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Development of the mineralisation of individual bones and bone regions in replacement gilts according to dietary calcium and phosphorus

P. Floradin [a,b], C. Pomar [b,c], M.P. Létourneau-Montminy [b], P. Schlegel [a,*]

[a] Agroscope, Swine Research Group, 1725 Posieux, Switzerland
[b] Department of Animal Sciences, Laval University, Quebec G1V 1A6, Canada
[c] Agriculture and Agri-Food Canada, Sherbrooke, Quebec J1M 1Z3, Canada

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ABSTRACT

Skeleton bones, distinguished by trabecular and cortical bone tissue content, exhibit varied growth and composition, in response to modified dietary calcium and phosphorus levels. The study investigated how gilts adapt their individual bone and bone region mineralisation kinetics in response to changing intake of Ca and P. A total of 24 gilts were fed according to a two-phase (Depletion (**D**) 60–95 and Repletion (**R**) 95–140 kg BW, respectively). During the D phase, gilts were fed either 60% (**D60**) or 100% (**D100**) of the estimated P requirement. Subsequently, during the R phase, half of the gilts from each D diet were fed either 100% (**R100**) or 160% (**R160**) of the estimated P requirement according to a 2 × 2 factorial arrangement. Bone mineral content (**BMC**) was assessed in the whole body, individual bones (femur and lumbar spine L2–L4), and bone regions (head, front legs, trunk, pelvis, femur, and hind legs) every 2 weeks using dual-energy X-ray absorptiometry (**DXA**). At 95 kg BW, gilts fed D60 showed reduced BMC and BMC/BW ratio in all studied sites compared to those fed D100 ($P < 0.001$). During the depletion phase, the allometric BW-dependent regressions slopes for BMC of D100 gilts remained close to 1 for all sites and did not differ from each other. In contrast, the slopes were lower in D60 gilts ($P < 0.05$), with an 18% reduction in the whole body, except for the front and hind legs, femur, and pelvis, which exhibited higher reductions ($P < 0.05$). At 140 kg BW, BMC and BMC/BW ratio of all studied sites were similar in gilts previously fed D60 and D100, but higher in R160 than in R100 gilts ($P < 0.05$), except for front and hind legs. During the repletion phase, the allometric BW dependent regressions slopes for BMC were lower ($P < 0.05$) in R100 than in R160 gilts (for whole body -10%; $P < 0.01$) except for front and hind legs, femur, and pelvis. In conclusion, bone demineralisation and recovery followed similar trends for all measured body sites. However, the lumbar spine region was most sensitive whereas the hind legs were least sensitive. These data suggest that using bone regions such as the head and forelegs that can be collected easily at the slaughterhouse may be a viable alternative to whole body DXA measurement.

Implications

In case of insufficient supply of dietary calcium and phosphorus, body reserves of these minerals are mobilised from the skeleton. It remains unclear if all bones or bone regions react to the same extent or not. Results show that all sites exhibit a similar pattern than the whole body, irrespective of whether diets are deficient, adequate, or excessive in calcium and phosphorus. The lumbar spine region is the most, whereas the hind legs are the least sensitive. The bone regions such as head or front legs may serve as practical proxies for assessing whole-body bone mineralisation in pigs.

Introduction

Data on bone mineralisation response to varying dietary supplies in calcium (Ca) and phosphorus (P) in gilts, as in growing pigs, have predominantly been assessed postmortem (Varley et al., 2011; Schlegel and Gutzwiller, 2020; Rieger et al., 2021). Measurement of bone mineralisation was conducted on various bones, such as the femur, metacarpus, and tibia employing diverse techniques including bone-breaking strength, bone ash percentage, and bone mineral density (Crenshaw et al., 1981; Schlegel and Gutzwiller, 2020). Dual-energy X-ray absorptiometry (**DXA**) a technique commonly used in human medicine enables longitudinal studies that monitor skeleton changes of various bones related to dietary supply and time, (Nordin et al., 1996; Åkesson et al., 1998; Mitchell et al., 2001).

* Corresponding author.
E-mail address: patrick.schlegel@agroscope.admin.ch (P. Schlegel).

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Animals exhibit resilience in overcoming certain mineral deficiencies by enhancing absorption and by increasing the efficiency of utilisation of the deficient nutrient (Ryan et al., 2011a; Létourneau-Montminy et al., 2014). As an example, dietary restriction of Ca and P (i.e. depletion) leads to increased intestinal absorption, renal reabsorption, and mobilisation in bone tissue (Lautrou et al., 2021). These adaptative mechanisms are stimulated by a set of genes encoding phosphate and Ca transporters in enterocytes, which are controlled by coordination between parathyroid hormone and 1,25-dihydroxyvitamin D_3 secretion (Just et al., 2018; Wubuli et al., 2019). The bone resorption is induced by the increased number and activity of osteoclasts (Stauffer et al., 1972; Taguchi et al., 1991; Åkesson et al., 1998). The deficit in bone mineralisation is recovered by increased number and activity of osteoblasts when dietary Ca and P are again sufficient (i.e. repletion) (Lautrou et al., 2021).

To enhance P efficiency and minimise P excretion, several experiments were performed in pigs. These confirmed that reducing dietary P and Ca levels triggered regulative response and enhanced P utilisation efficiency, to allow replenishment of bone mineralisation following a repletion period (Lautrou et al., 2021). For instance, Létourneau-Montminy et al. (2014) showed that the retention of vertebrae mineral content increased by 56% following a repletion period during the growing phase. However, these authors have highlighted that the efficiency of P use during depletion and the efficiency of recovery depends on factors such as the extent of bone depletion and the specific bone regions studied. Indeed, variations in growth and composition exist among skeleton bones, leading to different responses to modified dietary Ca and P levels (Crenshaw, 2001; Donnelly et al., 2012). This discrepancy is attributed to the non-uniform distribution of trabecular and cortical bone tissues across different bones. Trabecular bone tissue, with its greater relative surface area, tends to be more sensitive to mineral deficiency compared to cortical bone tissue (Kim and Park, 2013). Therefore, it is important to assess the ability of various bone regions, particularly those prone to metabolic bone disorders, as well as individual bones to serve as primary diagnostic sites for osteoporosis (Ryan et al., 2011a and 2011b).

The aims of the present study were thus to use DXA to (1) assess the time-dependent kinetics of mineralisation in specific bones (lumbar spine and femur) and bone regions (head, front legs, trunk, pelvis, femur, and hind legs) in gilts subjected to a dietary Ca and P depletion-repletion protocol and (2) study the extent of bone demineralisation and the recovery process, and (3) identify bones that can be easily collected and that would be representative to changes in the whole body mineralisation. It was hypothesised that, compared to the whole body, the response to a dietary Ca and P depletion-repletion protocol in bones and bone regions representing mainly trabecular bones (femur, legs) and mainly cortical bones (lumbar spine, trunk, pelvis) would differ from one to another.

Material and methods

Animals and diets

The experimental design, diet composition, and management procedures were previously described by Floradin et al. (2022). In summary, 24 Swiss Large White gilts from the Agroscope sow herd (mean BW of 55 kg) were grouped into six blocks of four animals each based on their BW. They were fed according to a two-phase feeding programme (60–95 kg BW and 95–140 kg BW), corresponding to a phase of depletion of 35 days and a phase of repletion of 56 days, respectively. During the depletion phase, two diets (Supplementary Table S1) were formulated: (1) a control diet (**D100**; 2.1 digestible P g/kg) providing 100% of the digestible P and total Ca requirements according to CVB (Bikker and Blok, 2017; optimised for 80 kg BW, daily 970 g BWG, 36 MJ and 5.75 g digestible energy and digestible P intake, respectively) and (2) a low – P diet (**D60**; 1.2 digestible P g/kg) providing 60% of the digestible P and total Ca of D100. Each diet was randomly assigned to two gilts within each block. At the beginning of the repletion phase, half of the gilts from each block and diet of the depletion phase were randomly assigned to one of two diets (Supplementary Table S1): (1) a control diet (**R100**; 2.1 digestible P/kg) providing 100% of the digestible P and total Ca requirements according to CVB (Bikker and Blok, 2017; optimised for 120 kg BW, daily 750 g BWG, 30 MJ and 5.06 g digestible energy and digestible P intake, respectively) and (2) a high-P diet (**R160**; 3.5 digestible P g/kg), providing 160% of the digestible P and total Ca of R100.

Overall, the experiment was set up according to two feeding sequences (D60-R100, D100-R100, D60-R160, and D100-R160), resulting in four treatments. All diets were formulated to contain a constant Ca/digestible P ratio of 2.8 with no supplemented phytase. The diets were offered *ad libitum* during the depletion phase but offered restrictively during the repletion phase to target a daily BWG of 700–750 g. The ingredients and chemical composition (as analysed value) of the diets including nutrient contents are detailed in Supplementary Material S1.

Body composition assessment

The bone mineral content (**BMC**) for each gilt was assessed at 2-week intervals by using DXA technology (Lunar i-DXA, GE Medical Systems, Glattbrugg, Switzerland). Prior to each scanning session, the gilts were fasted between 7 and 12 h and were then sedated with isoflurane (Attane, Piramal Critical Care, Inc., Bethlehem, PA, USA) before the DXA scanning. The calibration of the DXA was checked prior to each scanning session by using a calibration phantom according to the manufacturer's instructions. Once sedated, the gilts were placed on the DXA table in a prone position with their hind legs extended as detailed by Kasper et al. (2021). Two scans were performed per animal and processed using Encore software (version 16): (1) 'AP Spine – Thick' mode to capture images of the lumbar spine, and (2) 'Total Body – thick' mode to capture images of the whole body and bone regions.

The two scan images (spine, whole body; Fig. 1) were treated to remove artefacts (mask, ear tag) and were then examined to select suitable regions of interest (**ROI**). The BMC values were quantified using algorithms according to the ROI, provided by the manufacturer. For the spine scans, the standard ROI was placed to delimit L2, L3, and L4. For the total body scans, the standard total body ROI was placed as described by Kasper et al. (2021) to obtain whole-body data. In addition, six custom ROIs were defined within the total body scan mode: head, front legs, trunk, pelvis, femur, and back legs. The ROI for the head was delimited from the second cervical vertebrae. The ROI for the front legs included the blade (scapula), arm (humerus), foreshank (radius, ulna), and all metacarpal bones. The ROI for the back legs included the hind shank (tibia, fibula) and metatarsal bones. The ROI for the trunk included the cervical, thoracic, and lumbar vertebrae and ribs. The ROI for the pelvis included the ilium, ischium, and the pubis.

Statistical analysis

Data were analysed using a complete randomised block design including the time of each scan as the repeated measurement. The analysis was conducted using the mixed procedure of SAS (SAS Inst. Inc., Cary, NC, USA) employing the first-order autoregressive covariance structure [(AR)1]. Its selection was based on the Akaike

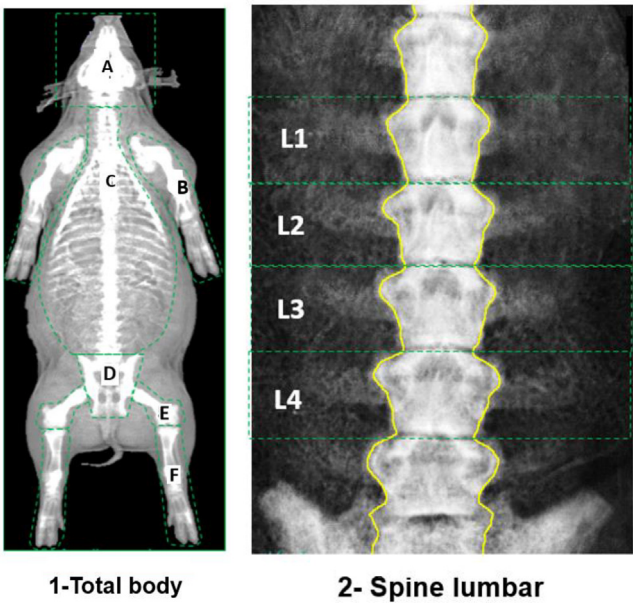

Fig. 1. Dual-energy X-ray absorptiometry images of gilts. Image 1 represents the total body, which includes the regions of interest for head (A), front legs (B), trunk (C), pelvis (D), femur (E) and hind legs (F). Image 2 represents the lumbar spine including the regions of interest for vertebrae L1, L2, L3, and L4.

information criterion value which favoured it over the compound symmetry and unstructured covariance structures. For the depletion phase (**D**), the 2 × 4 factorial model included depletion diets (D60, D100), time (scan number; 0–3), and the interaction between time and diets (D × Time) as fixed effects and block (1–6) as a random effect. For the repletion phase (**R**), the 2 × 2 × 4 factorial model included depletion diets (D60, D100), repletion diets (R100, R160), time (scan number; 3–7) and their interaction (D × time, R × time, D × R, D × R × time) as fixed effects and block as random effect. According to Floradin et al. (2022), no treatment effect was observed on BW. Consequently, for clarity and convenience, BW was utilised as the X-axis in the figures instead of Time. Differences between least square means were considered significant at $P < 0.05$ using the posthoc Tukey-Kramer test (Supplementary Material S1).

The following allometric relationship was used to assess the response kinetics of BMC in individual bones or bone regions in relation to BW development during the depletion and repletion phases, respectively:

$$Y = aX^b$$

For parameter estimation, the equation was linearised as follows:

$$\log Y = \log(a) + b\log X$$

where Y = BMC of each individual bone or bone region (g), and X = BW (kg), log*(a)* an intercept, and *b* the allometric growth coefficient that describes the relationship between the two body constituents. A unity of the allometric growth is assumed if $b = 1$; then, Y grows at the same proportional rate as X; if $b > 1$, Y grows proportionately faster than X, and the opposite is true if $b < 1$. The growth parameters log*(a)* and *b* were estimated using a PROC GLM procedure with SAS. The comparison focused on the *b* coefficient values as interpreting *b* as a ratio of a specific growth rate of Y/X is widely acknowledged. This approach was chosen because the interpretation of the *a* coefficient values had led to a large amount of inconclusive literature (Gould, 1971). To compare the slopes (*b* coefficient) between individual bones or bone regions, the CONTRAST statement in SAS was used. The SAS statements used are detailed in Supplementary Materials S2 and in Anzai et al. (2017).

Results

The initial BW (58.1 ± 1.62 kg; least square mean ± standard error of the mean, SEM) was similar among treatments. All animals exhibited the expected growth without any observed difference in growth performance (BW, average daily gain and feed intake, feed conversion ratio) between dietary treatments.

Development of bone demineralisation and remineralisation

Initial BMC and BMC/BW ratios of the whole body, individual bones, and bone regions were similar between dietary treatments (Figs. 2 and 3). The BMC in the front legs, femur, and pelvis increased with time ($P < 0.001$) and remained lower in D60 than in D100 gilts (Table 1; $P < 0.01$). The BMC in the whole body and lumbar spine were lower in D60 than in D100 gilts from the 4th week (scan 2) onwards and in the head and trunk from the 6th week (scan 3) onwards (D × Time, $P < 0.01$). Furthermore, from the 2nd week (scan 1) onwards until the end of the depletion phase (scan 3), BMC/BW ratio in the whole body, all individual bones, and bone regions were reduced in D60 compared to D100 (Figs. 2 and 3; D × Time, $P < 0.01$).

During the repletion phase, the BMC and BMC/BW ratio of the whole body and all individual bones and bone regions of D60 gilts gradually returned to similar values to those of D100 gilts. This occurred within 2 weeks (scan 4) for R160 gilts and within 4 weeks (scan 5) for R100 gilts (Figs. 2 and 3). However, throughout the repletion phase, the BMC of the whole body, individual bones, and bone regions (except front and hind legs) in R100 gilts remained lower than in R160 gilts regardless of the previous depletion phase (Table 2; Figs. 2 and 3; $P < 0.05$). At the end of the repletion phase, the BMC/BW ratio in the femur and lumbar spine was numerically higher by 5% in D60-R160 gilts compared to D100-R160 gilts ($P > 0.10$; Figs. 3E and 4).

Allometric regressions

During the depletion phase, the slopes (*b* coefficients) of the bones and bone regions BMC remained relatively close to 1 and did not differ in D100 gilts among bones and bone regions (Table 3 and Supplementary Table S2). However, in D60 gilts, the slope of the whole-body BMC was 18% ($P < 0.05$) lower than in D100 gilts. This slope reduction was more pronounced ($P < 0.05$) for the head, lumbar spine and pelvis, respectively, by 25, 28 and 26%.

During the repletion phase, the slopes of the whole body, the bones, and bone regions BMC were above 1 (Table 4 and Supplementary Table S3). Regardless of the depletion phase, the slope of the whole body was lower in R100 than in R160 gilts (-11% in D60-R100 vs D60-R160; -9% in D100-R100 vs D100-R160; $P < 0.01$). The slopes of bones and bone regions were also lower in R100 than in R160 ($P < 0.05$), except in the front and hind legs and femur. The slopes remained consistent across the bones and bone regions in R100 (D60-R100 and D100-R100) gilts, regardless of the depletion period. However, in R160 (D60-R160 and D100-R160) gilts, the slope of the trunk was 21% higher in D100-R160 gilts, while the slopes of the hind legs and femur were 21 and 22% lower than the slope of the whole body ($P < 0.05$). Additionally, D60-R160 gilts had an 18% higher slope for the trunk compared to the whole body ($P < 0.05$).

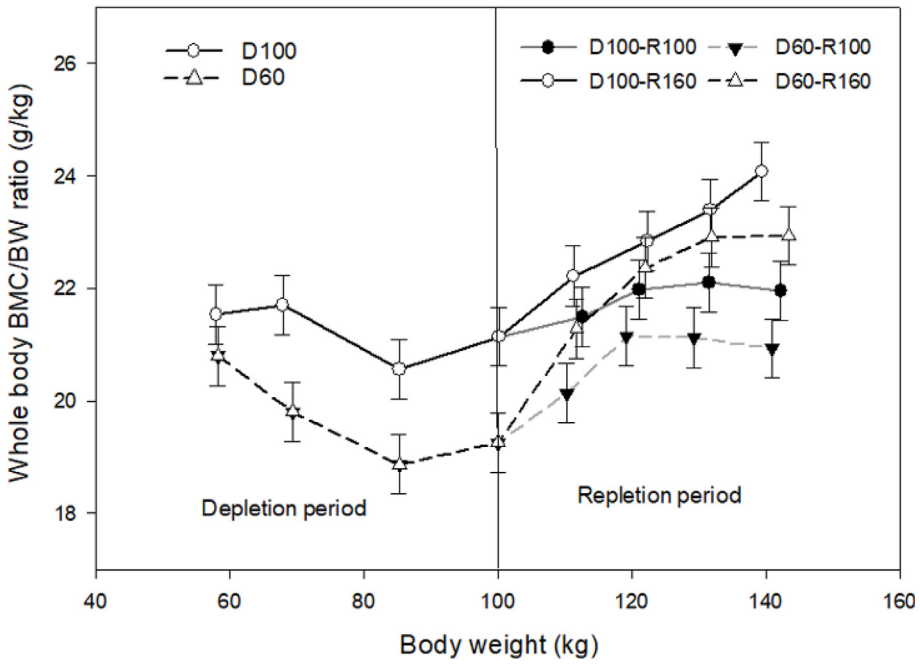

Fig. 2. Development of whole-body bone mineral content (BMC) relative to BW in gilts fed according to different calcium and phosphorus depletion (D, 60–95 kg) and repletion (R, 95–140 kg) phases. Values expressed as means ± SEM. D60 = depletion diet low in Ca and digestible P; D100 = depletion diet adequate in Ca and digestible P; R100 = repletion diet adequate in Ca and digestible P; R160 = repletion diet excessive in Ca and digestible P. Depletion phase: Diet effect ($P < 0.01$), Time effect ($P < 0.001$) and Diet × Time effect $P < 0.01$). Repletion phase: Diet effect ($P < 0.05$ and $P < 0.01$ for D and R respectively), Time effect ($P < 0.001$) and diet × Time effect ($P < 0.001$ for, R × Time).

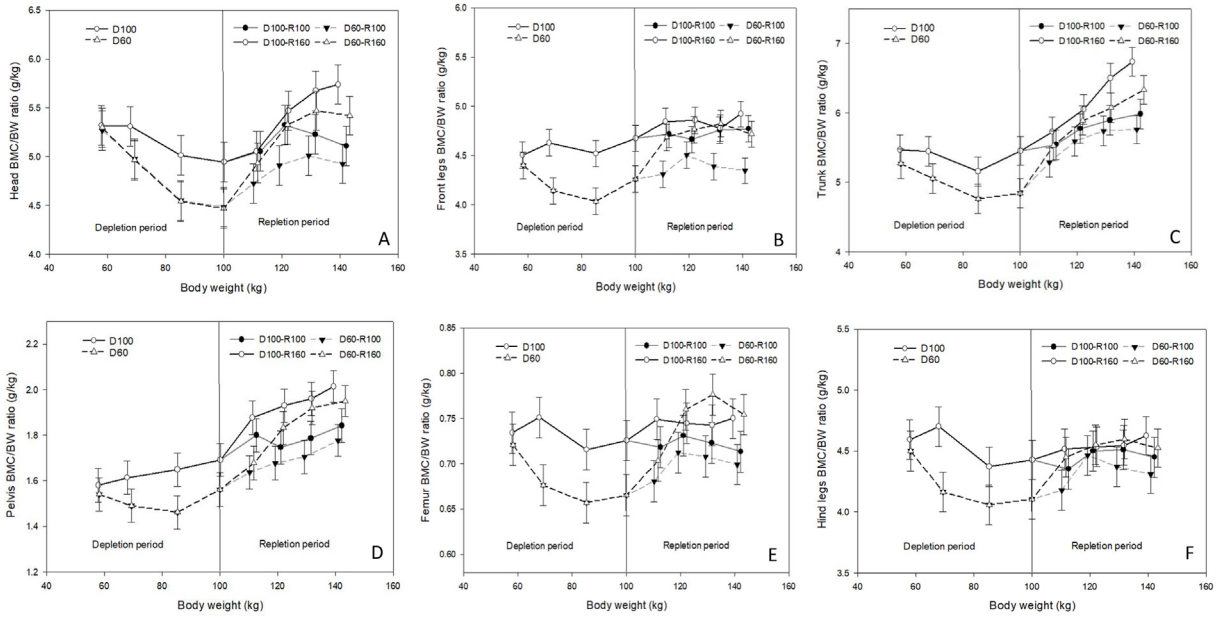

Fig. 3. Development of bone mineral content (BMC) in A) head, B) front legs, C) trunk, D) pelvis, E) femur, and F) hind legs relative to BW in gilts fed according to different calcium and phosphorus depletion (D, 60–95 kg) and repletion (R, 95–140 kg) phases. Values expressed as means ± SEM. D60 = depletion diet low in Ca and digestible P; D100 = depletion diet adequate in Ca and digestible P; R100 = repletion diet adequate in Ca and digestible P; R160 = repletion diet excessive in Ca and digestible P. Depletion phase: Diet effect ($P < 0.05$), Time effect ($P < 0.01$), Diets × Time ($P < 0.01$). Repletion phase: Diet effect ($P < 0.05$ for R), Time effect ($P < 0.001$) and Diet × Time effect ($P < 0.05$ for R × Time).

Discussion

Impact of depletion diets on the degree of bone demineralisation

During the depletion phase (60–95 kg BW), both dietary Ca and digestible P were reduced by 40% in D60 vs D100. As expected, by the end of the depletion phase, the D60 led to a notable decrease in BMC not only in the whole body but also across all individual bones and bone regions. This reduction in BMC aligns with findings from previous studies measured by DXA on live pigs following a deficient dietary P supply of 30–35% compared to a control (Gonzalo et al., 2018; Skiba et al., 2018; Schlegel and Gutzwiller, 2020). The present data further show that such a depletion can occur rapidly as observed from the 2nd week (scan 1). The time

Table 1
Effect of Ca and P depletion diets on bone mineral content (g) in the whole body, individual bones, and bone regions of gilts from 60 to 95 kg BW.

Time	0		1		2		3		SEM	*P*-value		
Diet	D100	D60	D100	D60	D100	D60	D100	D60		D	Time	D × Time
Whole-body	1 248[a]	1 209[a]	1 471[b]	1 367[b]	1 752[d]	1 604[c]	2 093[e]	1 865[d]	31.4	<0.001	<0.001	<0.001
Head	309[a]	306[a]	360[bc]	343[ab]	427[cd]	386[c]	489[e]	437[d]	9.7	0.032	<0.001	<0.001
Front legs	261	246	314	287	373	344	461	416	10.3	0.001	<0.001	0.114
Trunk	95[a]	88[a]	109[b]	106[ab]	140[cd]	124[bc]	172[e]	150[d]	4.4	0.010	<0.001	0.004
Pelvis	317	306	369	349	439	405	537	460	13.1	0.005	<0.001	0.287
Femur	43	40	51	47	61	56	72	64	1.0	<0.001	<0.001	0.090
Hind legs	266[a]	257[a]	318[b]	288[a]	372[c]	345[bc]	434[d]	400[cd]	7.6	0.002	<0.001	0.026
Lumbar spine	26[a]	25[a]	31[b]	28[a]	37[c]	33[b]	46[d]	39[c]	0.9	0.003	<0.001	0.002

Abbreviations: D60 = depletion (D) diet low in Ca and digestible P; D100 = depletion diet adequate in Ca and digestible P.
Main variables not shown. Bone mineral content in front legs, pelvis and femur differed between each Time of scan ($P < 0.001$).
[a-e]Values within a column not sharing a common superscript differ between dietary treatments and time (D × Time interaction, $P \leq 0.05$).

required for vertebral demineralisation response in 60 kg BW gilt was close to that in 20 kg BW piglets that required 7 days to respond when facing a similar dietary P deficiency (40%; Létourneau-Montminy et al., 2014). Moreover, the overall reduction in BMC in the present study is consistent with reduced bone mineral density in the whole body, spine, and fore and hind limbs of gilts between 30 and 70 kg BW (Skiba et al., 2018). In that study, pigs were fed a low P diet (1.6 vs 2.2 and 2.8 g digestible P/kg) for 28 days.

The present slopes of allometric regressions for the whole body in non-depleted gilts were similar to values reported by Mitchell et al. (2001). However, this consistency was not observed in depleted gilts where the slopes were lower and varied between individual bones and bone regions. This result confirms a demineralisation or decrease in the rate of BMC deposition across all examined bones in depleted gilts as BW increased, particularly notable in the lumbar spine, with a 28% difference (D60 vs D100), followed by the trunk (26%) and head (24%). In contrast, the remaining individual bones and bone regions were less affected (6–18% changes). Consistent with the present results, Gonzalo et al. (2018) reported a more pronounced BMC decrease in the lumbar spine (−18%) compared to the whole body (−5%) after gilts were fed over 28 days 13% less Ca (6.2 vs 5.4 g/kg) and 34% less digestible P (2.5 vs 1.7 g/kg) than the control. Additionally, Ryan et al. (2011b) demonstrated that a 43% reduction in digestible P resulted in a 31% decreased bone mineral density of the lumbar spine contrasting with a 21% decrease observed in the front leg. Thus, the lumbar spine bones are more sensitive to dietary Ca and P limitations than other bones, likely due in part of the higher proportion of trabecular bone tissue present in vertebrae bones. Bones and skeletal sites within bones exhibit varying ratios of cortical to trabecular bone contributing to differential responses to dietary factors. In humans, the vertebrae are composed of cortical to trabecular bone with a ratio of 25:75. This ratio is 50:50 in the femoral head and 95:5 in the radial diaphysis (Clarke, 2008). Cortical bone generally exhibits lower metabolic activity than trabecular bone. Bone remodelling triggered by osteoblasts and osteoclasts is more pronounced in trabecular bone with a high surface-to-volume ratio and a yearly volume turnover rate of 26%, in contrast to the 3% turnover rate of cortical bone (Jee et al., 1983). Consequently, a decline in bone mineralisation caused by nutrition may occur sooner in trabecular or spongy bones than in cortical or long bones (Maxson and Mahan, 1986; Underwood and Suttle, 1999). Additionally, fractures of the lumbar vertebrae are commonly associated with Ca and P deficiency in both sows (Mahan and Newton, 1995) and humans (Nordin et al., 1996), although no such occurrences were observed in the present study.

The bones within the trunk and head regions were the second most affected, with a change of about 25% in the rate of increase. The trunk region, primarily comprising vertebrae (cervical, thoracic, and lumbar) and ribs, contains a higher proportion of trabecular than cortical bone tissue (Mitchell et al., 2001; Kim and Park, 2013; Crenshaw and Rortvedt-Amundson, 2014). However, these bones reach peak bone mass earlier than the lumbar spine, making them less vulnerable to nutritional deficiency after 60 kg BW (Mitchell et al., 2001). Previous studies have reported that the beginning of the bone-building process within the embryonic development starts with the skull bones and extends along the bones within the trunk regions (Hammond and Appleton, 1932; McMeekan, 1940; Liu et al., 1999). Consequently, like the trunk, the growth rate of the skull regions low down later in life, particularly after reaching 60 kg BW, and undergoes less bone modelling and remodelling than lumbar spine bones (Mitchell et al., 2001; Matkovic et al., 2004). Similarly, by comparing normal and osteoporotic postmenopausal women, Nordin et al. (1996) reported a less pronounced reduction in BMC in the head than in the lumbar spine. This suggests that trunk and head bone regions were less sensitive to BMC mobilisation compared to the lumbar spine.

The finding that both pelvis and femur BMC were slightly less affected by depletion than the whole body BMC (12 and 13%, respectively) is consistent with Crenshaw et al. (2009), who showed that the femur served as a more accurate indicator of whole body bone mineralisation in pigs, particularly in response to dietary P. In addition, Walstra (1980) highlighted the sensitivity of the pelvis bone region to dietary mineral content suggesting a similar growth pattern as the femur, indicating a metabolic activity (Donnelly et al., 2012). This similarity in response to dietary P deprivation may be attributed, as discussed above, to a higher proportion of trabecular than cortical bone tissue, while pelvis bone regions exhibit a sandwich-like structure consisting mainly of trabecular bone tissue encased within a thin layer of cortical bone tissue (Donnelly et al., 2012;Kim and Park, 2013).

Impact of the repletion diets on recovery of bone mineralisation

Following the depletion phase, the R160 diet was evaluated to verify an eventual increase in speed rate and extent of bone remineralisation compared to R100. The R160 diet contained Ca and digestible P levels based on previous studies (Lagos et al., 2019; Vier et al., 2019) and to feeding recommendations for gilts (e.g., PIC, 2021). At the end of the repletion phase, when pigs reached 140 kg BW, the BMC and BMC/BW ratio in the whole body, individual bones, and bone regions remained similar among dietary treatments. The depleted BMC was rapidly compensated within the initial 2 weeks with R160 and 4 weeks with R100 following the onset of the repletion phase. These findings were confirmed by the allometric regressions performed throughout the repletion phase. Additionally, sites that were most severely affected during the depletion phase showed a more pronounced increase in slope coefficients, particularly evident in the trunk. This is consistent with previous studies indicating that growing pigs can recover within 5 weeks from a bone mineral deficit (lumbar spine,

Table 2
Effect of Ca and P repletion diets on bone mineral content (g) in the whole body, individual bones, and bone regions of gilts from 95 to 140 kg BW.

Time	0		1				2				3				4				*P*-value					
Diet	D100		D60		D100		D60		D100		D60		D100		D60									
	R160	R100	R160	R100	R160	R100	R160	R100	R160	R100	R160	R100	R160	R100	R160	R100	R160	R100	SEM	D	R	D × R	Time	D × Time	R × Time	D × R × Time
Whole body	2 097^{a}	2 134^{a}	1 948^{a}	1 897^{a}	2 474^{b}	2 420^{b}	2 378^{b}	2 215^{ab}	2 795^{cd}	2 661^{c}	2 728^{cde}	2 520^{c}	3 083^{e}	2 908de	3 024de	2 727^{cd}	3 355^{f}	3 128^{ef}	3 297f	2 967^{e}	62.6	0.012	0.003	0.349	<0.001	0.279	<0.001	0.989
Head	495^{a}	494^{a}	448^{ab}	446^{a}	584^{bc}	570^{b}	551^{bc}	518^{b}	669^{cd}	646^{c}	650^{cd}	583^{c}	747^{e}	688^{cd}	722de	645^{cd}	800^{f}	727de	778f	700de	20.6	0.048	0.027	0.648	<0.001	0.415	<0.001	0.485
Front legs	461	475	436	416	540	531	524	475	595	565	581	539	630	626	637	567	686	681	679	615	18.9	0.052	0.088	0.193	<0.001	0.501	0.078	0.316
Trunk	533^{a}	558^{a}	489^{a}	479^{a}	638^{ab}	625^{ab}	618^{b}	582^{ab}	740^{bc}	700^{b}	718^{cd}	666^{bc}	857^{d}	777^{cd}	802de	742^{cd}	941^{f}	851^{d}	912f	817^{e}	27.3	0.063	0.034	0.796	<0.001	0.557	<0.001	0.802
Pelvis	170	168	160	152	209	208	188	180	236	212	224	199	250	235	257	220	279	262	266	249	8.1	0.017	0.007	0.532	<0.001	0.282	0.053	0.734
Femur	72^{a}	73^{a}	68^{a}	65^{a}	83^{abc}	81^{ab}	79^{bc}	75^{bc}	91^{bcd}	90^{bc}	93^{cd}	86^{cd}	98^{cd}	95^{cd}	103de	88^{d}	105de	102de	108ef	97^{def}	2.8	0.194	0.028	0.183	<0.001	0.080	0.046	0.350
Hind legs	439^{a}	442^{a}	415^{a}	405^{a}	503^{bc}	490^{ab}	497^{b}	460^{ab}	555^{cd}	545^{bcd}	555^{bc}	533^{bc}	599de	601de	607^{cd}	553^{bc}	645^{ef}	637^{def}	650de	580^{cd}	21.6	0.069	0.423	0.104	<0.001	0.615	0.033	0.327
Lumbar spine	44^{a}	46^{a}	40^{a}	39^{a}	51^{abc}	53^{ab}	51^{bc}	46^{bc}	58^{bcd}	56^{bc}	60^{cde}	53^{cd}	63^{cde}	60^{cd}	67de	57^{cd}	69^{f}	67^{cd}	72ef	64^{def}	2.0	0.352	0.053	0.110	<0.001	<0.001	<0.001	0.774

Abbreviations: D60 = depletion (D) diet low in Ca and digestible P; D100 = depletion diet adequate in Ca and digestible P; R100 = repletion (R) diet adequate in Ca and digestible P; R160 = repletion diet excessive in Ca and digestible P.
Main variables not shown. Bone mineral content in front legs and pelvis differed between each Time of scan ($P < 0.001$).
[a-f] Values within a column not sharing a common superscript differ (D × Time and R × Time interaction, $P \leq 0.05$).

trunk, and femur) induced by 8 weeks of depletion (Mitchell, 2009; Weremko et al., 2013; Skiba et al., 2018). Gonzalo et al. (2018) also showed that growing pigs depleted for 28 days (from 68 to 100 kg BW) could recover their BMC deficit (whole body and lumbar spine) after 4 weeks of repletion.

Compensatory bone mineralisation, as indicated by bone mineral density, has previously been documented in the femur, fore, and hind limbs of gilts (Varley et al., 2011; Weremko et al., 2013; Skiba et al., 2018). However, complete restoration of bone mineral content following a period of depletion was not completely achieved in certain cases (Aiyangar et al., 2010; Ryan et al., 2011a). Several factors can influence bone mineralisation recovery, including dietary Ca and P, weight range, intensity and duration of the depletion and repletion periods, and parameters to measure bone mineralisation (Lautrou et al., 2021; Rieger et al., 2021). In terms of BW range, it has been observed that young pigs demineralised and remineralised the femur more rapidly and extensively than the whole body compared to older pigs (Aiyangar et al., 2010). In terms of repletion duration, growing gilts were unable to fully compensate for a bone mineralisation deficit after 35 days, following 8 weeks of depletion (Ryan et al., 2011a). Other factors, such as genetic line or physical activity, should be considered as well when evaluating bone mineralisation recovery in pigs (Tan et al., 2016).

After 6 weeks of repletion, the previously depleted R160 (D60-R160) gilts fully compensated and even exhibited greater BMC in the femur and lumbar spine than non-depleted R160 gilts (D100-R160). This observation suggests that a depletion period can result in compensatory bone mineralisation for certain bones. This is very interesting regarding the potential for sow longevity. The previously depleted R160 (D60-R160) gilts also compensated the front and hind legs in contrary to the previously depleted R100 (D60R100) gilts. Previous findings on growing pigs subjected to a depletion through a 30% reduction of dietary digestible P, followed by a subsequent 70-day repletion period with high P failed to achieve similar bone mineralisation in the front legs and hind legs than non-depleted pigs (Ryan et al., 2011a). The fact that the front and hind leg BMC were similar in D100-R100, D60-R160, and D100-R160 gilts at the end of the repletion phase suggests that peak bone mass was reached earlier (after 100 kg BW) for these bone regions compared to the others (Crenshaw and Rortvedt-Amundson, 2014). Finally, higher BMC was observed in R160 gilts compared to R100 gilts in various individual bones and bone regions, including the whole body, head, trunk, pelvis, and lumbar spine, aligns with maximal bone mineralisation obtained when pigs (100–130 kg BW) were fed 150–175% above digestible P requirement (Merriman et al., 2017).

The magnitude of bone demineralisation and remineralisation was more pronounced in the lumbar spine, making it the most sensitive site for detecting changes in bone mineralisation. However, its suitability for representing whole-body bone mineralisation is thus limited. According to Crenshaw and Rortvedt-Amundson (2014), the selection of a bone that best represents whole-body mineralisation should depend on how well that bone reflects changes at the time or age of measurement.

The findings suggest that the extent of bone demineralisation and subsequent recovery as assessed in the head, trunk, front and hind legs, pelvis, and femur were close to that of the whole body. Therefore, any of these individual bones or bone regions could serve as a viable alternative to whole-body DXA measurement. Bones in the extremities, such as the front and hind legs, are particularly convenient as proxy measurements of whole body bone mineralisation as they can be collected from a carcass without declassifying it. However, both head and whole-body assessments demonstrated a similar degree of demineralisation and remineralisation as previously observed by

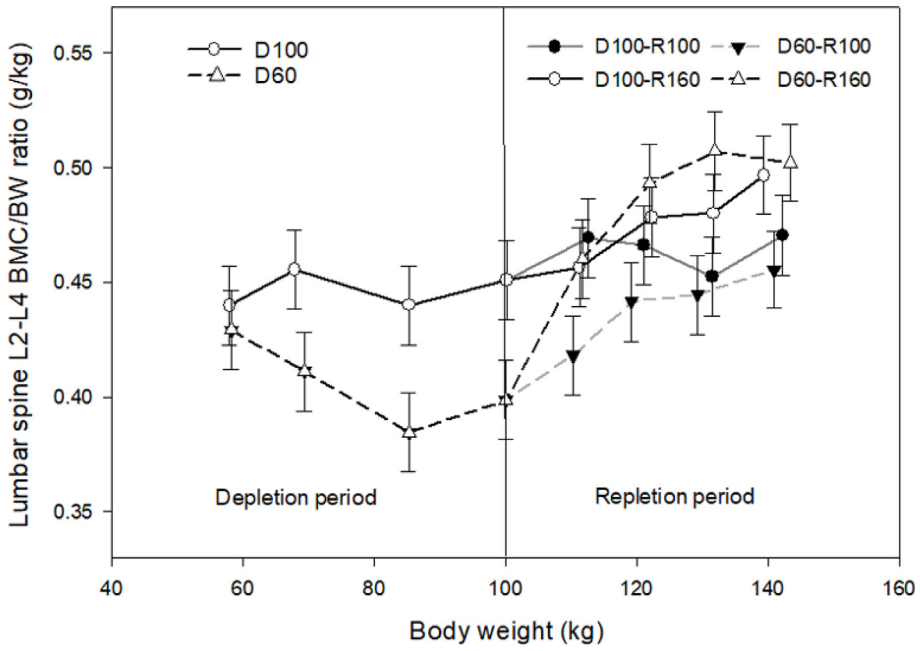

Fig. 4. Development of bone mineral content (BMC) in the lumbar spine relative to BW in gilts fed according to different calcium and phosphorus depletion (D, 60–95 kg) and repletion (R, 95–140 kg) sequences. Values expressed as means ± SEM. D60 = depletion diet low in Ca and digestible P; D100 = depletion diet adequate in Ca and digestible P; R100 = repletion diet adequate in Ca and digestible P; R160 = repletion diet excessive in Ca and digestible P. Depletion phase: Diet effect ($P < 0.01$), Time effect ($P < 0.01$) and Diet × Time effect ($P < 0.01$). Repletion phase: Diet effect ($P = 0.106$ for R), Time effect ($P < 0.001$) and Diet × Time effect ($P < 0.001$ and $P < 0.01$ for D × Time and R × Time, respectively).

Table 3
Allometric regression coefficients for bone mineral content (g) in the whole body, individual bones, and bone regions of gilts from 60 to 95 kg BW.

Bones	D100			D60			*P*-value
	b	SE	R^2	*b*	SE	R^2	
Whole body	0.94	0.06	0.87	0.76^{bc}	0.04	0.90	0.033
Head	0.85	0.08	0.75	0.64^{c}	0.07	0.65	0.010
Front legs	1.01	0.06	0.88	0.95^{a}	0.06	0.88	0.642
Hind legs	0.86	0.07	0.81	0.78^{bc}	0.05	0.84	0.274
Femur	0.94	0.05	0.90	0.82^{ab}	0.05	0.86	0.228
Lumbar spine	1.07	0.10	0.76	0.76^{b}	0.05	0.88	0.012
Pelvis	1.10	0.10	0.80	0.97^{a}	0.10	0.72	0.982
Trunk	0.97	0.09	0.70	0.72^{bc}	0.06	0.79	0.010

Abbreviations: D60 = depletion diet low in Ca and digestible P; D100 = depletion diet adequate in Ca and digestible P.
Allometric regression: BMC (g) = aBW^{b} where b-values represent the allometric growth coefficient (a value presented in Supplementary Table S2).
[a-c] Values within a column not sharing a common superscript differ between bone and bone region ($P \leq 0.05$).
P-value is for the difference of the *b* coefficient between D60 and D100 within bone and bone region.

Table 4
Allometric regression coefficients for bone mineral content (g) in the whole body, individual bones and bone regions of gilts from 95 to 140 kg BW.

Bones	R100						R160						*P*-value		
	D100			D60			D100			D60					
	b	SE	R^2	*b*	SE	R^2	*b*	SE	R^2	*b*	SE	R^2	D	R	D × R
Whole body	1.19	0.13	0.76	1.14	0.10	0.83	1.31^{b}	0.07	0.94	1.28^{ab}	0.07	0.93	0.705	0.004	0.547
Head	1.10	0.17	0.62	1.06	0.17	0.60	1.30^{b}	0.14	0.77	1.35^{ab}	0.15	0.77	0.941	0.014	0.842
Front legs	1.12	0.15	0.70	1.08	0.16	0.66	1.10^{bc}	0.10	0.84	1.04^{b}	0.09	0.84	0.770	0.336	0.676
Hind legs	0.99	0.29	0.32	0.98	0.15	0.65	1.03^{c}	0.07	0.89	1.03^{b}	0.09	0.85	0.940	0.248	0.784
Femur	1.06	0.15	0.65	1.06	0.14	0.69	1.02^{c}	0.08	0.88	1.15^{b}	0.14	0.72	0.359	0.179	0.205
Lumbar spine	1.19	0.22	0.53	1.27	0.15	0.62	1.27^{b}	0.11	0.89	1.40^{ab}	0.11	0.78	0.111	0.026	0.079
Pelvis	1.31	0.20	0.63	1.20	0.17	0.66	1.29^{b}	0.15	0.76	1.33^{ab}	0.13	0.82	0.879	0.081	0.692
Trunk	1.35	0.19	0.64	1.36	0.16	0.74	1.67^{a}	0.12	0.89	1.56^{a}	0.08	0.94	0.524	0.004	0.512

[a-e]Values within a column not sharing a common superscript differ between dietary treatments and time ($P \leq 0.05$).
Abbreviations: D60 = depletion (D) diet low in Ca and digestible P; D100 = depletion diet adequate in Ca and digestible P; R100 = repletion (R) diet adequate in Ca and digestible P; R160 = repletion diet excessive in Ca and digestible P. Allometric regression: BMC (g) = aBW^{b} where b-values represent the allometric growth coefficient (a value presented in Supplementary Table S3).
[a-c]Values within a column not sharing a common superscript differ between bone and bone region ($P \leq 0.05$).
P-value is for the difference of the *b* coefficients between dietary treatments and their interaction.

Létourneau-Montminy et al. (2017). This suggests that these sites could also serve as an effective proxy of bone mineralisation that could be sampled in a slaughterhouse if on-site DXA scanning is not feasible.

Conclusion

In conclusion, the results from this study confirm that both bone demineralisation (occurring within 2 weeks) and subsequent recovery (occurring within 2–4 weeks) followed similar patterns across the whole body, individual bones, and bone regions. However, variations in magnitude and dynamics were observed: the lumbar spine region displayed the highest sensitivity while the hind legs the least sensitivity. Using bone regions such as the head and fore legs which can be collected easily and cost-effectively at the slaughterhouse may serve as a viable alternative to whole-body DXA measurement.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101241.

Ethics approval

The experimental procedure was approved by the Office for Food Safety and Veterinary Affairs (2019_07_FR), and all procedures were conducted in accordance with the Swiss Ordinance on Animal Protection and Ordinance on Animal Experimentation Ordinance.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

Author ORCIDs

Piterson Floradin: https://orcid.org/0000-0002-5547-1454.
Marie-Pierre Létourneau-Montminy: https://orcid.org/0000-0001-5364-2662.
Candido Pomar: https://orcid.org/0000-0002-1686-0895.
Patrick Schlegel: https://orcid.org/0000-0001-5095-0889.

CRediT authorship contribution statement

P. Floradin: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **C. Pomar:** Writing – review & editing. **M.P. Létourneau-Montminy:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **P. Schlegel:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of interest

The authors report no conflicts of interest with any of the data presented.

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References

Aiyangar, A.K., Au, A.G., Crenshaw, T.D., Ploeg, H.L., 2010. Recovery of bone strength in young pigs from an induced short-term dietary calcium deficit followed by a calcium replete diet. Medical Engineering and Physics 32, 1116–1123.
Åkesson, K., Lau, K.H.W., Johnston, P., Imperio, E., Baylink, D.J., 1998. Effects of short-term calcium depletion and repletion on biochemical markers of bone turnover in young adult women. The Journal of Clinical Endocrinology & Metabolism 83, 1921–1927.
Anzai, H., Oishi, K., Kumagai, H., Hosoi, E., Nakanishi, Y., Hirooka, H., 2017. Interspecific comparison of allometry between body weight and chest girth in domestic bovids. Scientific Reports 7, 4817.
Bikker, P., Blok, M.C., 2017. Phosphorus and calcium requirements of growing pigs and sows (No. 59). Wageningen Livestock Research. Retrieved on 12 May 2022 from https://doi.org/10.18174/424780.
Clarke, B., 2008. Normal bone anatomy and physiology. Clinical Journal of the American Society of Nephrology 3, 131.
Crenshaw, T. D., Rortvedt-Amundson, L.A., 2014. Nutritionally induced cellular signals that affect skeletal integrity in swine. In: Proceedings 23rd International Pig Veterinary Society 1, 75-83.
Crenshaw, T.D., Peo Jr, E.R., Lewis, A.J., Moser, B.D., 1981. Bone strength as a trait for assessing mineralization in swine: a critical review of techniques involved. Journal of Animal Science 53, 827–835.
Crenshaw, T.D., 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In: Lewis, A.J., Southern, L.L. (Eds.), Swine Nutrition. 2nd ed. CRC Press, Washington DC, USA, pp. 187–212.
Crenshaw, T.D., Danielson, J.R., Hoffman, L.E., Schneider, D.K., 2009. Femurs are more accurate than fibulas as predictors of whole-body bone mineral content in growing pigs. Journal of Animal Science 87, 510.
Donnelly, E., Meredith, D.S., Nguyen, J.T., Boskey, A.L., 2012. Bone tissue composition varies across anatomic sites in the proximal femur and the iliac crest. Journal of Orthopaedic Research 30, 700–706.
Floradin, P., Pomar, C., Létourneau-Montminy, M.P., Schlegel, P., 2022. Development of bone mineralization and body composition of replacement gilts fed a calcium and phosphorus depletion and repletion strategy. Animal 16, 100512.
Floradin, P., 2023. Vers la maximisation des réserves minérales corporelles de la cochette afin d'optimiser les performances, la longévité et la durabilité. Ph.D. dissertation/thesis, Laval University, Quebec, QC, Canada.
Gonzalo, E., Létourneau-Montminy, M.P., Narcy, A., Bernier, J.F., Pomar, C., 2018. Consequences of dietary calcium and phosphorus depletion and repletion feeding sequences on growth performance and body composition of growing pigs. Animal 12, 1165–1173.
Gould, S.J., 1971. Geometric similarity in allometric growth: a contribution to the problem of scaling in the evolution of size. The American Naturalist 105, 113–136.
Hammond, J., and A. B. Appleton, 1932. Growth and the development of mutton qualities in the sheep. A survey of the problems involved in meat production. Biological monographs and manuals (no.10). Oliver and Boyd, London, UK.
Jee, W.S., Wronski, T.J., Morey, E.R., Kimmel, D.B., 1983. Effects of spaceflight on trabecular bone in rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 244, 310–314.
Just, F., Oster, M., Büsing, K., Borgelt, L., Murani, E., Ponsuksili, S., Wolf, P., Wimmers, K., 2018. Lowered dietary phosphorus affects intestinal and renal gene expression to maintain mineral homeostasis with immunomodulatory implications in weaned piglets. BMC Genomics 19, 1–11.
Kasper, C., Schlegel, P., Ruiz-Ascacibar, I., Stoll, P., Bee, G., 2021. Accuracy of predicting chemical body composition of growing pigs using dual-energy X-ray absorptiometry. Animal 15, 100307.

Kim, C., Park, D., 2013. The effect of restriction of dietary calcium on trabecular and cortical bone mineral density in the rats. Journal of Exercise Nutrition and Biochemistry 17, 123.
Lagos, L.V., Lee, S.A., Fondevila, G., Walk, C.L., Murphy, M.R., Loor, J.J., Stein, H.H., 2019. Influence of the concentration of dietary digestible calcium on growth performance, bone mineralization, plasma calcium, and abundance of genes involved in intestinal absorption of calcium in pigs from 11 to 22 kg fed diets with different concentrations of digestible phosphorus. Journal of Animal Science and Biotechnology 10, 1–16.
Lautrou, M., Narcy, A., Dourmad, J.Y., Pomar, C., Schmidely, P., Montminy, M.P.L., 2021. Dietary phosphorus and calcium utilization in growing pigs: requirements and improvements. Frontiers in Veterinary Science 8, 734365.
Létourneau-Montminy, M.P., Lovatto, P.A., Pomar, C., 2014. Apparent total tract digestibility of dietary calcium and phosphorus and their efficiency in bone mineral retention are affected by body mineral status in growing pigs. Journal of Animal Science 92, 3914–3924.
Létourneau-Montminy, M.P., Couture, C., Cloutier, L., Marcoux, M., Pomar, C., 2017. Révision de la méthode du bilan alimentaire simplifié en phosphore chez le porc charcutier dans le contexte canadien de production. Journées de Recherche Porcine 49, 263–264.
Liu, M.F., He, P., Aherne, F.X., Berg, R.T., 1999. Postnatal limb bone growth in relation to live weight in pigs from birth to 84 days of age. Journal of Animal Science 77, 1693–1701.
Mahan, D.C., Newton, E.A., 1995. Effect of initial breeding weight on macro-and micromineral composition over a three-parity period using a high-producing sow genotype. Journal of Animal Science 73, 151–158.
Matkovic, V., Landoll, J.D., Goel, P.R., Badenhop-Stevens, N., Eung-Jeong, H., Crncevic-Orlic, Z., Clairmont, A., 2004. Bone mineral density of the skull and lower extremities during growth and calcium supplementation. In: Burckhardt, D., Dawson-Hughes, B., Heaney, R.P. (Eds.), Nutritional Aspects of Osteoporosis. Elsevier Academic Press, Burlington, MA, USA, pp. 3–15.
Maxson, P.F., Mahan, D.C., 1986. Dietary calcium and phosphorus for lactating swine at high and average production levels. Journal of Animal Science 63, 1163–1172.
McMeekan, C.P., 1940. Growth and development in the pig, with special reference to carcass quality characters. II. the influence of plane of nutrition on growth and development. Journal of Agriculture Science 30, 387–436.
Merriman, L.A., Walk, C.L., Murphy, M.R., Parsons, C.M., Stein, H.H., 2017. Inclusion of excess dietary calcium in diets for 100-to 130-kg growing pigs reduces feed intake and daily gain if dietary phosphorus is at or below the requirement. Journal of Animal Science 95, 5439–5446.
Mitchell, A.D., 2009. Effect or ractopamine on growth and body composition of pigs during compensatory growth. Animal 3, 173–180.
Mitchell, A.D., Scholz, A.M., Pursel, V.G., 2001. Total body and regional measurements of bone mineral content and bone mineral density in pigs by dual energy X-ray absorptiometry. Journal of Animal Science 79, 2594–2604.
Nordin, B.E.C., Chatterton, B.E., Schultz, C.G., Need, A.G., Horowitz, M., 1996. Regional bone mineral density interrelationships in normal and osteoporotic postmenopausal women. Journal of Bone and Mineral Research 11, 849–856.
PIC. 2021. Nutrition and Feeding Guidelines. Retrieved on 13 October 2020 from www.pic.com.
Rieger, H., Ratert, C., Wendt, M., Schwennen, C., Kamphues, J., 2021. Comparative study on the chemical composition of different bones/parts of bones in growing pigs differently supplied with inorganic phosphorus and phytase. Journal of Animal Physiology and Animal Nutrition 105, 106–118.
Ryan, W.F., Lynch, P.B., O'Doherty, J.V., 2011a. Effect of dietary phosphorus on the development of bone mineral density of pigs assessed using dual energy X-ray absorptiometry. Livestock Science 137, 101–107.
Ryan, W.F., Lynch, P.B., O'Doherty, J.V., 2011b. Compensatory effect of dietary phosphorus on performance of growing pigs and development of bone mineral density assessed using dual energy X-ray absorptiometry. Livestock Science 138, 89–95.
Schlegel, P., Gutzwiller, A., 2020. Dietary calcium to digestible phosphorus ratio for optimal growth performance and bone mineralization in growing and finishing pigs. Animals 10, 178.
Skiba, G., Sobol, M., Raj, S., 2018. Bone mineralisation, mechanical properties and body phosphorus content in growing gilts as affected by protein or feed intake during depletion–repletion periods. Archives of Animal Nutrition 72, 478–491.
Stauffer, M., Baylink, D., Wergedal, J., Rich, C., 1972. Bone repletion in calcium deficient rats fed a high calcium diet. Calcified Tissue Research 9, 163–172.
Taguchi, A., Tanimoto, K., Ogawa, M., Sunayashiki, T., Wada, T., 1991. Effect of size of region of interest on precision of bone mineral measurements of the mandible by quantitative computed tomography. Dentomaxillofacial Radiology 20, 25–29.
Tan, F.P.Y., Kontulainen, S.A., Beaulieu, A.D., 2016. Effects of dietary calcium and phosphorus on reproductive performance and markers of bone turnover in stall-or group-housed sows. Journal of Animal Science 94, 4205–4216.
Underwood, E.J., Suttle, N.F., 1999. The mineral nutrition of livestock. CABI Publishing, CAB International, Oxon, UK.
Varley, P.F., Sweeney, T., Ryan, M.T., O'Doherty, J.V., 2011. The effect of phosphorus restriction during the weaner-grower phase on compensatory growth, serum osteocalcin, and bone mineralization in gilts. Livestock Science 135, 282–288.
Vier, C.M., Dritz, S.S., Tokach, M.D., DeRouchey, J.M., Goodband, R.D., Gonçalves, M. A., Orlando, U.A., Bergstrom, J.R., Woodworth, J.C., 2019. Calcium to phosphorus ratio requirement of 26 to 127kg pigs fed diets with or without phytase. Journal of Animal Science 97, 4041–4052.
Walstra, P., 1980. Growth and carcass composition from birth to maturity in relation to feeding level and sex in Dutch Landrace pigs. Wageningen University and Research, Wageningen, Netherlands (PhD thesis).
Weremko, D., Skiba, G., Fandrejewski, H., 2013. The effects of feed and protein restriction between 90 and 118 days of age on performance, bone growth and mineralization of pigs reared to 168 days of age. Animal Feed Science and Technology 182, 53–60.
Wubuli, A., Reyer, H., Muráni, E., Ponsuksili, S., Wolf, P., Oster, M., Wimmers, K., 2019. Tissue-wide gene expression analysis of sodium/phosphate co-transporters in pigs. International Journal of Molecular Sciences 20, 5576.