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Development of bone mineralization and body composition of replacement gilts fed a calcium and phosphorus depletion and repletion strategy

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ABSTRACT

This study investigated the ability of replacement gilts to adapt their calcium and phosphorus utilization and their kinetics in bone mineralization to compensate for modified intake of these nutrients by applying a novel Ca and P depletion and repletion strategy. A total of 24 gilts were fed according to a two-phase feeding program. In the first phase, gilts (60–95 kg BW) were fed *ad libitum* a depletion diet providing either 60% (**D60**; 1.2 g digestible P/kg) or 100% (**D100**; 2.1 g digestible P/kg) of the estimated P requirement. In the second phase, gilts (95–140 kg BW) were fed restrictively (aim: 700–750 g/d BW gain) a repletion diet. Half of the gilts from each depletion diet were randomly assigned to either a control diet or a high-P diet (**R100** and **R160**; with 2.1 and 3.5 g digestible P/kg, respectively) according to a 2 × 2 factorial design, resulting in four treatments: D60-R100, D60-R160, D100-R100 and D100-R160. Dual-energy X-ray absorptiometry was used to measure whole-body bone mineral content (**BMC**), bone mineral density (**BMD**) and lean and fat tissue mass on each gilt at 2-week intervals. The depletion and repletion diets, fed for 5 and 8 weeks, respectively, did not influence growth performance. The D60 gilts had a reduced BMC and BMD from the second week onwards and ended (95 kg BW) with 9% lower values than D100 gilts ($P < 0.001$). During repletion, D60 gilts completely recovered the deficit in bone mineralization from the second and fourth week onwards, when fed R160 (D60-R160 vs D100-R160) or R100 (D60-R100 vs D100-R100) diets, respectively (treatment × time interaction, $P < 0.001$); thus, the depletion diets did not affect these values at 140 kg BW. These results illustrate the rapid homeostatic counter-regulation capacity of dietary Ca and P, and they show the high potential to limit dietary digestible P concentration by completely excluding the use of mineral phosphates during the depletion phase, representative of the fattening period, without causing any detrimental effects to gilts at mating. The gilts were able to recover their BMC deficit between their selection at 95 kg BW and first mating at 140 kg BW by increasing their dietary Ca and P efficiency. Finally, excess dietary digestible P, requiring increased amounts of mineral phosphates, further increased the gilts' BMC.

Implications

To limit mineral phosphate use, optimizing dietary phosphorus for maximized growth, but not for maximum bone mineralization, is recommended in grower-finisher pigs. Some producers raise replacement gilts with fattening pigs until their breeding selection is around 100 kg BW. The results suggest that strategies to limit the use of non-renewable phosphorus sources, such as phosphate in grower-finisher pigs, could be applied by the industry, also if female pigs are selected for breeding at the end of the finishing phase. Replacement gilts would compensate for any bone mineralization deficit between selection and 1st mating when fed adequate calcium and phosphorus levels.

Introduction

Calcium and P are required to maintain skeletal development and bone mineralization (Crenshaw, 2001). Long-term deprivation of either Ca or P is associated to poor skeleton mineralization and predisposes animals to lameness; this was the primary cause for culling 10 and 25% of sows after 1st parity and 2nd parity,

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respectively (Mote et al., 2009; Kraeling and Webel, 2015). However, excess dietary Ca and P may reduce P utilization, resulting in increased P excretion, potentially leading to environmental concerns (Merriman et al., 2017; Lagos et al., 2019; Dourmad et al., 2020) if the manure is not applied properly in regard to the nutrient requirements of plants (Agroscope, 2017; Dourmad et al., 2020). Moreover, mineral P originates naturally in phosphate rock, which is an expensive, limited and non-renewable resource that could be depleted within a century if its use in agriculture as a fertilizer and feed supplement is not reduced (Cordell et al., 2011).

Reducing dietary Ca and P supply in pig diets is an effective way to optimize mineral P use (Oster et al., 2018; Schlegel and Gutzwiller, 2020). As an example, mineral P supply to grower-finisher pigs could be reduced by 65% and P excretion could be decreased by 41% without impaired growth performance, but with compromised bone mineralization (Schlegel and Gutzwiller, 2020). Létourneau-Montminy et al. (2014) showed that limited bone mineralization due to sub-optimal dietary Ca and P supply was recovered after fattening pigs were once again fed adequate amounts of Ca and P. Similarly, Gonzalo et al. (2018) noted that such recovery in bone mineralization was due to improved efficiency in Ca and P use. The underlying mechanism allowing pigs to recover from their bone mineral deficit was modulated by an improvement in the digestive and metabolic utilization of Ca and P and stimulated by a set of genes encoding phosphate and Ca transporters in enterocytes, which are controlled by a coordination between parathyroid hormone (**PTH**) and 1,25-dihydroxyvitamin D_3 secretion (Just et al., 2018; Wubuli et al., 2019).

In some production contexts, replacement gilts are raised with grower-finisher pigs until their breeding selection (around 100 kg BW) and they are eventually fed dietary P levels allowing maximal growth, without fulfilling the higher requirement for maximized bone mineralization. According to the previously mentioned studies on grower-finisher pigs, a recovery in bone mineralization may also be expected in gilts following their selection; however, restricted feeding, applied when their BW ranges from 100 to 140 kg so they can achieve BW and back-fat thickness targets for the first mating, may compromise this expected capacity. To ensure the welfare and longevity of replacement gilts, maximal bone mineralization and skeletal health should be achieved by controlling a range of factors, including nutrition and husbandry conditions, such as density and floor type (Mote et al., 2009; Whitney and Masker, 2010; Kraeling and Webel, 2015). To maximize the chances of achieving this goal, dietary P levels largely exceeding the recommendations are practiced when gilts reach 100 kg BW until first mating, although this may be detrimental to the environment.

The present study aimed to verify the ability and required time of replacement gilts to recover from a bone mineral deficit induced from 60 to 95 kg BW by a dietary Ca and P restriction when fed a diet containing normal or excessive amounts of Ca and P from 95 to 140 kg BW.

Material and methods

Animals and diets

A total of 24 large white Swiss gilts from the Agroscope sow herd were raised up to a 55 kg BW and were grouped into six blocks of four animals based on their initial BW to achieve similar BW across treatments. The experiment started when a block of gilts reached a mean of 60 kg BW (58.1 ± 1.62 kg, expressed as mean ± SD). All animals were housed in one pen (95 m^2) with deep straw. The pen was equipped with four computer-controlled feeding stations (Schauer Agrotronic GmbH, Prambachkirchen, Austria). The gilts had free access to one of the feeding stations and to freshwater.

The experiment included a two-phase feeding program consisting of a depletion phase from 60 to 95 kg BW and of a repletion phase from 95 to 140 kg BW. All diets were formulated to meet or exceed the current Swiss feeding recommendations for pigs (Agroscope, 2004), except for Ca and digestible P (Table 1). Two depletion diets were formulated: (1) a control diet (**D100**; 2.1 digestible P g/kg) providing 100% of the digestible P requirements according to Dutch CVB (Bikker and Blok, 2017; optimized for 80 kg BW, 970 g/d BW gain, 36 MJ/d and 5.75 g/d digestible energy and digestible P intake, respectively) and (2) a low-P diet (**D60**; 1.2 digestible P g/kg) supplying 60% of the digestible P of D100, which was similar to that used by Létourneau-Montminy et al. (2014) and Gonzalo et al. (2018). These diets were randomly assigned to two gilts from each block. At beginning of the repletion phase, half of the gilts from each depletion diet within each block were randomly assigned to two repletion diets: (1) a control (**R100**; 2.1 digestible P/kg) providing 100% of the P requirement based on Dutch CVB (Bikker and Blok, 2017; optimized for 120 kg BW, 750 g/d BW gain, 30 MJ/d and 5.06 g/d digestible energy and digestible P intake, respectively) and (2) a high-P diet (**R160**; 3.5 digestible P g/kg), providing 160% of the estimated digestible P requirement, based on the findings reported in previous studies (Sørensen et al., 2019; Vier et al., 2019; Lagos et al., 2019) and to genetic recommendation for gilts (PIC, 2016).

Overall, the experiment was set up according to a 2 × 2 factorial design with four treatments (D60-R100, D100-R100, D60-R160 and D100-R160). All diets were free of microbial phytase, formulated to contain a constant Ca/digestible P ratio of 2.8 and D60, D100, R100 and R160 diets were supplemented with 0.0, 5.1, 5.4 and 13.3 g/kg monocalcium phosphate, respectively, to achieve the desired digestible P levels. The diets were offered *ad libitum* during the depletion phase, but restricted during the repletion phase to aim for 700–750 g/d BW gain, as recommended prior to first mating (Kraeling and Webel, 2015). The software Allix2 (A-Systems S.A., Versailles, France) was used to formulate the experimental diets, based on the analyzed CP, crude fiber, crude fat, ash, Ca and P concentrations of each feedstuff. The other mineral concentrations, digestible energy contents, digestible amino acid profiles and coefficients for digestible P of each feedstuff were formulated according to the open-access Swiss national reference table values (Agroscope, 2019). All diets were manufactured at the onsite experimental feed mill and were offered in a pelleted form (60 °C, 4 mm diameter).

Sample collection and measurements

Diet samples were collected weekly, then pooled into three samples per treatment diet to obtain a representative composite sample from the beginning, middle and end of each experimental phase. A blood sample was collected between 0700 and 0900 h from the jugular vein and a urine sample was collected between 0400 and 0600 h according to the tampon technique, as described by Nickel et al. (2017) from each gilt at the end of each experimental phase. Blood samples were then centrifuged (3000*g*, 15 min) within 1 h after collection, and serum was decanted and stored at –20 °C for mineral and at –80 °C for the biomarker analysis.

Feed intake was recorded at each individual visit and totaled each day. Body weight was recorded weekly. Whole-body bone mineral content (**BMC**), bone mineral density (**BMD**), lean tissue mass and fat tissue mass were measured on each gilt at 2-week intervals using dual-energy X-ray absorptiometry (**DXA**, Lunar i-DXA, GE Medical Systems, Glattbrugg, Switzerland). The calibration was checked and passed before each scanning session by scanning a calibration phantom according to the manufacturer's

Table 1
Ingredient and chemical composition of diets supplied to gilts during a Ca and P depletion phase (60–95 kg BW) and a Ca and P repletion phase (95–140 kg BW).

Items (g/kg as fed)	Feeding phases			
	Depletion diets		Repletion diets	
	D60	D100	R100	R160
Barley	521	508	596	550
Oats			100	100
Wheat	299	299	71.6	95.7
Soybean meal, expelled	71.6	71.6		
Rapeseed meal, expelled	56.5	56.5	95.0	95.0
Sugar beet pulp, dehydrated	20.0	20.0	50.0	50.0
Wheat bran	15.0	15.0	11.6	11.6
Apple pomace, dried			43.2	43.2
Fat (tallow and lard)		4.72		7.00
L-Lysine-HCl	1.66	1.71	2.30	2.35
L-Threonine			0.46	0.46
Calcium carbonate	5.51	8.71	7.61	14.2
Monocalcium phosphate		5.11	5.78	13.8
Sodium chloride	2.50	2.50	3.56	3.56
Binder[1]	3.00	3.00	3.00	3.00
Maize cob meal[2]			6.00	6.00
Vitamin and mineral premix[3]	4.00	4.00	4.00	4.00
Analyzed nutrient composition per kg, as fed[4]				
DM (g)	890	890	887	894
Digestible energy (MJ)[5]	13.3	13.3	12.6	12.6
CP (g)	150	146	131	131
Fat (g)	26.8	29.8	34.4	38.5
Crude fiber (g)	41.1	43.3	68.1	68.7
Ash (g)	37.6	48.4	45.4	56.9
Calcium (g)	3.83	6.73	6.52	10.6
Phosphorus (g)	4.10	5.14	5.33	7.19
Phytic acid (g)	2.32	2.31	2.14	2.49
Phytase activity (FTU)[6]	285	285	133	209
Digestible phosphorus (g)[5]	1.20	2.10	2.10	3.50
Calcium/ digestible phosphorus	3.19	3.19	3.10	3.02

Abbreviations: D60 = depletion diet low in Ca and digestible P; D100 = depletion diet with adequate Ca and digestible P; R100 = repletion diet with adequate Ca and digestible P; R160 = repletion diet in excess Ca and digestible P.
[1] Pellan (Mikro-Technik, Bürgstadt, Germany).
[2] Mikrogrit (Microtracers, San Francisco, CA, USA.) colored meal for visual identification of the repletion diets.
[3] Supplied per kg diet: 4 mg Cu; 20 mg Fe; 10 mg Mn; 45 mg Zn; 0.15 mg I; 0.15 mg Se; 4 000 IU vitamin A; 400 IU vitamin D3; 200 mg choline; 2 mg vitamin B1; 3 mg vitamin B2; 3 mg vitamin B6; 15 mg niacin; 0.02 mg vitamin B12; 15 mg pantothenic acid; 0.05 mg biotin; 0.5 mg folic acid; 65 mg vitamin E; 1 mg vitamin K3.
[4] Values analyzed in duplicate, except DM and ash.
[5] Values calculated according to Agroscope (2019).
[6] From plant phytase.

instructions. Before scanning, the animals were fasted for at least 16 h and were subjected to short-lasting sedation by mask inhalation of isoflurane (max. 5% in oxygen, Isoflo, Abbott Laboratories, North Chicago, USA) to prevent movement during the scanning procedure. After the onset of sedation, the animals were placed on the DXA table in the prone position with their hind legs extended; their front legs were positioned along the side, but kept away from the body using two wedges of foam plastic. The whole-body scan was carried out using the total body thick mode with enCORE software (version 16). The obtained scan images were processed to remove artifacts (mask and tube of sedation apparatus and ear tag), and the regions of interest positions were placed as described by Kasper et al. (2021).

Chemical analysis

The DM content of the feed samples were determined by heating at 105 °C for 3 h (AOAC International, 2005; method no: 930.15) and ash content was subsequently determined after incineration at 550 °C (prepAsh, Precisa Gravimetrics AG, Dietikon, Switzerland) until a constant weight was attained. Crude protein content in the diets was determined as 6.25 × N, where N content was determined using the Dumas method (ISO 16634-1) on an automated analyser (TruMac CNS, Leco, Mönchengladbach, Germany). Crude fiber content in the diets was determined using Fibretherm FT-12 (C. Gerhardt GmbH & Co. KG, Königswinter, Germany) after the samples were digested successively with H_2SO_4 and KOH, washed with acetone, dried at 130 °C and then ashed (VDLUFA, method 6.1.4). Crude fat content in the diets was determined as the petrol ether extract after acidic hydrolysis in boiling HCl for 1 h (VDLUFA, method 5.1.1) using Hydrotherm HT-6 (C. Gerhardt GmbH & Co. KG, Königswinter, Germany) and Speed Extractor E-916 (Büchi Labortechnik AG, Flawil, Switzerland). The Ca and P contents were analyzed, according to the European Standard EN 155510:2008, using an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 7300 DV, Perkin-Elmer Schwerzenbach, Switzerland) after microwave (UltraClave MLS, Egrolyt) digestion with a solution of nitric acid. Phytic acid content and phytase activity in the diets were determined as previously described by Schlegel and Gutzwiller (2020).

Blood serum and urine samples were assayed using commercially available kits according to the manufacturer's instructions on a BT1500 autoanalyzer (Biotecnica Instruments S.p.A., Roma, Italy) to determine the concentrations of creatinine, total Ca and inorganic P (Greiner Diagnostic GmbH, Langenthal, Switzerland). Bone formation marker, carboxy-terminal propeptide of type I collagen (**PICP**), was measured using an ELISA kit (MicroVue PICP EIA, Quidel) and C-terminal telopeptides of type I collagen (**CTX**) was used as a marker for bone degradation and analyzed with an ELISA kit (Novus Biologicals, Centennial, USA). The lowest detection limit

was 0.2 ng/ml for PICP and 0.19 ng/ml for CTX. The determined intra- and interassay CVs were 5 and 7% for PICP and 5.65 and 5.63% for CTX, respectively. All analyses were performed in the onsite laboratories in duplicate, except for DM and ash.

Calculations and data analysis

The empty body protein, lipid, P and Ca contents were calculated according to Kasper et al. (2021) using the following equations: Protein = −784.77 + 0.2 × lean tissue mass; Lipid = −294.21 + 0.986 × fat tissue mass + 0.042 × lean tissue mass; P = −9.819 + 0.145 × BMC + 0.003 × lean tissue mass; Ca = −2.807 + 0.407 × BMC. Deposition of protein, lipid and bone mineral, retention of P and Ca, as well as, daily BW gain were individually obtained by subtracting the final values from the initial values of an experimental phase or of a two-week DXA scan interval, divided by the duration. Feed conversion ratio was calculated as the ratio between the daily feed intake and BW gain. Excretion and retention efficiency of P and Ca were respectively calculated by subtracting or dividing the amount of P and Ca retained by the amount of P and Ca ingested.

For the statistical analysis, the individual gilt was considered as experimental unit. Non-repeated urinary and blood serum data, growth performance data, Ca and P balance data, including the required BMC and protein mass, were analyzed for each feeding phase using a generalized mixed model with PROC MIXED from SAS (SAS Inst. Inc., Cary, NC, USA) after normality of the variables had been checked using the Shapiro-Wilks test. For the depletion phase, the model included depletion diets (D60, D100) as a fixed effect and block (1–6) as a random effect (Supplementary Material S1). For the repletion phase, the 2 × 2 factorial model included the depletion diets (D60, D100), the repletion diets (R100, R160) and their interaction as fixed effects and block (1–6) as a random effect (Supplementary Material S1). Data measured more frequently, namely BW, composition (lipid, protein, BMC, BMD and BMC/protein) and deposition (protein, lipid and bone mineral), were analyzed for the overall experimental period as repeated measurements using the mixed procedure of SAS. The model included the block, the time (scan number; 0–7), the treatments (D60-R100, D60-R160, D100-R100, D100-R160) and the interaction between time and treatment as fixed effects and the animal as repeated factor (Supplementary Material S1). Logarithmic transformation was performed for body protein and lipid deposition, as not normally distributed. Differences between least square means were considered as significant at $P \leq 0.05$, and tendencies were noted at $P < 0.10$ using posthoc test of Tukey-Kramer.

Results

All animals exhibited the expected growth of the Agroscope herd and required 35 days to reach 95 kg BW and 56 days to reach 140 kg BW. No major health problems were observed throughout the entire experiment. However, two gilts (D60-R100 and D100-R100) had joint inflammation on their right foot. These animals were isolated for six days and treated with an anti-inflammatory medication until they were completely healed. Data from these gilts were not extreme or aberrant, and no residual effects were noted in the statistical analysis.

The analyzed Ca and P concentrations in D60 and D100 diets were in agreement with the expected values. However, they were slightly higher than expected in R100 and R160 diets, with an increase in P of 6.3 and 8.2%, respectively, and an increase in Ca of 9.5 and 7.3%, respectively (Table 1).

Depletion phase (60–95 kg BW)

At start of the experiment, BW, body protein mass, BMC and the BMC/protein ratio were similar among treatments (Table 2). At the end of the depletion phase, BW, body protein mass and growth performances (Total feed intake, daily feed intake, daily BW gain, feed conversion ratio and body protein deposition) remained similar among treatments. However, gilts that were fed D60 had lower BMC (−9%; $P < 0.001$) and BMC/protein (−8%, $P < 0.001$) than those fed D100. Finally, the daily bone mineral deposition was drastically lower (−18%; $P < 0.001$) in D60 gilts than in D100 gilts. As expected, D60 gilts consumed less Ca (−41%; $P < 0.001$; Table 3) and P (−17%; $P < 0.001$) than D100 gilts, resulting in lower Ca (−63%; $P < 0.001$) and P (−21%; $P < 0.001$) excretion, urinary P concentration (−66%; $P = 0.012$) and body Ca (−17%; $P < 0.001$) and P (−11%; $P < 0.001$) retention. This resulted in a higher body Ca retention efficiency (+41%; $P < 0.001$) in D60 gilts than D100 gilts. Serum Ca, P and PICP concentrations remained unchanged between the two treatments. However, CTX ($P = 0.096$) and PICP/CTX ratio ($P = 0.071$) tended to be lower in D60 gilts than in the D100 gilts.

Table 2
Effect of Ca and P depletion diets on growth performance and body composition in gilts from 60 to 95 kg BW.

Response criteria[1]	Depletion diets		SEM	*P*-value
	D100	D60		
Initial conditions				
BW (kg)	58.0	58.2	1.62	0.806
Body protein (kg)	9.60	9.57	0.256	0.895
BMC (g)	1 248	1 209	31.9	0.145
BMC/protein ratio (g/kg)	127	124	2.7	0.296
Final conditions				
BW (kg)	99.7	99.8	1.78	0.891
Body protein (kg)	15.9	15.7	0.31	0.488
BMC (g)	2 114	1 922	37.6	<0.001
BMC/protein ratio (g/kg)	127	118	2.7	0.001
Overall Performances				
Total Feed intake (kg)	107.9	111.2	5.37	0.532
Feed intake (g/d)	3 055	3 119	91.6	0.427
BW gain (g/d)	1 099	1 087	23.2	0.673
Feed conversion ratio	2.78	2.87	0.154	0.717
Body protein deposition (g/d)	162	159	4.6	0.449
Bone mineral deposition (g/d)	22.5	18.5	0.64	<0.001

Abbreviations: D60 = depletion diet low in Ca and digestible P; D100 = depletion diet adequate in Ca and digestible P; BMC = Bone mineral content.
[1] BMC was obtained using dual-energy X-ray absorptiometry (DXA), and body protein was predicted based on DXA lean (Kasper et al., 2021).

Table 3
Effect of Ca and P depletion diets on P and Ca balance and on blood serum and urine concentrations in gilts from 60 to 95 kg BW.

Response criteria	Treatments		SEM	*P*-value
	D100	D60		
Phosphorus balance				
Total P intake (g)	550	456	25.5	<0.001
P body retention (g)[1]	220	195	9.9	<0.001
P excretion (g)	331	260	31.0	<0.001
Total P Efficiency (%)[2]	40.8	43.7	3.61	0.176
Calcium balance				
Ca intake (g)	723	426	29.2	<0.001
Ca body retention (g)[1]	352	291	16.7	<0.001
Ca excretion (g)	370	135	35.9	<0.001
Total Ca efficiency (%)[2]	49.7	69.9	5.05	<0.001
Blood serum minerals at 95 kg BW (mmol/l)				
Calcium	2.58	2.58	0.029	0.916
Phosphorus	2.64	2.77	0.069	0.209
Blood serum bone markers at 95 kg BW (ng/ml)				
PICP	37.0	37.3	1.94	0.889
CTX	1.22	1.63	0.22	0.096
Urine concentration at 95 kg BW (mol/mol creatinine)				
Ca	0.144	0.174	0.307	0.569
P	0.678	0.232	0.114	0.012

Abbreviations: D60 = depletion diet low in Ca and digestible P; D100 = depletion diet adequate in Ca and digestible P. PICP = carboxy-terminal propeptide of type I collagen; CTX = carboxy-terminal cross-linking telopeptide of type 1 collagen.
[1] Body P and Ca retention was calculated from the difference between initial and final Body P and Ca obtained from body bone mineral content and body lean according to Kasper et al. (2021).
[2] Calculated as the amount of total P and Ca intake divided by the amount of total P and Ca retained.

Repletion phase (95–140 kg BW)

At the end of the repletion phase, BW, body protein mass and growth performances (Total feed intake, daily feed intake, daily BW gain, feed conversion ratio and protein deposition) remained similar among dietary treatments (Table 4). Furthermore, no interaction was observed between the depletion and repletion diets. However, regardless of the treatment diets fed during the depletion phase, BMC ($P = 0.001$), BMC/protein ($P < 0.001$) and bone mineral deposition ($P < 0.001$) were lower in R100 gilts than in R160 gilts. Moreover, there was a trend indicating that the previously depleted D60 gilts fed R100 or R160 diets deposited more bone mineral ($P = 0.086$) than the non-depleted D100 gilts. The intake, retained and excreted P and Ca values were lower in R100 gilts than in R160 gilts ($P < 0.001$; Table 5), and these findings were independent from the depletion diets (no interaction). The body P retention tended to be higher ($P = 0.068$) in depleted gilts than in non-depleted gilts and this is independent of the repletion diets (no interaction). The body Ca and P retention efficiency was higher in R100 gilts than in R160 gilts ($P < 0.001$). The serum Ca and P, PICP and CTX concentrations and urinary Ca and P concentrations remained similar ($P > 0.10$) between dietary treatments.

Bone mineralization and body composition development throughout the entire experiment (60–140 kg BW)

The time-dependent development in body composition and bone mineralization (BW, body protein, body lipid and BMC, BMD, BMC/protein) and deposition of body protein, body lipid and bone mineral are presented in Table 6 and Supplementary Table S1, respectively. There was no interaction between treatment and time for BW, body composition and deposition of protein and lipid mass, which increased with time ($P < 0.001$). However, a treatment × time interaction ($P < 0.001$) was found for BMC, BMC/protein ratio (Fig. 1), bone mineral deposition (Fig. 2) and BMD (Table S1). From the second week (scan 1) of the depletion phase onwards until the end of the depletion phase (scan 3), BMC, BMD, BMC/protein ratio and bone mineral deposition were lower in D60 (D60-R100, D60-R160) gilts than in D100 (D100-R100, D100-R160) gilts. From the second week (scan 4) and fourth week (scan 5) of the repletion phase, BMC ($P < 0.001$), BMC/protein ($P < 0.001$) and bone mineral deposition ($P < 0.05$) values of D60 gilts returned to similar values than those of D100 gilts, when gilts were fed R160 (D60-R160, D100-R160) and R100 (D60-R160, D100-R160), respectively. From the fourth week (scan 5) of the repletion phase, BMC ($P < 0.001$) values and from the sixth week (scan 6) of the repletion phase, BMD ($P < 0.001$), BMC/protein ($P < 0.001$) and bone mineral deposition ($P < 0.05$) values of D60-R160 and D100-R160 gilts were higher than D60-R100 and D100-R100 gilts, respectively.

Discussion

Impact of a P and Ca depletion strategy to remove mineral phosphates for finisher pigs

Feeding fattening pigs with a P level that maximizes growth without fulfilling the requirement for maximum bone mineralization offers the opportunity to reduce the use of dietary mineral phosphates and the amount of P excretion. However, in the context of gilts, in order to ensure and sustain maximal bone mineralization, a nutritional practice should be implemented at the start of the gilt's breeding period (Kraeling and Webel, 2015). The present study tested a strategy of mineral status depletion with the aim of inducing regulations to increase the use of dietary Ca and P. This was then followed by a repletion phase with normal or high Ca and P intakes to recover and maximize bone mineralization before the first mating and under targeted daily BW gain conditions. A similar strategy was successfully achieved in grower-finisher pigs (Varley et al., 2011; Létourneau-Montminy et al., 2014; Gonzalo et al., 2018).

Under non-limiting conditions, it has long been recognized that the partitioning of Ca and P in bone is proportional to the amount

Table 4
Effect of Ca and P repletion diets on growth performance and body composition in gilts from 95 kg BW to 140 kg BW.

Response criteria[1]	Treatments				SEM			
	D60		D100			*P*-value		
	R100	R160	R100	R160		Depletion	Repletion	Interaction
Initial conditions								
BW (kg)	99.6	101.0	101.3	99.1	2.14	0.894	0.697	0.316
Body protein (kg)	16.2	16.7	16.8	16.5	0.39	0.485	0.717	0.158
BMC (g)	1 897	1 948	2 132	2 097	49.6	0.001	0.857	0.352
BMC/Protein ratio (g/kg)	118	117	127	127	3.5	<0.001	0.755	0.448
Final conditions								
BW (kg)	142.3	144.1	142.8	139.1	1.55	0.282	0.391	0.065
Body protein (kg)	22.4	22.8	22.7	21.6	0.47	0.358	0.496	0.152
BMC (g)	2 989	3 314	3 133	3 356	62.9	0.159	0.001	0.426
BMC/protein ratio (g/kg)	134	146	138	156	4.4	0.097	0.004	0.512
Overall performances								
Total Feed intake (kg)	183.1	188.4	178.3	187.2	7.88	0.681	0.388	0.789
Feed intake (g/d)	3 101	3 081	3 030	3 084	16.4	0.517	0.337	0.236
BW gain (g/d)	718	737	712	686	37.5	0.461	0.927	0.554
Feed conversion ratio	4.37	4.25	4.26	4.60	0.304	0.790	0.477	0.336
Body protein deposition (g/d)	96.7	97.2	92.9	83.4	8.51	0.314	0.604	0.560
Bone mineral deposition (g/d)	18.7	23.4	17.0	21.4	1.04	0.086	<0.001	0.879

Abbreviations: 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. BMC = Bone mineral content.
[1] BMC was obtained using dual-energy X-ray absorptiometry (DXA), and body protein was predicted based on DXA lean (Kasper et al., 2021).

Table 5
Effect of Ca and P repletion diets on P and Ca balance and on blood serum and urine concentrations in gilts from 95 to 140 kg BW.

Response criteria	Treatments				SEM[1]			
	D60		D100			*P*-value		
	R100	R160	R100	R160		Depletion	Repletion	Interaction
Phosphorus balance								
Total P intake (g)	976	1 359	947	1 342	50.9	0.720	<0.001	0.824
P body retention (g)[1]	245	283	227	254	16.5	0.068	0.014	0.722
P excretion (g)	730	1 067	720	1 087	56.3	0.917	<0.001	0.744
Total P Efficiency (%)[2]	25.3	21.1	24.1	19.1	2.08	0.157	<0.001	0.761
Calcium balance								
Ca intake (g)	1 193	1 985	1 158	1 973	71.4	0.744	<0.001	0.873
Ca body retention (g)[1]	444	555	407	512	31.6	0.121	<0.001	0.893
Ca excretion (g)	749	1 430	751	1 462	83.3	0.806	<0.001	0.827
Total Ca efficiency (%)[2]	37.3	28.2	35.3	26.0	3.06	0.265	<0.001	0.933
Blood serum minerals at 140 kg BW (mmol/l)								
Calcium	2.28	2.38	2.29	2.39	0.104	0.305	0.773	0.583
Phosphorus	2.49	2.42	2.36	2.38	0.052	0.061	0.840	0.971
Blood Serum bone biomarkers at 140 kg BW (ng/ml)								
PICP	30.7	23.9	22.1	23.9	2.33	0.109	0.348	0.112
CTX	1.49	1.49	1.05	1.26	0.253	0.180	0.667	0.657
Urine concentration at 140 kg BW (mol/mol creatinine)								
Ca	0.173	0.194	0.212	0.196	0.048	0.956	0.636	0.681
P	1.50	1.49	1.67	1.45	0.172	0.533	0.727	0.568

Abbreviations: 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.; PICP = carboxy-terminal propeptide of type I collagen; CTX = carboxy-terminal cross-linking telopeptide of type 1 collagen.
[1] Body P and Ca retention was calculated from the difference between initial and final Body P and Ca obtained from body bone mineral content and lean according to Kasper et al. (2021).
[2] Calculated as the amount of total P and Ca intake divided by the amount of total P and Ca retained.

of soft tissues (Crenshaw, 2001; Misiura et al., 2020). However, when the diet is deficient in Ca and P, soft tissue deposition is given priority, which is equivalent to saying that the Ca and P requirements for bone mineral deposition are secondary to those associated with soft tissue growth (Létourneau-Montminy et al., 2015; Misiura et al., 2020). Consequently, the short-term restriction of Ca and P can lead to a decrease in bone mineralization, but not in the growth of soft tissue and of BW (Ryan et al., 2011; Skiba et al., 2018; Misiura et al., 2020). In accordance with these observations, the present study's results revealed that feeding gilts a diet that provides 60% of the estimated Ca and digestible P requirements for 35 days did not decrease their growth performance in the gilts with a BW ranging from 60 kg to 95 kg BW. This finding is also in agreement with previous studies on piglets (Skiba et al., 2017; Schlegel, 2019) and grower-finisher pigs (Hastad et al., 2004; Schlegel and Gutzwiller, 2020; Misiura et al., 2020). Thus, the animal would attempt to maintain its maximum lean tissue retention because the bone could handle a small deficit, as suggested by Létourneau-Montminy et al. (2015).

As expected, based on whole-body BMC and BMD, the low supply of Ca and digestible P (D60) led to a decrease in bone mineralization (−9% vs D100), which, according to the repeated measure

Table 6
Development in body composition of gilts (60–140 kg) according to different Ca and P feeding strategies.

Response criteria	Time (scan number with a two-week interval, each)								SEM	*P*-value		
	0	1	2	3	4	5	6	7		Treatment	Time	Treatment × time
BW (kg)												
D60-R100	58.0	69.1	85.0	99.3	110.2	118.6	128.9	141	1.93	0.707	<0.001	0.969
D60-R160	58.4	69.7	85.6	101.8	111.7	122.1	132.1	143				
D100-R100	59.0	68.4	86.4	101.3	113.0	121.3	132.4	142				
D100-R160	56.9	67.4	84.2	99.0	111.4	121.6	131.6	139				
Body protein (kg)												
D60-R100	9.5	11.2	13.5	15.4	16.8	18.1	19.2	20.9	0.36	0.271	<0.001	0.705
D60-R160	9.6	11.4	13.8	15.9	17.6	19.0	20.3	21.6				
D100-R100	9.8	11.2	14.0	16.0	17.6	18.8	20.1	21.4				
D100-R160	9.4	11.1	13.7	15.7	17.5	19.0	20.2	20.7				
Body lipid (kg)												
D60-R100	7.1	9.9	14.4	19.4	23.0	25.5	30.2	33.1	0.79	0.257	<0.001	0.735
D60-R160	7.0	9.5	13.9	18.1	20.9	23.9	27.2	32.3				
D100-R100	6.7	8.9	13.4	18.4	21.6	24.2	27.9	32.4				
D100-R160	6.4	8.4	12.5	17.5	20.5	24.1	27.6	32.8				
BMC (g)												
D60-R100	1 198	1 369^{a}	1 593^{a}	1 886^{a}	2 215^{a}	2 520^{a}	2 727^{a}	2 966^{a}	54.1	0.011	<0.001	<0.001
D60-R160	1 219	1 366^{a}	1 615^{a}	1 928^{a}	2 378^{b}	2 728^{c}	3 024^{bc}	3 287^{bc}				
D100-R100	1 252	1 483^{b}	1 773^{b}	2 131^{b}	2 420^{b}	2 661^{ab}	2 908^{ab}	3 128^{ab}				
D100-R160	1 244	1 458^{b}	1 730^{b}	2 104^{b}	2 474^{b}	2 795^{c}	3 083^{c}	3 350^{c}				

Abbreviations: 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; BMC = Bone mineral content;
[a–c]Values within a column not sharing a common superscript differ (treatment × time interaction, $P \leq 0.001$).

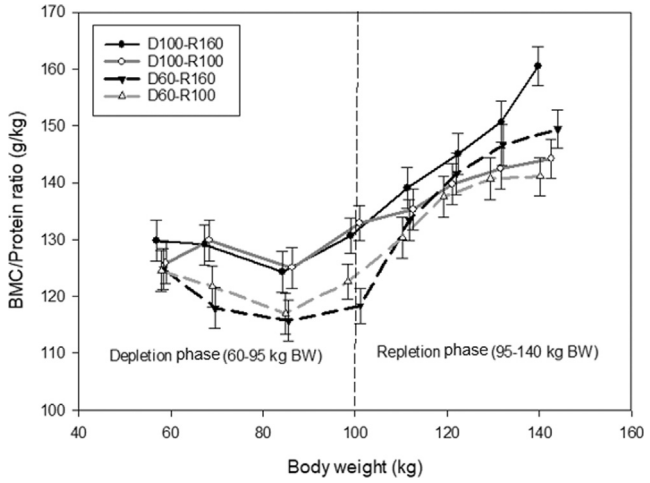

Fig. 1. Ratio of whole-body bone mineral content (BMC) to protein content, obtained by dual-energy X-ray absorptiometry in gilts fed according to different calcium and phosphorus depletion (60–95 kg) and repletion (95–140 kg) phases. D60 = depletion diet low in Ca and digestible P; D100 = depletion diet adequate in Ca and digestible P; R100 = repletion diet adequate in Ca and digestible P; R160 = repletion diet excessive in Ca and digestible P. Protein = −784.77 + 0.2 × lean tissue mass. Values expressed as means ± SEM.

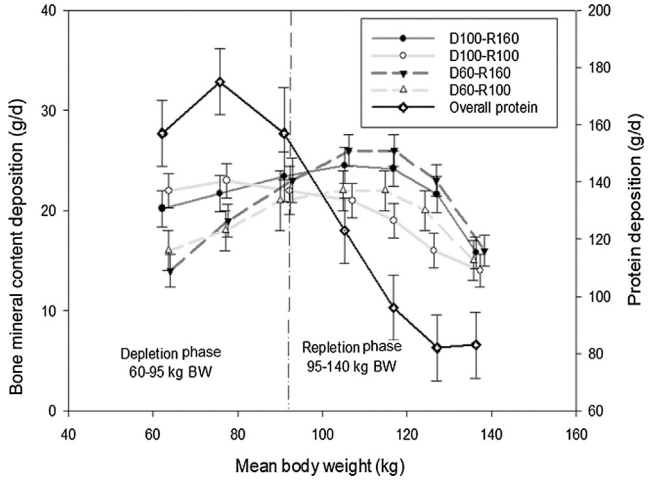

Fig. 2. Whole-body deposition of bone mineral and of protein (treatments combined) obtained by dual-energy X-ray absorptiometry in gilts fed according to different calcium and phosphorus depletion (60–95 kg) and repletion (95–140 kg) phases. D60 = depletion diet low in Ca and digestible P; D100 = depletion 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. Protein = −784.77 + 0.2 × lean tissue mass. Values expressed as means ± SEM.

analysis, had already occurred within 2 weeks during the depletion phase. This finding is supported by the results reported in previous studies showing that a low Ca and digestible P diet resulted in reduced bone mineralization (Pokharel et al., 2017; Skiba et al., 2018; Schlegel and Gutzwiller, 2020). This negative effect on bone mineralization confirms the hypothesis that Ca and P requirements for maximizing bone mineralization are higher than those required for maximizing growth performance, as previously demonstrated (Crenshaw, 2001; National Research Council, 2012). However, the magnitude of bone demineralization in the present study was lower than that reported in other studies. In grower pigs (30–70 kg BW), Ryan et al. (2011) showed that whole-body BMD was reduced by 17% when dietary P decreased from 2.2 to 1.6 g of digestible P/kg over a 28-day period. Similarly, in finisher pigs (70–100 kg BW), Gonzalo et al. (2018) found that whole-body BMC was reduced by 15% when a diet providing 13% less Ca (6.2 vs 5.4 g/kg) and 34% less digestible P (2.5 vs1.7 g/kg) than the control was fed to the animals for 28 days. This is probably explained by the fact that pigs may adapt their bone demineralization depending on their age at the time of depletion, the husbandry conditions and their genetic background (Hittmeier et al., 2006; Alexander et al., 2008; Pokharel et al., 2017).

The depletion phase resulted in an improved Ca utilization of 40%. In a similar type of study design, but with finisher pigs (70–100 kg BW), Gonzalo et al. (2018) investigated a depletion phase that was longer than 28 days; they reported that the P efficiency

improved, but the Ca efficiency did not, probably due to a limited dietary Ca restriction (<20 vs 40% in the present study). In the present study, gilts fed D60 had a lower urinary P concentration and a tendency for an increased blood serum CTX concentration than gilts fed D100. These observations indicate the presence of a homeostatic regulation response to cope with a deficient P intake by increased renal P re-absorption and by bone demineralization, since CTX is a bone-derived degradation product of type I collagen C-telopeptides.

These metabolic processes are probably related to phosphocalcic regulations through the coordinated action of PTH and 1,25-dihydroxyvitamin D_3 that act on specific organs such as kidneys and intestines as well as bones to maintain Ca and P extracellular concentrations (Just et al., 2018; Wubuli et al., 2019). Indeed, Ca and P deficiencies can lead to momentary decreases in serum concentration of both Ca and P, although their homeostasis is tightly regulated (Crenshaw, 2001). As serum Ca concentration falls, a Ca-sensing receptor stimulates the secretion of PTH in the parathyroid gland, which in turn stimulates bone resorption, indicated by CTX, by acting directly on osteoblasts and then indirectly to increase differentiation and function of osteoclasts to release Ca (Just et al., 2018). In the kidneys, PTH also stimulates the synthesis of $1,25(OH)_2D_3$, which increases the presence of Ca and P transporters and intervenes by favoring intestinal absorption and renal re-absorption of Ca and P (Just et al., 2018; Wubuli et al., 2019). Increased renal P re-absorption under dietary P deficiency was linked with an improved mRNA expression of Na-dependent phosphate transporters in the kidney (Pokharel et al., 2017). In the end, the absence of dietary effects on serum Ca and P concentrations may be due to the establishment of digestive and metabolic regulations. Finally, the results of this study also highlight the possibility of completely removing the use of non-renewable mineral phosphate sources in the finisher diet without affecting finisher pigs' growth performance. These findings go even further than the results reported by Schlegel and Gutzwiller (2020), who concluded that maximization of performance and soft tissue growth was possible in finisher pigs without the addition of mineral phosphate to the diet, but with the addition of microbial phytase resulting to a higher digestible P concentration.

Impact of a P and Ca repletion strategy to maximize bone mineralization before the first mating

Two types of repletion diets were studied: (1) R100, a repletion diet that fulfilled the Ca and P requirements to maximize growth performance while optimizing bone mineralization of gilts and (2) R160, a strategy supplying 160% of the dietary requirements to ensure maximum bone mineralization, which is frequently practiced in North America (PIC, 2016) and also observed in Switzerland. As expected, given the growth performance results during the depletion phase, neither the depletion from 60 to 95 kg BW nor the diet supplying 100 or 160% of the dietary requirements affected animals' growth performance during the repletion phase. Interestingly, bone mineralization, measured in terms of whole-body BMC and BMD, was similar in the depleted and non-depleted gilts from 60 to 95 kg BW. Although the relative balance efficiencies of body Ca and P was not affected by dietary treatments, the tendency for improved bone mineral deposition and P retention in the depleted gilts indicated that they were able to deposit more P into their skeleton than non-depleted gilts did to recover their bone mineralization. Similar to the results demonstrated in the present study on gilts, pigs previously depleted for a period of 28–56 days beginning with a BW ranging from 15 to 25 kg (Létourneau-Montminy et al., 2014) or from 100 to 130 kg (Gonzalo et al., 2018) could also recover from their bone mineral deficit, as indicated by an increase in bone mineral deposition and an increase in P retention. To more comprehensively investigate the underlying mechanisms of bone mineralization recovery, it is necessary to obtain samples of physiological fluids, such as blood and urine more frequently than was done in the present study. Nevertheless, it is well known that PTH and $1,25(OH)_2D_3$ are involved (Rousseau et al., 2016; Just et al., 2018) which stimulate the expression of a set of genes encoding phosphate and Ca transporters in enterocytes promoting an increase in intestinal and renal re-absorption, as previously showed in depletion-repletion strategies in broilers (Rousseau et al., 2016; Valable et al., 2018). According to the repeated measures analysis, the magnitude of this response appears to be progressively reduced with the compensatory response in the deficit of bone mineralization. Indeed, Varley et al. (2011) observed that the retention of P reached its maximal level during the first weeks after depletion and plateaued when bone recovery was observed. Similarly, when depleted gilts were fed an adequate diet over two, 28-day repletion phases, Skiba et al. (2018) observed a higher rate of mineral deposition during the first half of the repletion phase than in the second half.

Finally, in the present study, in comparison to the non-depleted gilts, compensatory response in bone mineralization was observed in the depleted D60 gilts, as there was a tendency for an increased serum PICP, which might indicate a higher activity of bone formation, especially in those fed D60-R100. This result suggests that although compensation of bone mineralization of the depleted D60 gilts was achieved in regards to DXA values, the physiological responses were still active at 140 kg BW. In reproductive sows, Grez-Capdeville and Crenshaw (2021) found that plasma PICP and the PICP/CTX ratio tended to increase with P intake (4.0–4.8 g of total P/kg) in late lactation, which may be due to recovery of osteoblastic activity to compensate for bone mineralization deficit during lactation. Similarly, Sipos et al. (2011) observed a relative increase in serum PICP when multiparous sows were restrictively fed over 10 months and re-alimented in comparison to those that were fed adequately.

The present study also showed that in comparison to gilts fed R100, gilts fed R160 had increased bone mineralization, deposition, Ca and P balance both in terms of quantities retained and efficiencies. This suggests that the Ca and P requirements to maximize bone mineralization in gilts (between 95 and 140 kg BW) would exceed current recommendations based on the factorial method (eg. Jondreville and Dourmad, 2005; National Research Council, 2012; Bikker and Blok, 2017) when considering the effective daily BW gain and feed intake of replacement gilts under a controlled feeding (aim: 700–750 g/d BW gain between 100 and 140 kg BW). In accordance with these results, Merriman et al. (2017) indicated that bone ash from pigs (100–130 kg BW) was maximized when, respectively, fed 152% and 173% of the digestible P and Ca requirements established by the National Research Council (2012), which are set to provide 85% of the requirements for maximum P retention. It is important to note that all these models were developed for grower-finisher pigs under *ad libitum* conditions. Nevertheless, the higher BMC in the gilts undergoing the 160% treatment indicates that the requirement should be reviewed.

Development of protein and bone mineral deposition in replacement gilts

As previously indicated, some studies have shown that bone growth is independent of protein deposition, especially after gilts reach 60–80 kg BW (Ruiz-Ascacibar et al., 2019; Lautrou et al., 2020). This was also observed in the present study with a BMC/protein ratio that increased with increasing BW, especially from 85 kg BW onwards. When looked at separately, the protein depo-

sition peaked at a mean BW of 77 kg and then decreased rapidly, while bone mineral deposition peaked later at around 110 kg BW and then decreased slowly. This is in agreement with Couture et al. (2018) and Lautrou et al. (2020), who showed that bone mineral deposition linearly increased with BW, while protein deposition followed a Gompertz function that peaked at around 70 kg BW. The fact that bone mineral deposition decreased during the repletion phase even when gilts were fed R160 was probably closely linked to the progressive decline of daily BW gain in each two-week period to reach a mean of 713 g. Although the impact of energy restriction on bone metabolism has not been extensively explored in growing animals, gilts reduced bone mineralization when fed only 85% of their usual energetic intake (Arthur et al., 1983). In humans, energy restriction caused by intensive exercise resulted in an uncoupling of bone turnover, increasing bone resorption and decreasing bone formation, especially in women (Ihle and Loucks, 2004; Papageorgiou et al., 2018). Similarly, in sheep, a decrease in estrogen due to weight loss slowed bone accretion and intestinal Ca and P absorption (Fleet and Schoch, 2010). These results indicate that bone mineral deposition will slow down when energy is restricted. Further research is needed to understand the underlying mechanisms and to ensure that bone mineralization is optimal for replacement gilts in the swine industry.

Conclusion

The results of this study confirm the possibility of limiting the use of phosphate during the finisher phase in pigs without inducing adverse effects on growth performance. The resulting limitation in bone mineralization could be recovered within 2 and 4 weeks, respectively, in 100 kg BW replacement gilts fed a diet containing either adequate or excessive amounts of Ca and P. Highest bone mineralization was achieved in the gilts fed 160% of recommended Ca and digestible P indicating that the requirement for maximal bone mineralization in gilts should be further investigated and reviewed.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2022.100512.

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.

Data and model availability statement

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

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Declarations of interest

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

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