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Effects of phosphorus and calcium depletion on growth performances and bone mineralisation in growing pigs

M. Lautrou [a,b,1,*], C. Pomar [c], P. Schmidely [b], M.P. Létourneau-Montminy [a]

[a] Département des sciences animales, Université Laval, Québec, QC G1V 0A6 Canada
[b] UMR Modélisation Systémique Appliquée aux Ruminants, INRA, AgroParisTech, Université Paris-Saclay, 75005 Paris, France
[c] Agriculture et Agroalimentaire Canada, Sherbrooke, QC J1M 1Z3 Canada

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ABSTRACT

The use of P nowadays is raising environmental (eutrophication) and sustainability (limited resource) concerns in the swine industry, but initial trials have shown that similar growth performance can be achieved between pigs fed on a requirement basis and those fed using a P depletion-repletion strategy. To optimise the use of dietary P by pigs, three feeding strategies were studied according to a 3-phase feeding programme: (1) C–C–C providing 100% of the P and Ca requirements, (2) C-L_{Normal}-C, providing 100% of the P and Ca requirements in phases 1 and 3 (C) with a depletion in phase 2 with 60% of the P requirements combined with a normal Ca:digP ratio of 2.6 (L_{Normal}), and 3) C-L_{High}-C, providing 100% of the P and Ca requirements in phases 1 and 3 (C) with a depletion in phase 2 with 60% of the P requirements combined with a high Ca:digP ratio of 3.3 (L_{High}). Bone mineral content (**BMC**) and BW were measured at the beginning and end of each phase. BMC gain, average daily gain, average daily feed intake and feed efficiency were calculated for each phase. In phase 1, all pigs received the same diet. At the end of phase 2, the C-L_{Normal}-C and C-L_{High}-C groups had similar BMCs compared to the C–C–C group. Finally, at the end of phase 3, the BMC gain was numerically higher in the C-L_{Normal}-C group than in the C–C–C group (25.4 vs 18.7 g/d, $P = 0.10$). Although depletion did not cause a decrease in BMC in the C-L_{Normal}-C and C-L_{High}-C groups (versus C–C–C), it did result in better P use during repletion. These results demonstrate the value of a depletion-repletion strategy to reduce P intake and excretion without compromising the final performance.

Implications

Optimising the use of phosphate is crucial for sustainable agriculture, and this may include improving the use of P in pig diets. We therefore tested a depletion-repletion strategy in P and Ca to increase the efficiency of dietary utilisation of these minerals by growing pigs. The results confirm similar growth performance and bone mineral content between the depleted and control groups, fed at phosphorus and calcium requirements. This strategy reduced P excretion by 10%, confirming its promise for sustainable pig production.

Introduction

The decline in the number of pig farms and the increase in pork consumption have led to intensification of livestock production in some regions. The density of the pig farms in these areas has introduced various problems, particularly environmental issues due to P in manure. As P is mainly available as phytate in cereal-based diets, a form that is poorly available to pigs, more than half of the ingested P is excreted (Jondreville and Dourmad, 2006; Selle and Ravindran, 2008). For this reason, pigs receive a mineral form of phosphate, but it is a limited resource (Van Enk et al., 2011; Cordell and White, 2013), supplied by only a few countries and therefore subject to price volatilely, as currently observed (Investing News Network, 2022). Therefore, the efficient use of P remains a sustainability issue in the swine industry. Pigs can increase P utilisation by digestive and metabolic adaptations when fed a P-deficient diet (Breves and Schröder, 1991). This restriction can reduce mineral P supply, feed cost and environmental consequences. When a depletion period, using deficient diet, early in life (i.e. between 15 and 50 kg of BW) is followed by a repletion period (diet satisfying P requirements), the increased P utilisation is maintained (Létourneau-Montminy et al., 2014; Gonzalo et al., 2018; Aiyangar et al., 2010), resulting into a 20% reduction in P intake and excretion. Previous trials with P and Ca depletion and repletion

* Corresponding author.
E-mail address: marion.lautrou@agroscope.admin.ch (M. Lautrou).
[1] Present address: Agroscope, Swine Research Group, La Tioleyre 4, 1725 Posieux, Switzerland.

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sequences have confirmed the possibility of achieving the same final level of bone mineralisation in pigs fed a diet meeting the requirements, in addition to maintaining a similar growth performance (Létourneau-Montminy et al., 2014; Gonzalo et al., 2018; Varley et al., 2011). However, this strategy does not always achieve the same bone mineral content (**BMC**) level as those fed a diet meeting the requirements, securing skeletal quality (Gonzalo, 2017; Aiyangar et al., 2010).

Another way to optimise P utilisation is to precisely estimate Ca and P requirements. Current P requirement models (e.g. National Research Council NRC, 2012; van Milgen et al., 2008) suggest that empty BW and BW can be estimated from protein and lipid body mass, based on Quiniou and Noblet (1995), with water and ash embedded in these two body constituents. This modelling approach implies that body ash (all body minerals) is related to body protein, and therefore, that body ash mass and growth are driven by the growth of body protein mass. Previous authors have demonstrated the differential growth of body soft tissues and bone in growing pigs (Gonzalo et al., 2018; Couture et al., 2018), while others have shown that depleted pigs can rapidly replace bone mass through compensatory bone mineralisation (Ryan et al., 2011; Varley et al., 2011; Gonzalo et al., 2018; Létourneau-Montminy et al., 2014). Based on these results, a new model allowing to simulate the growth of bone and body protein independently was developed (Lautrou et al., 2020). This model showed an increase in bone mineral deposition with increasing BW, while the protein deposition reached a plateau or a maximum around 60 kg. Therefore, the period before 60 kg, when protein deposition in soft tissue is high and when the depletion–repletion strategy was implemented, seems dangerous for bone health.

The objective of this study was twofold: (1) to validate the model of Lautrou et al. (2020) by simulating the control diet to maximise bone mineralisation and (2) to determine whether a Ca and P depletion–repletion protocol would improve pigs' utilisation of the Ca and P minerals without affecting growth performance and slaughter bone mineralisation levels. Our hypothesis is that the digestible P and total Ca requirements estimated by the model of Lautrou et al. (2020) would maximise the bone mineralisation and that during a depletion-repletion protocol, the bone mineral growth of pigs could be temporarily reduced but that thanks to hormonal regulations, their P utilisation efficiency would be improved, leading to a maintain of final bone mineralisation level without compromising the growth performances.

Material and methods

Animals were housed and treated in accordance with the guidelines established by the Canadian Council on Animal Care (CCAC, 2009), and the experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee at the Laval University Québec, QC, Canada.

Experimental diets

The pigs were fed according to a three-phase feeding programme, each phase lasting 39, 27 and 28 d, respectively (expected 25–55 kg, 55–90 kg and 90–125 kg). In this trial, the diets met all the pigs' nutritional requirements (NRC, 2012), except for P and Ca. Animals were fed daily *ad libitum* and had continuous and unlimited access to fresh water. The first group (C–C–C) was fed a diet meeting 100% of the total Ca and digestible P requirements for maximum bone mineralisation during the three feeding phases, namely 4.3, 2.6 and 2.2 g/kg of digestible P (apparent total tract digestible), with total Ca to digestible P ratios of 2.3, 2.4 and 2.6 for phases 1, 2 and 3, respectively according to the requirements from Lautrou et al. (2020; Table 1). A depletion–repletion strategy was applied to the C-L_{Normal}-C and C-L_{High}-C groups, meeting all the nutritional requirements in phases 1 and 3, but in phase 2, only 60% of the digestible P requirement and 65 or 80% of the Ca requirement for maximal bone mineralisation were covered. In phase 2, the total Ca:digestible P ratio was planned to be normal in the L_{Normal}-feed group (2.7) and high in the L_{High}-feed (3.3) group. During the first phase, the diet was in meal form but in steam-pelleted form in the second and third phases, because of technical issues on farm (agglomeration). During the first phase, no pigs were fed a depletion diet, because this period is associated with increased protein deposition (before 60 kg of BW) and low bone mineral deposition. Therefore, to avoid any risk of fractures or lameness, all animals received a control diet maximising BMC deposition. This also maintained Ca and P levels prior to depletion, as the pigs were not raised on the farm before the start of the trial.

Growth trial

The experimental diet distribution started when the mean BW of the pigs was about 30.3 ± 3.90 kg. A total of 144 castrated pigs (276 or 275 fast female × PIC 800 Duroc) were randomly assigned to treatments and housed in pens of six pigs 4 days before the start of the study. Individual BW was recorded at the beginning of the experiment and at the end of each phase. The delivered feed was weighted daily per pen and refusal was recorded at the end of the phase. Body composition of 8 animals per treatment (one animal per pen, randomly selected) was measured at the beginning of the experiment and at the end of each phase using dual-energy X-ray absorptiometry (DXA, Hologic Discovery W, Hologic Inc., Waltham, MA, USA) to quantify the BMC, bone mineral density and lean and fat tissue mass (Mitchell et al., 2002). Animals received an injection of azaperone (2.2 mg/kg BW; Stresnil; Jansen-Cilag, Neuss, Germany) for calming and were then anaesthetised by mask inhalation of sevoflurane (Sevorane; Abbott Laboratories, North Chicago, IL, USA) at an oxygen concentration of 7% until sleepiness. Sevoflurane was then replaced by isofluorane (IsoFlo; Abbott Laboratories) at an oxygen concentration of 5% to maintain anaesthesia during scans. Animals were scanned in prone position, with the front legs alongside the body and using the whole-body mode. At the end of phase 3, the animals were too heavy to be scanned, so only their heads, recovered from the slaughterhouse, were scanned. At the beginning of the experiment and at the end of phases 1 and 2, blood samples were collected to the jugular vein on all scanned animals (d-1; d37-38; d66-68), after fasting for 12 h, in dry and sodium heparinised vacutainer tubes (Becton Dickson, Franklin Lakes, NJ) to obtain serum and plasma after centrifugation at 2 000g for 20 min. The plasma and serum samples were frozen at −80 °C until analysis.

Metabolic trial

A total collection of faeces and urine was organised during phase 2. On day 58 (phase 2) of the experiment, at approximately 100 kg, 6 animals randomly selected from each group (among the 144 pigs) were placed in individual metabolic cages for 5 days, including 1 day of adaptation. For each pig, all faeces and 20% of the daily urine were collected separately over 4 consecutive days and then frozen at −20 °C. Thawed faeces were homogenised with water, and samples were then frozen at −20 °C and freeze-dried before analysis. Thawed urine was filtered (Ashless, grade 42, Whatman), and samples were frozen at −20 °C before analyses.

Table 1
Ingredients and chemical compositions of diets with varying dietary Ca and P concentrations (as-fed basis) offered to growing pigs.

	Phase 1	Phase 2			Phase 3
Treatments	C[1]	C[1]	L_{Normal}[1]	L_{High}[1]	C[1]
Ingredients[2], g/kg					
Corn	713.1	575	575	575	575
Wheat		245.1	260.6	257.4	233.4
Soybean meal 47%	222.6	144.5	141.8	142	158.5
Soy oil	15.8	4.9	1	1.6	6.7
Limestone	14.1	9.4	6.5	8.9	9.6
Monocalcium phosphate	18.7	8.5	2.6	2.6	6.3
Salt	5.4	4.9	4.9	4.9	7.1
L-Lysine	4.4	3.73	3.79	3.78	1.57
DL-Methionine	1.29	0.54	0.52	0.52	
L-Threonine	1.47	1.13	1.14	1.13	0.22
L-Tryptophan	0.56	0.29	0.29	0.29	
L-Valine	0.51				
Vitamin-mineral premix	2	2	2	2	1.6
Formulated chemical composition					
DM (%)	87.69	87.30	87.16	87.20	87.32
CP (%)	16.62	14.29	14.33	14.31	14.50
Crude fat (%)	4.23	2.94	2.57	2.62	3.12
Net energy (kcal/kg)	2 475.0	2 475.0	2 478.4	2 475.0	2 475.0
Lysine SID[3] (%)	1.064	0.842	0.843	0.842	0.705
Calcium (g/kg)	9.72	6.12	4.02	4.94	5.81
Total phosphorus (g/kg)	7.22	4.98	3.76	3.76	4.57
Digestible phosphorus[4] (g/kg)	4.3	2.6	1.5	1.5	2.2
Magnesium (g/kg)	1.6	1.4	1.3	1.3	1.4
Vitamin D (UI/kg)	1 000	1 000	1 000	1 000	800
Total Ca/digestible P	2.3	2.4	2.7	3.3	2.6
Analysed chemical composition[4]					
DM (%)	89.1	86.1	85.9	87.2	89.1
Total calcium (g/kg)	8.0	5.8	3.1	3.9	3.8
Total phosphorus (g/kg)	7.1	5.3	3.9	4.0	4.7
Total Ca/digestible P	1.9	2.2	2.1	2.6	1.7

[1] C: Control diet at 100% of the total Ca and digestible P requirements for maximal bone mineralisation; L_{Normal}: diet at 60% of the digestible P requirements and 65% of the total Ca requirements; L_{High}: diet at 60% of the digestible P requirements and 80% of the total Ca requirements.
[2] During the first phase, the feed was in mashed form and in pelleted form during the second and third phases.
[3] SID = Standardised ileal digestible.
[4] DM and total P and Ca were analysed, while digestible P (apparent total tract digestible) and total Ca to digestible P ratio were calculated from NRC (2012).

Calculations

Due to the large number of animals, the measurements (scan, weighing and refusal of feed) could not all be performed in a single day. Thus, corrections were applied by linear regression to bring the data to the same phase duration, the feeding phase. Average daily feed intake (**ADFI**), average daily gain (**ADG**) and the gain–to–feed ratio (**G:F**) were calculated per pen and phase. At the end of phase 3, whole-body BMC was estimated according to Létourneau-Montminy et al. (2017), based on the head. However, the amount of lean and fat tissues could not be determined. To enable comparison of results at the beginning and end of phase 3, the same whole-body BMC calculations were performed based on the head region from the beginning of phase 3. Daily BMC gain was calculated per pig as the difference in BMC between the end and beginning of the phase divided by the number of days in the phase. Body lean and fat contents were used to determine whole–-body protein and lipid contents as proposed by Pomar and Rivest (1996). Whole-body Ca and P contents were calculated as the sum of Ca and P in bone, muscle, and fat. The percentage of Ca and P in bone represents approximately 39 and 18%, respectively, of the whole-body BMC (Crenshaw, 2001). P and Ca concentrations in protein and lipid were estimated, as those associated with lean and adipose tissue, respectively, as per Lautrou et al. (2020). The retention efficiency coefficients of total analysed Ca and digestible P were calculated by dividing the daily body gains of P and Ca by the daily ingested quantities estimated by phase in phases 1 and 2. This calculation was not possible in phase 3, because the body protein and lipid composition were not available.

Chemical analysis

Feed samples were taken at the beginning of the phase to analyse Ca and P concentrations. DM was analysed in feed samples thereafter using the Association of Official Analytical Chemists (1990) standard method (Method 930.15). Ca and P in feed were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES, Actlab-Agriculture, Ancaster, ON, Canada). Ca, P and Mg in plasma were analysed by inductively coupled optical plasma emission spectrometry (ICP-OES, Agilent 5110 ICP-OES, Agilent Technologies, Inc., Ca, USA, method 990.08; AOAC International, 2006). Serum procollagen I C-terminal Propeptide (**PICP**) and cross-linked C-telopeptide of type I collagen (**CTX**) concentrations were measured using ELISA kits (Porcine PICP, Elabscience, Houston, TX, USA, sensitivity: 37.5 pg/mL, inter-assay: CV < 10%, intra-assay: CV < 10% and Porcine CTX-1 ELISA, Novus biologicals, Toronto, Canada, sensitivity: 0.19 ng/mL, inter-assay: CV < 5.6%, intra-assay: CV < 5.63%). The serum osteocalcin (**OC**) concentration was analysed using an ELISA kit (Porcine OC/BGP (Osteocalcin/Bone Gla Protein), Elabscience, Houston, TX, USA, sensitivity: 0.47 ng/mL, inter-assay: CV < 10%, intra-assay: CV < 10%). The calcitriol precursor (25(OH)-vitamin D_3) was measured in serum using an ELISA kit (Porcine 25-Hydroxy-Vitamin-D3, ELISA Kit, MyBioSource, San Diego, CA, USA, sensitivity: 1.0 ng/ml,

inter-assay: CV < 12%, intra-assay: CV < 10%). Faeces samples were freeze-dried at −85 °C, and Ca and P in faeces and urine were analysed by inductively coupled optical plasma emission spectrometry (ICP-OES, Actlab-Agriculture, Ancaster, ON, Canada).

Statistical analysis

Most results were analysed with linear mixed models using the R software (R version 4.2.2), where the experimental unit was the pen for BW, ADG, ADFI and G:F ratio and was the pig for BMC, protein, lipid and blood samples. The effects of dietary treatments were evaluated separately for each feeding phase using models including the dietary treatment as a fixed effect and the block as a random effect. For the metabolic trial, the experimental unit was the pig, and the effect of dietary treatment was evaluated using a model including the dietary treatment as a fixed effect. In phase 1, the treatments were the same in each group, whereas in phases 2 and 3, the differences were considered significant when $P \leq 0.05$ and a value of $P \leq 0.10$ denotes a statistical trend. When differences were significant, means were separated using a modified Tukey's highly significant difference test (package emmeans, R version 4.2.2).

Results

The analysed values of Ca were different than expected in phase 1: −18% in phase 1, −5% in C diet, −13% in the L_{Normal} diet and −11% in the L_{High} diet in phase 2 and −34% in the C diet in phase 3. The analysed values of P were consistent with expectations: −2% in phase 1, +6% in the C diet in phase 2, +4% in the L_{Normal}, +6% in the L_{High} diets and +3% in the C diet in phase 3. During the adaptation phase, three animals died of unknown causes.

Common and depletion phases

During the first phase, all pigs received the same diet, with the aim of maximising BMC. The trial started when the pigs weighed about 30 kg, and the first period ending 39 days later with pigs at 73.1 kg (Table 2). Thanks to this long common period, the initial conditions (BW, body protein, lipid, P and Ca, BMC, blood mineral status) of the pigs were the same at the beginning of the depletion period (Table 3). During the second phase, two groups were given a depletion diet. BW, body protein and lipid, and growth performances (ADG, ADFI, G:F ratio) were the same in the three groups (Table 3). BMC, body P and Ca were also the same, due to similar BMC, P and Ca gains. The plasma Ca level tended to be higher in the C-L_{Normal} group than in the others ($P = 0.08$). The plasma P level was higher in the C-L_{Normal} group than in the C–C group ($P < 0.05$), while the Mg level did not differ. The plasma Ca:P was the same in all groups. Pigs in each group had the same serum CTX, PICP and OC levels. The vitamin D_3 concentration tended to be lower in the C–C and C-L_{Normal} groups than in the C-L_{High} group (16.73, 19.63 and 25.00 ng/mL, respectively; $P = 0.10$). The digestible P efficiency was lower in the C–C group than in the C-L_{Normal} and C-L_{High} groups (52.6 vs 77.1%, $P < 0.01$). The Ca efficiency was lower in the C–C group than in the C-L_{Normal} (35.6 vs 53.9%, $P < 0.05$).

The P intake during the balance trial was higher in the C–C than in the depletion groups ($P < 0.001$; Table 4). However, faecal P excretion was the same in the C–C and C-L_{Normal} groups and lower than in the C-L_{High} group ($P < 0.01$). The amount of P absorbed was limited in the depletion groups compared to the C–C group: 12.72, 5.49 and 5.39 g/d in the C–C, C-L_{Normal} and C-L_{High} groups, respectively ($P < 0.001$). Therefore, P absorption efficiency was the lowest in the C-L_{High} group, intermediate in the C-L_{Normal} group and the highest in the C–C group ($P < 0.001$). Urinary P excretion was the same in all three groups, resulting in a higher amount of P retained in the C–C group than in the depletion groups ($P < 0.001$). In addition, P retention efficiency was lower in C-L_{Normal} pigs than in the others ($P < 0.05$). Ca intake was the highest in the C–C group (17.42 g/d), intermediate in the C-L_{High} group (11.34 g/d) and the lowest in the C-L_{Normal} (7.52; $P < 0.001$). Faecal Ca excretion was lower in the C-L_{Normal} group than in the C–C (7.19 versus 3.92 g/d; $P < 0.05$). Meanwhile, urinary Ca concentration was undetectable in all groups. The amount of Ca absorbed was higher in the C–C group (10.23 g/d) than in the C-L_{High} and C-L_{Normal} groups (4.24 g/d and 3.60 g/d, respectively; $P < 0.01$). Finally, Ca absorption efficiency tended to be higher in the C–C group ($P = 0.06$).

Table 2
Growth performance and mineral status of pigs in feeding phase 1.

Item	C	SEM
Initial conditions[1]		
BW, kg	30.3	0.23
Bone mineral content, g	524	11.9
Protein, g	4 102	128.0
Lipid, g	7 405	191.9
Phosphorus, g	141	3.3
Calcium, g	206	4.7
Final conditions[1]		
BW, kg	73.1	0.47
Bone mineral content, g	1 374	29.9
Protein, g	11 408	227.2
Lipid, g	15 006	422.6
Phosphorus, g	362	7.1
Calcium, g	539	11.7
Performances[1,2]		
ADG, kg/d	1.10	0.009
ADFI, kg/d	2.51	0.023
Gain to feed, kg	0.438	0.0037
BMC gain, g/d	21.8	0.58
P gain, g/d	5.7	0.12
Ca gain, g/d	8.6	0.23
digP efficiency, %	53.2	1.19
Ca efficiency, %	42.9	1.14

[1] The experimental unit for the BW, ADG, ADFI and gain to feed was the pen (n = 24), for the bone mineral content, protein, lipid, phosphorus and calcium content and gain and efficiency it was the animal (one animal per pen, n = 24).
[2] ADG: average daily gain; ADFI: average daily feed intake; BMC: bone mineral content; digP: digestible phosphorus (apparent total tract digestible).

Repletion phase

At the end of the repletion phase, the BW tended to be higher in the C-L_{Normal}-C and C-L_{High}-C groups than in the C–C–C group ($P = 0.06$). The pigs reached the same BMC in the three groups and the growth performances (ADG, ADFI, G:F ratio and BMC gain) were the same (Table 5).

Discussion

Validation of the model

Digestible P and total Ca requirements for C diets were estimated according to the mechanistic model of Lautrou et al. (2020), with the aim of validating the model to maximise bone mineralisation. This model is the first to predict daily P and Ca requirements in growing pigs with requirements for bone mineral deposition independent of protein or soft tissue deposition. Fig. 1 shows the potential BMC gain as a function of BW according to Lautrou et al. (2020), and the average BMC gain per group as a function of the average BW during the three feeding phases. During phase 1, when all pigs received the C diet, the average BMC

Table 3
Growth performance and mineral status of pigs fed with different phosphorus and calcium levels during feeding phase 2.

	Treatment[1]				
Item	C–C	C-L_{Normal}	C-L_{High}	SEM	*P*-values[2]
Initials conditions[3,4]					
BW, kg	72.7	73.1	73.6	1.09	0.66
Bone mineral content, g	1 319	1 394	1 410	56.7	0.44
Protein, g	11 251	11 291	11 683	482.8	0.71
Lipid, g	14 565	14 641	15 812	950.8	0.42
Phosphorus, g	351	365	371	12.9	0.51
Calcium, g	518	547	553	22.2	0.44
Ca plasma, mg/dL	11.61	10.46	10.86	0.562	0.26
P plasma, mg/dL	8.88	8.11	8.20	0.453	0.41
Mg plasma, mg/dL	1.80	1.66	1.77	0.094	0.39
Ca:P	1.32	1.29	1.33	0.049	0.73
CTX, ng/mL	6.56	3.18	3.39	1.673	0.072
PICP, pg/mL	145.88	194.94	208.48	70.102	0.74
OC, ng/mL	6.37	7.83	8.25	3.062	0.70
Vitamin D_3, ng/mL	13.83	12.49	14.48	1.334	0.32
Final conditions[5]					
BW, kg	108.9	110.2	109.6	1.41	0.70
Bone mineral content, g	1 835	1 817	1 850	105.8	0.98
Protein, g	15 400	15 336	15 197	561.3	0.96
Lipid, g	28 442	31 283	31 294	2 513.9	0.31
Phosphorus, g	482	483	488	21.5	0.98
Calcium, g	720	713	726	41.4	0.98
Ca plasma, mg/dL	11.55	12.78	11.47	0.474	0.08
P plasma, mg/dL	8.17[a]	10.20[b]	8.65[ab]	0.627	*
Mg plasma, mg/dL	1.57	1.63	1.58	0.058	0.67
Ca:P	1.41	1.26	1.36	0.069	0.12
CTX, ng/mL	7.11	3.23	3.17	2.822	0.21
PICP, pg/mL	53.50	113.18	187.33	68.762	0.25
OC, ng/mL	3.57	3.66	3.99	0.756	0.88
Vitamin D_3, ng/mL	16.73	19.63	25.00	3.712	0.10
Performances[5,6]					
ADG, kg/d	1.31	1.35	1.32	0.039	0.57
ADFI, kg/d	3.51	3.65	3.64	0.089	0.35
Gain to feed, kg	0.375	0.370	0.363	0.0063	0.32
BMC gain, g/d	18.4	15.9	16.3	2.76	0.69
P gain, g/d	4.7	4.4	4.3	0.56	0.78
Ca gain, g/d	7.2	6.3	6.4	1.08	0.69
digP efficiency, %	52.6[a]	77.9[b]	76.3[b]	5.80	**
Ca efficiency, %	35.6[a]	53.9b	45.5[ab]	6.54	*

[1] C–C = Control diet during phases 1 and 2; C-L_{Normal} = Control diet at 100% of the total Ca and digestible P requirements for maximal bone mineralisation during phase 1 and diet at 60% of the digestible P requirements and 65% of the total Ca requirements during phase 2; C-L_{High} = Control diet at 100% of the total Ca and digestible P requirements for maximal bone mineralisation during phase 1 and diet at 60% of the digestible P requirements and 80% of the total Ca requirements during phase 2.
[2] Data were analysed using ANOVA linear models that included the effect of diet. **: $P < 0.01$; *: $P < 0.05$.
[3] The experimental unit for the BW was the pen (n = 8 per treatment), it was the animal for the bone mineral content, protein, lipid, phosphorus and calcium content (one animal per pen, n = 8 per treatment).
[4] CTX = cross-linked C-telopeptide of type I collagen; PICP = procollagen I C-terminal Propeptide; OC = osteocalcin
[5] The experimental unit for the BW, ADG, ADFI and gain to feed was the pen (n = 8 per treatment), The experimental unit for the bone mineral content, protein, lipid, phosphorus and calcium content and gain and efficiency was the animal (one animal per pen, n = 6 for the C–C group, n = 7 for the C-L_{Normal} and C-L_{High} groups). Some data are missing because of a malfunction of the DXA scan.
[6] ADG: average daily gain; ADFI: average daily feed intake; BMC: bone mineral content; digP: digestible phosphorus (apparent total tract digestible).
[a,b] Values with different superscript letters differ ($P < 0.05$) from each other, as analysed by Tukey's tests.

gain was slightly higher than the potential (20.0 versus 21.8 g/d, Fig. 1). This suggests that the pigs reached their maximum BMC gain potential. However, during the two subsequent feeding phases, the average BMC gain of the pigs fed the C diets was also below the potential. The conversion from digestible Ca to total Ca requirements (to be in line with current formulation principles) is based on a strong global digestibility assumption: 70% (Stein, personal communication, Lautrou et al., 2020), which is quite high compared to the 58% adopted by Bikker and Blok (2017), for example. If the chosen digestibility is too high, the absorbable Ca intake is limited, and Ca becomes therefore limiting for mineral deposition in bones. To convert the daily total Ca requirements provided by the model (Lautrou et al., 2020) into a phase value, we chose to apply the maximum total Ca requirement value of the phase. The requirement decreases quickly during phase 1 (Fig. 2); therefore, Ca was distributed in excess and the pigs could achieve the potential BMC gain (Fig. 1). During the second phase, the requirements were quite stable, meaning that the possible Ca excess with phase feeding is weaker than in phase 1. The total Ca inputs were above the requirements for phases 1 and 2, with the hypothesis of Ca digestibility at 70% (Fig. 2). In the last phase, the inputs were below the requirements because of the 34% missing Ca compared to the formulation value. When the model simulated the total Ca requirements with a Ca digestibility set at 58% (Bikker and Blok, 2017), the inputs were below the requirements during the first part of phase 1 and during all phases 2 and 3 (Fig. 2). Moreover, the Ca balance measurements taken during the second feeding phase showed that urinary Ca was undetectable, even in the C–C group, confirming the possibility that Ca was limiting for the bone mineral deposition.

Table 4
Phosphorus and calcium balance in pigs measured during the feeding phase 2.

Item	Treatments[1]			SEM	*P*-values[2]
	C–C	C-L_{Normal}	C-L_{High}		
Phosphorus[3]					
Intake, g/d	15.74[a]	9.32[b]	11.63[b]	1.084	***
Faeces, g/d	3.02[a]	3.83[a]	6.24[b]	0.708	**
Urine, g/d	0.82	0.90	0.38	0.201	0.21
Absorbed, g/d	12.72[a]	5.49[b]	5.39[b]	1.059	***
Retained, g/d	12.18[a]	4.75[b]	5.20[b]	1.008	***
Absorption, % intake	80.74[a]	58.79[b]	46.88[c]	4.142	***
Retention, % absorbed	95.76	83.60	96.55	5.788	*
Calcium[3]					
Intake, g/d	17.42[a]	7.52[b]	11.34[c]	1.200	***
Faeces, g/d	7.19[a]	3.92[b]	7.10[ab]	1.265	*
Urine, g/d	Undetectable[4]				
Absorbed, g/d	10.23[a]	3.60[b]	4.24[b]	1.522	***
Retained, g/d	10.19[a]	3.53[b]	4.24[b]	1.526	***
Absorption, % intake	58.68	47.44	37.51	7.291	0.06
Retention, % absorbed	100				

[1] C–C = Control diet during phases 1 and 2; C-L_{Normal} = Control diet at 100% of the total Ca and digestible P requirements for maximal bone mineralisation during phase 1 and diet at 60% of the digestible P requirements and 65% of the total Ca requirements during phase 2; C-L_{High} = Control diet at 100% of the total Ca and digestible P requirements for maximal bone mineralisation during phase 1 and diet at 60% of the digestible P requirements and 80% of the total Ca requirements during phase 2.
[2] Data were analysed using ANOVA linear models that included the effect of diet. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$.
[3] The experimental unit for the phosphorus and calcium measurements was the animal (n = 6 per treatment).
[4] Urine calcium concentration was under the detection limit (<0.01%).
[a,b,c] Values with different superscript letters differ ($P < 0.05$) from each other as analysed by the Tukey's tests.

Table 5
Growth performance and mineral status of pigs fed with different phosphorus and calcium levels during feeding phase 3.

Item	Treatment[1]			SEM	*P*-values[2]
	C–C–C	C-L_{Normal}-C	C-L_{High}-C		
Initial conditions[3]					
BW, kg	108.9	110.2	109.6	1.41	0.70
Bone mineral content[4], g	1 956	1 865	1 979	109.4	0.64
Final conditions[5]					
BW, kg	131.6	135.2	134.6	1.45	0.06
Bone mineral content[3], g	2 342	2 558	2 509	96.9	0.20
Performances[5,6,7]					
ADG, kg/d	1.11	1.22	1.20	0.059	0.30
ADFI, kg/d	3.88	4.04	4.06	0.097	0.21
Gain to feed, kg	0.286	0.302	0.296	0.0161	0.62
BMC gain, g/d	18.7	25.4	17.5	3.77	0.19

[1] C–C–C = Control diet during phases 1, 2 and 3; C-L_{Normal}-C = Control diet at 100% of the total Ca and digestible P requirements for maximal bone mineralisation during phases 1 and 3 and diet at 60% of the digestible P requirements and 65% of the total Ca requirements during phase 2; C-L_{High}-C = Control diet at 100% of the total Ca and digestible P requirements for maximal bone mineralisation during phases 1 and 3 and diet at 60% of the digestible P requirements and 80% of the total Ca requirements during phase 2.
[2] Data were analysed using ANOVA linear models that included the effect of diet.
[3] The experimental unit for the BW was the pen (n = 8 per treatment), The experimental unit for the bone mineral content, was the animal (one animal per pen, n = 6 for the C–C–C group, n = 7 for the C-L_{Normal}-C and C-L_{High}-C groups). Some data are missing because of a malfunction of the DXA scan.
[4] The bone mineral content of the whole body was evaluated according to the bone mineral density of the head Létourneau-Montminy et al. (2017).
[5] The experimental unit for the BW, ADG, ADFI and gain the feed was the pen (n = 8 per treatment), The experimental unit for the bone mineral content was the animal (one animal per pen, n = 6 for the C–C–C and C-L_{High}-C groups, n = 7 for the C-L_{Normal}-C group). Some data are missing because we did not receive all the heads from the slaughterhouse.
[6] The experimental unit for the BMC gain was the animal (one animal per pen, n = 5 for the C–C–C and C-L_{High}-C groups, n = 6 for the C-L_{Normal}-C group). Some data are missing because of a malfunction of the DXA scan and the heads that we did not receive from the slaughterhouse.
[7] ADG: average daily gain; ADFI: average daily feed intake; BMC: bone mineral content.

Consequences of depletion–repletion sequences

During phase 1, animals had a greater feed intake than expected based on in-field growth performances. Given that the energy level was as expected, it is possible that the experimental conditions allowed better performance. This increase in ADFI resulted in heavier pigs than expected: about 70 kg at the end of the first phase instead of 55 kg. During the other phases, the ADFI was as expected. The depletion in digestible P and Ca intake had no effect on growth performances, in agreement with Pomar et al. (2006) and Létourneau-Montminy et al. (2014), who did not observe a decrease in pig performance despite P deficiency. Nevertheless, in the current study, we expected that the growth performances might be reduced by the C-L_{High} diet, which was supposed to induce greater P deficiency with low P, combined with a high Ca: P ratio (Reinhart and Mahan, 1986). It is known that a phosphocal-

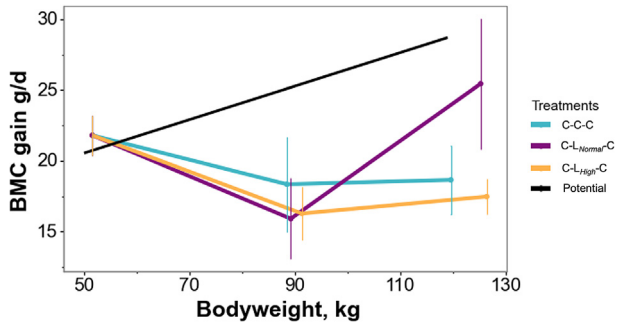

Fig. 1. Realised bone mineral content gain (g/d) per phase in each group of pigs and potential bone mineral content gain (g/d) according to Lautrou et al. (2020). C–C–C = Control diet during phases 1, 2 and 3; C-L$_{Normal}$-C = Control diet at 100% of the total Ca and digestible P requirements for maximal bone mineralisation during phases 1 and 3 and diet at 60% of the digestible P requirements and 65% of the total Ca requirements during phase 2; C-L$_{High}$-C = Control diet at 100% of the total Ca and digestible P requirements for maximal bone mineralisation during phases 1 and 3 and diet at 60% of the digestible P requirements and 80% of the total Ca requirements during phase 2; BMC = Bone mineral content.

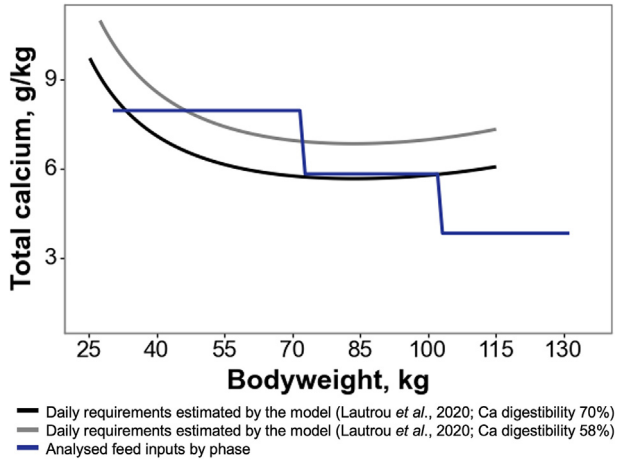

Fig. 2. Estimated total calcium requirements of pigs and realised total calcium inputs per phase (g/kg of feed).

cic imbalance with an excess of Ca in the gut can lead to the formation of insoluble Ca–P complexes (Heaney and Nordin, 2002; Létourneau-Montminy et al., 2012) that exacerbate the P deficiency and could decrease the ADFI and then the growth performances (Suttle, 2010). On the other hand, as explained above, we suspect a lack of absorbable Ca in the diets, which means that the imbalance between P and Ca that we wanted to achieve may not have been present, which is why there was no effect on growth performance.

Depletion had no effect on BMC, which goes against Létourneau-Montminy et al. (2014) and Gonzalo et al. (2018). This suggests that Ca and P levels were not low enough to induce a bone mineralisation deficit, despite a decrease of 42% in digestible P and from 32 to 46% of Ca in the L$_{High}$ and L$_{Normal}$ diets, respectively, in comparison to the C diet. Ca was undetectable in urine, and its absorption efficiency was the same in all groups, confirming that Ca was limiting for bone retention in all diets, even the C–C. This lack of Ca could lead to lower BMC gains in C–C pigs in the second feeding phase compared to the first feeding phase (18.4 versus 21.8 g/d, Fig. 1) whereas the BMC gain usually increases with BW when animals are fed adequate P and Ca (Couture et al., 2018; Lautrou et al., 2020). Therefore, because bone mineralisation was not maximised in the C–C group, the difference with the depleted groups could have been smaller. Meanwhile, Ca deprivation triggers the parathormone pathway, which first stimulates Ca reabsorption in the kidneys and Ca and P release from bones. Then, calcitriol synthesis by the kidneys can enhance Ca and P intestinal absorption and renal reabsorption (Schröder and Breves, 2006). The synthesis of calcitriol (1,25(OH)$_2$-vitamin D$_3$) results from the hydroxylation of calcidiol (25(OH)-vitamin D$_3$, Holick 2006), the circulating form of vitamin D, which tended to be lower in the C–C and C-L$_{Normal}$ groups than in the C-L$_{High}$ group. Moreover, the higher plasma Ca and P concentrations in C-L$_{Normal}$ pigs are likely the result of greater absorption and reabsorption due to parathormone and calcitriol (Schröder and Breves, 2006; Riccardi and Brown, 2010). Finally, C-L$_{Normal}$ and C-L$_{High}$ pigs were depleted enough to exhibit an increased P utilisation, although bone mineralisation was not altered.

The depletion experienced by C-L$_{High}$ pigs was supposed to be more acute because of the higher Ca:P ratio. Consequently, no activation of the parathormone pathways was expected because of a higher Ca status (Sommerville et al., 1985). This is confirmed by the fact that serum 25(OH)-vitamin D$_3$ concentration was not decreased in C-L$_{High}$ pigs, suggesting no calcitriol synthesis. Despite similar levels of digestible P in the C-L$_{High}$ and C-L$_{Normal}$ diets, the high Ca supply in C-L$_{High}$ resulted in lower intestinal absorption (46.9 vs 58.8%), due to their antagonism in the small intestinal lumen. Besides, given the higher Ca level in C-L$_{High}$ compared to C-L$_{Normal}$, the retention of over-absorbed P is much higher in C-L$_{High}$ (97 vs 84%) because of the Ca available to retain the P absorbed in bone. It is interesting to note that PICP, a marker of osteoblast activity and consequently bone formation (Eklou-Kalonji et al., 1999; Delmas et al., 2000) increased numerically in the serum of both C-L$_{Normal}$ and C-L$_{High}$, compared to the C–C group, indicating a higher capacity to deposit mineral into bone. This result, combined with the lower 25(OH)-vitamin D$_3$ level in the C-L$_{Normal}$ group, could mean that bone formation increased at the end of the depletion phase, as if the pigs were finally in a repletion phase. This suggests that C-L$_{High}$ and C-L$_{Normal}$ pigs experienced a period of deprivation, probably at the beginning of the depletion phase. This increased bone formation, allowing the pigs to recover from a bone mineral deficit prior to the scan at the end of the depletion phase.

Repletion had no effect on growth, in accordance with Varley et al. (2011), Létourneau-Montminy et al. (2014) and Gonzalo et al. (2018). As in the second phase, the BMC gain in the C–C–C group was lower than expected: 21.6 versus 28.3 g/d (Fig. 1). At the end of phase 3, the pigs could not be scanned because of their heavy weight. Therefore, BMC was estimated according to Létourneau-Montminy et al. (2017), based on heads. The BMC at the beginning of phase 3 was also recalculated to limit the error in the BMC gain, but this method is certainly less precise than measuring whole-body BMC directly. The non-significant difference, despite the important gap in BMC gain between C-L$_{Normal}$-C and C–C–C pigs, could be related to the reduced number of heads received: 5–6 per group, compared with eight animals scanned at other times. Létourneau-Montminy et al. (2014) and Gonzalo et al. (2018) observed increased P and Ca utilisation efficiency until 56 days of repletion. Therefore, the numerically superior BMC gain in the C-L$_{Normal}$-C group (+6.8 g/d compared to the C–C–C group) confirms a depletion that was short, but long enough to induce a reaction to the deficiency and improved P and Ca utilisation, even more than 28 days postdepletion. The higher BMC gain resulted in a numerically higher BMC in the C-L$_{Normal}$-C group at the end of the trial. A longer third feeding phase might have allowed the detection of not only a numerical but also a statistical difference in bone mineralisation.

Conclusion

The requirements estimated using the model of Lautrou et al. (2020) did not allow the BMC gain potential to be reached. Bone mineralisation was therefore not maximised, probably due to an overestimation of Ca digestibility. It is possible to achieve the same level of bone mineralisation and comparable growth performance using different strategies. The depletion period in this trial was shorter than expected but sufficient to increase the absorption and metabolic use of P and Ca. A decrease of approximately 9.5% in total dietary P was observed while maintaining the same level of bone mineralisation. Conversely, considering P utilisation efficiencies, excretion would have been reduced by 24% during the depletion phase in the depletion–repletion groups (C-L_{Normal}-C and C-L_{High}-C). Finally, the success of the depletion–repletion strategy mainly depends on the Ca and P requirement estimation of pigs, on the precise measure of the nutrients in feed and on the method of estimating digestible part of the total P and Ca.

Ethics approval

Animals were housed and treated in accordance with the guidelines established by the Canadian Council on Animal Care (CCAC, 2009). The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at the Laval University Québec, QC, Canada (2019069-1).

Data and model availability statement

None of the data were deposited in an official repository. The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable 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

M. Lautrou: https://orcid.org/0000-0002-6648-8497.
C. Pomar: https://orcid.org/0000-0002-1686-0895.
P. Schmidely: https://orcid.org/0000-0002-7566-7868.
M.P. Létourneau-Montminy: https://orcid.org/0000-0001-5364- 2662.

CRediT authorship contribution statement

M. Lautrou: Writing – original draft, Methodology, Formal analysis, Data curation. **C. Pomar:** Writing – review & editing, Conceptualization. **P. Schmidely:** Writing – review & editing, Supervision, Conceptualization. **M.P. Létourneau-Montminy:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of interest

None.

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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. https://doi.org/10.1016/j.medengphy.2010.08.001.
AOAC, 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, VA, USA.
AOAC International, 2006. Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, VA, USA.
Bikker, P., Blok, M.C., 2017. Phosphorus and calcium requirements of growing pigs and sows, CVB documentation report nr. 59. Wageningen Livestock Research, Wageningen, NL. https://doi.org/10.18174/424780.
Breves, G., Schröder, B., 1991. Comparative aspects of gastrointestinal phosphorus metabolism. Nutrition Research Reviews 4, 125–140. https://doi.org/10.1079/nrr19910011.
CCAC, 2009. Guidelines on: the care and use of farm animals in research, teaching and testing. Canadian Council on Animal Care, Ottawa, ON, Canada.
Cordell, D., White, S., 2013. Sustainable Phosphorus measures: strategies and technologies for achieving phosphorus security. Agronomy 3, 86–116. https://doi.org/10.3390/agronomy3010086.
Couture, C., Chiasson, R., Pomar, C., Letourneau, M.-P., 2018. Évolution de la teneur en protéine corporelle et du contenu minéral osseux des porcs charcutiers nourris avec différents niveaux de phosphore et calcium alimentaires. Journées Recherche Porcine 50, 167–168.
Crenshaw, T.D., 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In: Lewis, A.J., Southern, L.L. (Eds.), Swine Nutrition. second edition. CRC Press, Boca Raton, FL, USA, pp. 187–212.
Delmas, P.D., Eastell, R., Garnero, P., Seibel, M.J., Stepan, J., 2000. The use of biochemical markers of bone turnover in osteoporosis. Osteoporosis International 11, S2–S17. https://doi.org/10.1007/s001980070002.
Eklou-Kalonji, E., Zerath, E., Colin, C., Lacroix, C., Holy, X., Denis, I., Pointillart, A., 1999. Calcium-regulating hormones, bone mineral content, breaking load and trabecular remodeling are altered in growing pigs fed calcium-deficient diets. The Journal of Nutrition 129, 188–193. https://doi.org/10.1093/jn/129.1.188.
Gonzalo, E., 2017. Consequences of a dietary phosphorus and calcium depletion and repletion strategy in growing-finishing pigs. Laval University, Québec, QC, Canada (PhD thesis).
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. https://doi.org/10.1017/S1751731117002567.
Heaney, R.P., Nordin, B.E.C., 2002. Calcium effects on phosphorus absorption: Implications for the prevention and co-therapy of osteoporosis. Journal of the American College of Nutrition 21, 239–244.
Holick, M.F., 2006. Resurrection of vitamin D deficiency and rickets. Journal of Clinical Investigation 116, 2062–2072. https://doi.org/10.1172/JCI29449.
Investing News Network, 2022. Phosphate Outlook 2022: Geopolitics to be a Key Market Mover. Retrieved on 29 November 2023 from: https://investingnews.com/phosphate-outlook-2022/.
Jondreville, C., Dourmad, J.Y., 2006. Phosphorus in pig nutrition. Productions Animales 18, 183–192.
Lautrou, M., Pomar, C., Dourmad, J.-Y., Narcy, A., Schmidely, P., Létourneau-Montminy, M.-P., 2020. Phosphorus and calcium requirements for bone mineralisation of growing pigs predicted by mechanistic modelling. Animal 14, 313–322. https://doi.org/10.1017/S1751731120001627.
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 en post-sevrage dans le contexte canadien de production. Journées Recherche Porcine 49, 261–262.
Létourneau-Montminy, M.P., Jondreville, C., Sauvant, D., Narcy, A., 2012. Meta-analysis of phosphorus utilization by growing pigs: effect of dietary phosphorus, calcium and exogenous phytase. Animal 6, 1590–1600. https://doi.org/10.1017/S1751731112000560.
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. https://doi.org/10.2527/jas.2013-7320.
Mitchell, A.D., Scholz, A., Pursel, V., 2002. Prediction of the in vivo body composition of pigs based on cross- sectional region analysis of dual energy X-ray absorptiometry (DXA) scans. Archives Animal Breeding 45, 535–545.
National Research Council (NRC), 2012. Nutrient requirements of swine. National Academies Press, Washington, DC, USA.
Pomar, C., Rivest, J., 1996. The effect of body position and data analysis on the estimation of body composition of pigs by dual energy x-ray absorptiometry (DEXA). Proceedings of the 46th Annual Conference of the Canadian Society of Animal Science, 7–11 July 1996, Lethbridge, AB, Canada, p. 26.

Pomar, C., Jondreville, C., Dourmad, J., Bernier, J., 2006. Influence du niveau de phosphore des aliments sur les performances zootechniques et la rétention corporelle de calcium, phosphore, potassium, sodium, magnésium, fer et zinc chez le porc de 20 à 100 kg de poids vif. Journées Recherche Porcine 38, 209–216.

Quiniou, N., Noblet, J., 1995. Prediction of tissular body composition from protein and lipid deposition in growing pigs. Journal of Animal Science 73, 1567–1575. https://doi.org/10.2527/1995.7361567x.

Reinhart, G.A., Mahan, D.C., 1986. Effect of various calcium:phosphorus ratios at low and high dietary phosphorus for starter, grower and finishing swine. Journal of Animal Science 63, 457–466. https://doi.org/10.2527/jas1986.632457x.

Riccardi, D., Brown, E.M., 2010. Physiology and pathophysiology of the calcium-sensing receptor in the kidney. American Journal of Physiology-Renal Physiology 298, F485–F499. https://doi.org/10.1152/ajprenal.00608.2009.

Ryan, W.F., Lynch, P.B., O'Doherty, J.V., 2011. 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. https://doi.org/10.1016/j.livsci.2010.12.006.

Schröder, B., Breves, G., 2006. Mechanisms and regulation of calcium absorption from the gastrointestinal tract in pigs and ruminants: comparative aspects with special emphasis on hypocalcemia in dairy cows. Animal Health Research Reviews 7, 31–41. https://doi.org/10.1017/S1466252307001144.

Selle, P.H., Ravindran, V., 2008. Phytate-degrading enzymes in pig nutrition. Livestock Science 113, 99–122. https://doi.org/10.1016/j.livsci.2007.05.014.

Sommerville, B., Maunder, E., Ross, R., Care, A., Brown, R., 1985. Effect of dietary calcium and phosphorus depletion on vitamin d metabolism and calcium binding protein in the growing pig. Hormone and Metabolic Research 17, 78–81. https://doi.org/10.1055/s-2007-1013456.

Suttle, N.F., 2010. Mineral nutrition of livestock, 4th ed. CABI, Wallingford, Oxfordshire, UK. https://doi.org/10.1079/9781845934729.0000.

Van Enk, R.J., Acera, L.K., Schuiling, R.D., Ehlert, P., De Wilt, J.G., De Vries, C.K., Van Haren, R.J.F., 2011. The phosphate balance: current developments and future outlook. Innovation Network Reports, Utrecht, the Netherlands. ISBN 978-90-5059-414-1.

van Milgen, J., Valancogne, A., Dubois, S., Dourmad, J.-Y., Sève, B., Noblet, J., 2008. InraPorc: a model and decision support tool for the nutrition of growing pigs. Animal Feed Science and Technology 143, 387–405. https://doi.org/10.1016/j.anifeedsci.2007.05.020.

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. https://doi.org/10.1016/j.livsci.2010.07.025.