin adjusting its minerals homeostatic regulation. Since neither birth weight nor gestational dietary Ca and P affected piglets' serum bone markers, this suggests the influence of other factors such as passive transfer of Ca from milk or colostrum (Institute of Medicine (US) Committee, 2011) or increased efficiency of intestinal Ca absorption. It is also possible that due to the variation in blood collection time, this may have increased the analytical variability of the results. Therefore, to minimize some of this limitation, the timing of sample collection should be more controlled.

Although the levels of bone markers in piglets serum at birth and prior to weaning were not affected by gestational dietary Ca and P, normal-size piglets had higher serum Ca and P concentrations compared to the smaller piglets prior to weaning, The levels of serum Ca and P however, were within the range of previously published reference values for piglets

at weaning (Friendship et al., 1984; Witschi et al., 2011). These results may indicate that adaptive mechanisms of the piglets maintained the serum mineral levels despite the differences in piglet body weight at weaning. The greater concentration of the bone resorption marker and lower concentration of the bone formation marker at birth relative to weaning may be attributed to the abrupt loss of a Ca source at birth, and a result of the newborn piglets' attempt to maintain a normal serum Ca level and at the same time, continuing Ca accretion for skeletal growth (Kovacs and Kronenberg, 1997).

The femur has been used by others to assess mineralization in bones of young pigs of approximately 8 wk of age (Crenshaw et al., 1981). In agreement with the values reported here, the percentage of Ca and P, and the Ca/P ratio of femur bone ash of newborn piglets was unaffected by differing dietary Ca and P treatments fed to gestating gilts (Forsyth et al., 1972). The Ca content and Ca/P ratio however, were lower than the values stated in a review by Crenshaw (2001). Piglet age however, is not clearly stated in their study. Serum Ca values in the current study are comparable to values reported by Witschi et al. (2011) in piglets at lactation d 21 and 33 (just before weaning). According to Hayes (1976) as cited by Crenshaw (2001), total bone ash may change in response to nutrient status but the composition of Ca and P in ash will remain constant. This was confirmed in the current study where gestational dietary Ca and P did not influence bone ash % but small-sized pigs had a tendency for a higher bone ash content compared to normal-sized piglets.

Volumetric density acquired from pQCT provides a better expression of BMD than area density measurements obtained by the more common technique, dual-energy X-ray absorptiometry (DXA) that is influenced by bone size (Ferretti, 1999 and Wilnecker, 2003). The same authors stated that as pQCT takes into consideration the volume rather than size, the measured cortical density would not be affected by the size of the bone. Peripheral quantitative computed tomography is therefore a more suitable tool to determine bone parameters in children or growing animals (Binkley and Specker, 2000; Willnecker, 2003). In the current study, the total area, cortical area and cortical BMC of the normal-sized piglets were higher than the small-sized piglets.

Sow dietary Ca and P during gestation did not influence the area measurements, but an effect of diet on cortical density was observed. An older study by Forsyth et al. (1972) reported that the density of the humerus in new born piglets increased as dietary Ca intake of sows increased. The current study also showed a higher cortical density of piglets from sows fed the high Ca diet compared to the control diet. Surprisingly, however, the highest cortical density was seen in piglets from sows fed the low Ca diet. It is not clear why this may have

occurred. It is unlikely that the low cortical density seen in the femurs from piglets from sows fed the control diet was caused by the underestimation of density due to partial volume effect (PVE), one of the disadvantages of pQCT when cortical thickness is below 2 mm (Binkley, 2007). A pQCT scan is divided into square area units (pixels). As each bone slice has a predetermined thickness, the pixels in the image are actually the volumetric equivalent (voxels). In PVE, the voxels located close to the bone edge (cortical bone) usually represent bone and soft tissue (connective tissue such as tendons, ligaments, fibrous tissue and fat and non-connective tissue such as muscle). These voxels with overlapping bone and soft tissue will have lower density resulting in an underestimation of the real density of the bone (Binkley and Specker, 2000; Leonard and Bachrach, 2012). The cortical thickness of the femur bones in our study had an average of 1.9 mm regardless of treatment; thus, the presence of PVE-derived errors would have been similar across treatments. In human fetal bone mass, a poor correlation between bone density and conceptual age suggested the presence of individual variability during intrauterine growth (Panattoni et al., 1999). Even so, our statistical analysis showed a significant difference in cortical density due to diet, despite the possibility of a high variability between individual piglets. Bone resorption occurs through a reduction in bone density or bone wall thickness (Taylor et al., 2009) and large increases in bone density tend to correlate with reductions in markers of bone resorption such as C-terminal telopeptide (CTX) (Ettinger et al., 1999; Faulkner, 2000). In the current study, PYD concentration, a marker of bone resorption, in piglets was not affected by the dietary treatments fed to sows during gestation. Although Kovacs (2006) stated that in humans, the main source of Ca for the fetus is via placental transfer, the author also mentioned other possible routes including renal Ca reabsorption, Ca reabsorption from urine or amniotic fluid and Ca resorption from the developing skeleton. As bone resorption of piglets from sows fed the low Ca diets was not any higher than other dietary treatments, it can be assumed that the fetuses utilized other mechanisms involving the intestine or renal to maintain Ca homeostasis. Moreover, the different histological structure of the placenta between humans and pigs suggests differences in placenta permeability which modifies maternal transfer of nutrients to the fetus (Furukawa et al., 2014). Several lines of evidence in rats (Yeh and Aloia, 1986; Ferretti et al., 1978) and human (Mathis et al., 2013) studies suggest that glucocorticoids (such as cortisol) produced by the adrenal gland may alter Ca metabolism by increasing bone resorption and reducing intestinal Ca absorption. Cortisol levels were not measured in this study, however, as bone resorption did not differ between treatments, it would be likely that the hormone levels were not different between treatments.

In our study, serum Ca and P levels of sows at d 100 gestation were unaffected by dietary Ca and P, suggesting a regulation of maternal adaptive mechanisms to maintain Ca and P levels. Moreover, studies in the past showed that inadequate dietary Ca and P for sows does not negatively affect the concentration of these minerals in the developing fetus or in the milk (Mahan and Vallet, 1997) and the maternal skeleton is known as a potential source of Ca to meet demands of the developing fetus (Liesegang et al., 2005; Kovacs, 2006). The current study was conducted over only one reproductive cycle; therefore it is not known if the dietary Ca and P fed during gestation would alter the bone metabolism of sows if treatments had been carried out over several parities. Maxson and Mahan (1986) showed that parity had a greater influence on the sow's mineral skeletal reserves than dietary Ca and P, with older sows having greater bone mineral loss compared to the younger sows. Studies have shown minimal evidence of compromised bone mineral density in multiparous women (Henderson III et al., 2000; Lenora et al., 2009), however, lower bone density may be associated with age at first pregnancy (Sowers et al., 1992).

In the current study, bone strength of the femur in piglets was unaffected by dietary Ca and P of sows during gestation. Older studies in growing pigs have reported increased bone strength when pigs were fed higher levels of Ca and P (Miller et al., 1962; 1964; Cromwell et al., 1970; Nimmo et al., 1980; Crenshaw et al., 1981), although the response differed among bones and age groups of pigs (Crenshaw et al., 1981). Those studies involved feeding the dietary Ca and P treatments directly to the growing pigs whereas the current study was designed to examine whether the effect of dietary Ca and P fed to sows during gestation would be detected in their fetuses. The pQCT-derived bone strength (BSI) or stress-strain index (SSI) is a commonly used index to assess bone strength. Studies in rats and mice have shown that BSI, a product of BMD and the cross sectional moment of inertia, is a better predictor of bone strength than BMD or BMC (Siu et al., 2003). Kontulainen et al. (2008) reported that 77 % of the variation in bending strength of the tibial diaphysis could be explained by strength index, SSI. In contrast, BSI is more often used to measure bone strength at distal sites on bones, which are loaded more in compression than in bending (Kontulainen et al., 2003; Wetzsteon, 2007). The SSI was therefore used in the current study to assess femoral bone strength at midshaft. Bone mineral density provides a measure of bone mass however, analysis of bone mass such as BMD or BMC should also determine if it will be adequate for bone functions such as providing strength for physical support (Schoenau et al., 2004). Bone material properties and bone geometry are important for bone strength (Cointry et al., 2004). There is very little variation in bone material properties between

individuals (Cointry et al., 2014), whereas bone geometry, is highly variable among individuals and has been shown to be correlated with bone strength (Willnecker, 2003; Cointry et al., 2014). This is important in determining bone strength because a larger bone would be stronger than a smaller bone even if they had comparable mineral density (Clarke, 2008). This is agreement with the current study, where even though cortical density was not affected by piglet size, the larger piglets, with larger bone area measurements had stronger bones than the small-sized piglets.

No correlation was observed between dietary Ca and P concentration in the sows' diets and bone parameters of the piglets. The bone ash percentage in the piglets however, was positively correlated with the total litter weight on d 3 and had a tendency for a positive correlation with litter size. This may imply that, apart from the number of piglets per litter, the total litter weight may also contribute to the bone ash percentage rather than the individual birth weight of piglets. Apart from the bone density measurements, the pQCT results (cortical area, cortical mineral content and SSI) are consistent with the lack of an effect of gestational dietary Ca and P on bone area, cortical mineral content or bone strength. The absence of correlation further supports the theory that fetal skeletal development is maintained even if there is a deficiency in dietary Ca and P levels during gestation. Cortical density was correlated with birth weight, cortical mineral content, SSI, and bone ash. Similar correlations between bone density and bone ash were reported in laying hens (Schreiweis et al., 2003) and growing pigs (Liesegang et al., 2002), which possibly indicates bone resorption should a reduction in bone density was seen. However, regression analysis showed birth weight, cortical area and bone ash % were the best predictors of cortical density with bone ash explaining 19 % of the variation in cortical density. The combination of these factors explained 41 % of the variance of cortical density, indicating the involvement of other factors.

Results from this study suggest that a moderate increase or decrease to dietary Ca and P levels for young, reproducing sows during gestation do not negatively affect the bone metabolism and skeletal development of the newborn piglets. Other minerals, such as Mg, Zn, Cu, Mn and Fe and vitamins B and D are also involved in this process but to a reduced extent (van Riet et al., 2013). Additionally, an older study has reported that the levels of F in the maternal diet did not influence the Ca and P content of the piglets' skeleton (Forsyth et al., 1972). In the current study, all diets were formulated to include minerals and vitamins as premixes to meet or exceed the requirements as recommended by NRC (2012); hence an effect of these nutrients is not expected.

Based on this study, the smaller piglets at birth are not at a higher risk of mineral deficiency. Although small piglets from sows fed the control diet had lower serum Ca at birth, serum Ca was similar regardless of the size of piglets at weaning. On the other hand, although serum P was similar between the small and normal-sized piglets at birth, small-sized piglets had lower serum P at weaning. These results, combined with the lack of significance on the bone markers would suggest that apart from mobilization of Ca and P from the bones, other adaptive mechanisms such as increased intestinal Ca absorption and reduced renal Ca excretion may play a role in maintaining the levels of Ca and P in the. Estimates of bone density from the pQCT results suggest that the gestational diets did not negatively affect the cortical density of the newborn piglets. In fact, sows fed the low Ca diet produced piglets with the highest cortical density. This result further supports the current understanding that fetal skeletal development and serum Ca concentration will not be compromised unless maternal Ca intake is severely deficient. Femoral bone analysis showed that cortical density of the small piglets were similar to the normal-sized piglets despite the size differences thus, suggesting that the smaller piglets at birth did not appear to have a higher risk of mineral deficiency.

Based on this study, it can be concluded that moderate changes to dietary Ca and P levels for young, gestating sows did not negatively affect the growth and skeletal development of their piglets and smaller birth-weight piglets did not appear to be at a greater risk of mineral deficiency. Therefore, the current recommended levels of dietary Ca and P for gestating sows are adequate for the young, high-producing sows of the modern genetics. The gestational diets, however, were fed for a single parity and further research is required to examine if similar results will be obtained over consecutive parities. This study provides information to better understand the role of gestational dietary Ca and P, apart from other nutritional factors, that may influence the events that occur during gestation which have an impact on fetal growth and development and thus, important to achieve overall improved efficiency.

5 GENERAL DISCUSSION AND CONCLUSIONS

These studies have provided important information on dietary Ca and P requirements in modern, highly prolific sows during gestation. These studies were particularly useful as the evaluation of dietary Ca and P was also compared for gestating sows in two different housing systems. The first study focused on the effects of the current recommended levels of Ca and P during gestation and lactation on sow reproductive performance and bone metabolism and whether the housing system affects these requirements. The second study determined the effects of these minerals in the diet of younger sows during gestation on their piglets' skeletal development and whether the smaller birth weight piglets are at a higher risk for mineral deficiency than their heavier littermates.

The results of these studies confirm that moderate alterations to the current recommendations (NRC 2012) for dietary Ca and P levels in gestation diets do not affect sow reproductive performance. These results agree with older studies even though some of these studies used an even wider range of Ca and P intakes and others were conducted over several parities. As suggested by Maxson (1985), the Ca and P buffer provided to the fetus from the sows' diet or even bone tissue, are adequate over a wide range of Ca and P intake, suggesting that reproductive performance exclusively, may not be the best tool for evaluating Ca and P needs. The improvement in reproductive performance of sows housed in groups compared to stalls suggests that group housing does not negatively affect the sow reproductive performance and in fact, may be advantageous. However, a few factors, such as the feeding system, and the number of parities evaluated in these studies require consideration. In the current study, sows that were assigned to stalls or groups were manually fed in their individual feeders without the need to compete for food while sows assigned to group housing were subsequently locked-out of their stalls about 1 h after feeding. This method of feeding allows for the dietary treatment and housing system to be studied without the confounding effect of other variables such as feed rationing due to competition for food among the sows. Therefore, if different results were to be obtained from other future studies, this may be attributable to the differences in feeding system suggesting that a different approach may have to be employed to meet the Ca and P needs of gestating sows. Extrapolating this data to systems that utilize competitive feeding system in a group housing requires caution.

In general, concentrations of Ca and P or bone markers in sow serum in both studies were not affected by dietary treatment. An older study reported that fewer sows completed 5

parities when fed reduced levels of Ca and P (Kornegay et al., 1973). However, in a separate study, the number of sows that completed 3 parities were not improved when sows were fed higher than recommended levels of Ca and P during their initial growth period (Kornegay et al., 1985). The current study was conducted over only 1 parity and the potential effect of the reduced serum Ca and P in group-housed sows fed low Ca on sow longevity requires further investigation. Although studies in humans and rats have shown that maternal skeletal Ca can be restored after weaning, the extent of restoration differed between the species, indicating that extrapolation of results between species needs to be done cautiously (Kovacs and Kronenberg, 1997). In most modern production systems the sow has only a few days between weaning and rebreeding. The lack of an effect of the different housing systems on serum Ca and P concentration and bone markers obtained in this study may be because housing system had no effect on sow posture and movement. Future studies should consider recording the sows physical activity more frequently or provide enrichment to encourage more movement in the group housing.

In both humans and rats, the maternal adaptive mechanisms for regulating Ca differs between gestation and lactation. With a normal Ca intake, intestinal Ca absorption, which is partially mediated by $1,25(OH)D_3$, is the major maternal adaptation used during gestation whereas in lactating women, increased bone turnover is the primary mechanism allowing increased Ca output in milk. In rats, however, increased bone turnover and intestinal absorption of Ca both occur during lactation (Kovacs and Kronenberg, 1997; Kovacs, 2005). Results from our study indicate that in swine receiving adequate levels of dietary Ca and P, the serum Ca and P and bone marker levels are influenced by reproductive stage rather than the dietary Ca and P levels. The lower concentrations of both bone markers during late gestation, relative to early gestation, indicated a decreased bone turnover, suggesting that the serum Ca and P levels were maintained using other adaptive mechanisms, similar to the situation in humans and rats. The gradual increase in bone turnover in late lactation, therefore, would likely involve mobilization of skeletal Ca. The extent of the involvement of each adaptive strategy in pigs remains to be determined.

Our study showed that varying dietary Ca and P for gestating sows, did not negatively influence the serum Ca and P or bone marker levels in newborn piglets, even those born to younger sows. Our results also showed that the blood Ca of newborn piglets is maintained regardless of the maternal blood Ca level, suggesting that maternal skeletal Ca reserves, as a source of Ca for the piglets during developmental stage, may be mobilized should a deficiency in dietary Ca occur in sows during gestation. Although the serum Ca levels

differed among treatments in the smallest birth-weight piglets at birth, the levels were similar at weaning. The milk Ca output may influence the serum Ca levels in the piglets, however in our study, the number of piglets in each litter was standardized according to the number of functional teats and potential variation in milk production among individual teats was not considered. The greater increase in bone formation in sows prior to weaning compared to mid-lactation however, would suggest that in order to allow for continued bone mineralisation, maintenance of the serum mineral levels would likely be at the expense of other possible adaptive mechanisms.

The consistent percentage of bone ash, ash Ca and ash P observed in our study, regardless of treatment, suggests that the current recommended levels of dietary Ca and P for gestating sows are adequate for fetal skeletal development. This conclusion is supported by the estimations of cortical density and bone strength obtained from the pQCT bone analyses. Although sows fed high Ca and P diets produced piglets with higher femoral cortical density compared to the control, the highest cortical density seen in piglets from sows fed the low Ca and P diet suggests that apart from mobilization of Ca from the bones, other adaptive mechanisms such as increased intestinal Ca absorption and reduced renal Ca excretion are involved to maintain the serum Ca level. In the current study, despite similar cortical densities, the greater bone strength, measure by SSI, seen in average piglets compared to the smaller piglets may be due to the need to sustain a heavier load, as suggested by Clarke, 2008. The assessment of dietary Ca and P requirements however, may be affected by the bone used for analysis (Crenshaw et al., 1981).

Our study has important limitations that must be acknowledged. Vitamin D and parathyroid hormone, also associated with Ca and P regulation were not measured. Although serum bone markers selected for this study were of those not affected by feed intake, it is unclear if serum Ca and P (postprandial) will be affected. Further, the housing system used in this study did not require that the sows walk any distance or compete for feed; hence extrapolation of the results have to be done in caution if a different feeding system is utilized. In conclusion, based on sow reproductive performance, bone metabolism and fetal skeletal development, the current recommended levels of dietary Ca and P for gestating sows are adequate for the modern, high-producing sow, regardless of housing. These diets, however, were fed for a single parity and further research is required to determine if similar results would be obtained over multiple parities. Eventhough the dietary Ca and P treatments did not negatively affect the skeletal development of the newborn piglets, future studies can also evaluate the effects of the piglets bone status on future breeding performance.

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APPENDIX A

Table A-4: Formulated ingredient and nutrient composition used as lactation diet[1] (as-fed basis)

Ingredient	Lactation, %
Wheat white soft 12 %	48.62
Barley 10.3 %	15.00
Peas 21.13 %	12.44
Wheat 13 %	10.00
Soybean meal	8.58
Limestone	1.85
Tallow	1.34
Monocalcium P (21.1 % P)	0.85
Salt	0.48
Lysine	0.40
Threonine 98.5 %	0.15
Sow Micro 1.5 Phytase[2]	0.15
Choline chloride	0.08
Methionine	0.05
Formulated nutrient	
DM, %	87.69
DE, Mcal/kg	3.23
Crude protein, %	15.78
SID Lys, %	0.90
Ca, %	0.90
Total P, %	0.53
Available P, %	0.45

[1] A standard lactation diet used at Prairie Swine Centre Inc., Saskatoon, SK, Canada was provided ad libitum to all sows during lactation period.
[2]Provided (per kg of diet): Zn, 100 mg as zinc sulphate; Fe, 80 mg as ferrous sulphate; Cu, 50 mg as copper sulphate; Mn, 25 mg as manganous sulphate; I, 0.50 mg as calcium iodate; Se, 0.10 mg as sodium selenite, Vitamin A, 8250 IU; Vitamin D, 825 IU; Vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg; Vitamin B_{12}, 25 ug.