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Dynamics of bone mineralization in primiparous sows as a function of dietary phosphorus and calcium during lactation

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ABSTRACT

To maximize the efficiency of dietary P utilization in swine production, understanding the mechanisms of P utilization in lactating sows is relevant due to their high P requirement and the resulting high inorganic P intake. Gaining a better knowledge of the Ca and P quantities that can be mobilized from bones during lactation, and subsequently replenished during the following gestation, would enable the development of more accurate P requirements incorporating this process of bone dynamics. The objective was to measure the amount of body mineral reserves mobilized during lactation, depending on dietary digestible P and phytase addition and to measure the amount recovered during the following gestation. Body composition of 24 primiparous sows was measured by dual-energy x-ray absorptiometry 2, 14, 26, 70 and 110 days after farrowing. Four lactation diets were formulated to cover nutritional requirements, with the exception of Ca and digestible P: 100% (**Lact100**; 9.9 g Ca and 3.0 g digestible P/kg), 75% (**Lact75**), 50% without added phytase (**Lact50**) and 50% with added phytase (**Lact50 + FTU**). The gestation diet was formulated to cover the nutritional requirements of Ca and digestible P (8.2 g Ca and 2.6 g digestible P/kg). During the 26 days of lactation, each sow mobilized body mineral reserves. The mean amount of mobilized bone mineral content (**BMC**) was 664 g, representing 240 g Ca and 113 g P. At weaning, the BMC (g/kg of BW) of Lact50 sows tended to be lower than Lact100 sows (−12.8%, linear Ca and P effect × quadratic time effect) while the BMC of Lact50 + FTU sows remained similar to that of Lact100 sows. During the following gestation, BMC returned to similar values among treatments. Therefore, the sows fed Lact50 could recover from the higher bone mineral mobilization that occurred during lactation. The P excretion was reduced by 40 and 43% in sows fed Lact50 and Lact50 + FTU, respectively, relative to sows fed Lact100. In conclusion, the quantified changes in body composition during the lactation and following gestation of primiparous sows show that bone mineral reserves were mobilized and recovered and that its degree was dependent on the dietary P content and from phytase supplementation during lactation. In the future, considering this potential of the sows' bone mineralization dynamics within the factorial assessment of P requirement and considering the digestible P equivalency of microbial phytase could greatly limit the dietary use of inorganic phosphates and, thus, reduce P excretion.

Implications

The risk of pollution exists when phosphorus supply over slurry exceeds crop requirement. Aligning phosphorus intake with requirement mitigates excretion. During lactation, 14 g calcium and 6 g phosphorus are mobilized daily from sow body reserves fed at 50% of the requirement, meeting 36, 22 and 64% of calcium, phosphorus and digestible phosphorus. Even with higher inorganic phosphate levels or with phytase, a limited mobilization persists. During subsequent gestation, bone mineralization is recovered. The potential exists to decrease dietary digestible phosphorus by considering body mineral mobilization and thus to limit the use of inorganic phosphates leading to lower phosphorus excretion.

Introduction

The minerals Ca and P are essential nutrients that play a major role in bone constitution (Crenshaw, 2001). Therefore, dietary Ca

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and P should be sufficient to meet animals' requirements. However, a dietary excess of these nutrients increases P in manure, potentially causing pollution if fertilization exceeds crop requirements (Agroscope, 2017; Dourmad et al., 2020). Sow diets are optimized to achieve target levels of digestible P content, mainly by adapting the inclusion of non-renewable sources of inorganic phosphates. In the context of nutrient efficiency, agronomic nutrient cycling and limited inorganic P resources, research in animal nutrition is focused toward maximizing the efficiency of P use while minimizing the reliance on inorganic phosphates. During their reproductive cycle, sows undergo a short lactation characterized by a high mineral requirement associated with milk production, followed by a longer gestation with a low mineral requirement. In periods of deficient Ca and/or P intake relative to the animals' requirements, it is hypothesized that the highly efficient phosphocalcic homeostatic regulation uses Ca and P stored in bones as mobile reserves for compensation. Indeed, a decrease in plasma Ca concentration to below-normal levels induces parathyroid hormone secretion, which increases bone resorption (González-Vega and Stein, 2014). The activity of bone resorption or accretion can be characterized in blood by measuring the presence of bone biomarkers (Allen, 2003; Sørensen et al., 2018). Based on bone resorption marker, Grez-Capdeville and Crenshaw (2021b) suggested that bone demineralization occurs during sow's lactation and Floradin et al. (2022) confirmed that the concentration of bone resorption markers in the blood increased when gilts decreased their bone mineral content. Recovery of bone mineral content, following a demineralization induced by sub-optimal dietary Ca and P intake, was successful in gilts (Floradin et al., 2022) and in finishing pigs (Létourneau-Montminy et al., 2014), compared to animals that had not experienced a period of deficiency. Finally, it can be concluded that the pig is able to regulate the phosphocalcic homeostasis by using stores of Ca and P in bones.

It is well known that the content of fatty acids and proteins in milk is supported by the mobilization of body lipids and proteins (Tokach et al., 2019). However, the mobilization of bone reserves is not yet considered in the factorial determination of sows' dietary Ca and P requirements throughout their cycle (NRC, 2012; Bikker and Blok, 2017; Quiniou et al., 2019). This is mainly due to a lack of confirmation that this process occurs in sows and, if so, the unknown Ca and P quantities that bone mobilization can provide. The future consideration of Ca and P mobilization would allow the development of new feeding strategies that could substantially decrease, during lactation when the requirement is high, the dietary P intake thereby reducing inorganic phosphates. The recovery of bone reserves, which were mobilized during lactation, can be compensated during the first stage of the following gestation characterized by low Ca and P requirements, which are to a great extent covered by the natural Ca and P contents of feed ingredients. Another strategy to reduce the use of inorganic phosphates is increasing dietary P digestibility using the enzyme phytase (Simões Nunes, 1993; Simões Nunes and Guggenbuhl, 1999; Jongbloed et al., 2000). This approach has been successful for growing pigs to improve bone mineralization, but it is not well documented for sows.

The study's objective was to quantify the kinetics of bone mineralization during lactation and subsequent gestation as a function of dietary digestible P content in lactation, modified by the supply of inorganic phosphates and exogenous phytase. The hypotheses were that during lactation, the sow's bone mineral reserves are mobilized and that this amount is dependent on digestible dietary P content. Then, the sow's bone mineral reserves are recovered during the following gestation.

Material and methods

Animals, housing, and diets

A total of 24 primiparous Large White Swiss sows were selected from the Agroscope herd. During the lactation (the 110th day of gestation to the 26th day of lactation), the sows were housed in individual 7.1 m^2 pens (5.9 m^2 solid concrete floor and 1.2 m^2 slatted concrete floor). The sows could move freely at any time in their pen, and their piglets had separate access to a covered and heated area (0.8 m^2). The pen was equipped with an automatic feeder (Schauer Spotmix, Agrotonic GmbH, Prambachkirchen, Austria) allowing the provision of predefined amounts of feed. On the second day after farrowing, the litters were equalized to 13 piglets. Following weaning, the sows were housed for 10 days, in a breeding pen (12.7 m^2 indoor slatted concrete floor and 56 m^2 outdoor concrete floor) grouping 10–14 animals from the same farrowing series. The sows were blocked in individual 2.9 m^2 breeding pens for 3 days during feeding (less than 1 h per day), followed by seven complete days. Following breeding, the sows from the same farrowing series were housed in group pens (28.5 m^2 concrete floor, 6.5 m^2 slatted floor and 16.0 m^2 straw-bedded floor). The pen was equipped with an automatic feeder (Schauer Compident VI, Agrotonic GmbH, Prambachkirchen, Austria) identifying the sow and weighing the feed in the trough prior to and following a visit.

The sows were allocated to one of four dietary treatments on the 110th day of gestation, according to their expected farrowing date, BW (226 ± 11.9 kg) and age at mating (268 ± 26.6 days). From the day of farrowing until the 26th day of lactation (weaning), the sows had *ad libitum* access to one of the experimental lactation diets (Table 1). The experimental diets were formulated to cover nutritional requirements (Agroscope, 2004) with the exception of Ca and digestible P. These diets comprised: a control diet without exogenous phytase (**Lact100**; 9.0 g Ca, 3.0 g digestible P/kg and 3:1 Ca to digestible P ratio), according to Bikker and Blok (2017); a 25% deficient diet without exogenous phytase (**Lact75**; 6.7 g Ca, 2.3 g digestible P/kg and 3:1 Ca to digestible P ratio); a 50% deficient diet without exogenous phytase (**Lact50**; 4.5 g Ca, 1.5 g digestible P/kg and 3:1 Ca to digestible P ratio); and 50% deficient diet with exogenous phytase (**Lact50 + FTU**). The Lact50 + FTU diet was the same as the Lact50 diet, but supplemented with 500 FTU/kg of phytase (Quantum® Blue 5G, AB Vista, Marlborough, United Kingdom, analyzed at 8 100 FTU/g). The indigestible marker celite was included at 20 g/kg in each experimental diet to allow for an assessment of apparent nutrient digestibility.

From the day of weaning until the end of the experiment (110 days after farrowing, corresponding to the 80th day of gestation), all sows were fed the same diet restrictively, according to their BW, backfat thickness (measured by ultrasound at 6.5 cm on either side of the midline, at the level of the last rib) and body condition score at weaning. The gestation diet was formulated to cover nutritional requirements (Agroscope, 2004; Bikker and Blok, 2017, for Ca and digestible P) and did not contain exogenous phytase. The sows had free access to water and straw throughout the experiment.

The Allix3 software (A-Systems S.A., Versailles, France) was used to formulate the lactation diets, based on each feedstuff's analyzed CP, crude fiber, crude fat, ash and Ca and P concentrations. Each feedstuff's other mineral concentrations, digestible energy contents, digestible amino acid profiles and digestible P coefficients were formulated according to the values of the freely available Swiss national reference table (Agroscope, 2019). The lactation and gestation diets were produced at an on-site experimental feed mill and formulated as pellets (conditioning temperature max. 60 °C, 4 mm pellet diameter).

Table 1
Ingredients and chemical composition of lactation and gestation diets for sows.

Phase	Lactation			Gestation
Treatment	Lact100	Lact75	Lact50[1]	
Item (g/kg as fed)				
Barley	450	450	450	312
Oats				100
Wheat				100
Maize	220	220	220	
Wheat starch	5.00	19.5	34.1	
Fat (tallow and lard)	33.5	27.8	22.0	15.8
Potato protein	45.0	45.0	45.0	
Soybean meal, extruded	109	108	107	58.1
Rapeseed meal, expelled	50.0	50.0	50.0	50.0
Linseed meal, expelled				20.0
Wheat bran				100
Sugar beet pulp, dehydrated	30.0	30.0	30.0	140
Apple pomace, dehydrated				75
L-Lysine-HCl	2.72	2.74	2.76	1.17
L-Threonine	0.58	0.59	0.60	0.71
L-Tryptophane	0.23	0.24	0.24	
Monocalcium phosphate	10.95	6.83	2.71	5.91
Calcium carbonate	13.18	9.25	5.32	11.24
Sodium chloride	3.80	3.80	3.80	3.61
Binder[2]	2.00	2.00	2.00	2.00
Indigestible marker, celite	20.0	20.0	20.0	
Vitamin and mineral premix[3]	4.00	4.00	4.00	4.00
Analyzed nutrient composition per kg, as fed[4]				
DM (g)	898	899	900	896
Digestible energy (MJ)[5]	14.1	14.1	14.1	12.1
CP (g)	174	175	177	142
Fat (g)	59.2	54.1	51.3	48.4
Crude fiber (g)	41.3	39.6	40.0	84.2
Ash (g)	67.7	64.40	57.3	55.4
Calcium (g)	9.9	8.3	6.0	8.2
Phosphorus (g)	5.9	5.3	4.3	5.7
Phytic P (g)	2.6	2.6	2.2	3.0
Digestible P (g)[5]	3.0	2.3	1.5	2.6
Ca: Digestible P	3.3	3.6	4.0	3.2
Phytase activity (FTU)	218	208	198	378

Abbreviations: Lact100 (control diet); Lact75 (75% of the digestible P and total Ca level of Lact100); Lact50 (50% of the digestible P and total Ca level of Lact100); FTU: phytase activity unit defined as the amount of phytase that liberates 1 mmol of inorganic phosphate per minute from 0.0051 mol/l sodium phytate at pH 5.5 and 37 °C.
[1] Lact50 + FTU (Lact50 with 500 FTU of phytase, Quantum Blue 5G, AB Vista, Marlborough, United Kingdom); analyzed 6.0 g Ca, 4.2 g P, 2.4 g phytic P, 849 FTU/kg.
[2] Pellan (Mikro-Technik, Bürgstadt, Germany).
[3] Supplied per kg of diet: 7 mg Cu; 40 mg Fe; 20 mg Mn; 55 mg Zn; 0.55 mg I; 0.20 mg Se; 8 000 IU vitamin A; 800 IU vitamin D3; 300 mg choline; 2 mg vitamin B1; 5 mg vitamin B2; 4 mg vitamin B; 20 mg niacin; 0.02 mg vitamin B12; 21 mg pantothenic acid; 0.10 mg biotin; 1.5 mg folic acid; 40 mg vitamin E; and 2.0 mg vitamin K3.
[4] Values analyzed in duplicate.
[5] Values calculated according to Agroscope (2019).

Sample collection and measurements

A diet sample was collected during each week of lactation. The samples were pooled per farrowing series, totaling 19 samples (Lact100: four samples; Lact75: five samples; Lact50: four samples; Lact50 + FTU: six samples). A diet sample was collected at each of the 26 gestation diet productions spread over the experimental period. Feed intake was recorded at each visit to the feeder during gestation (except the first 7 days with manual weighing). During lactation, feed refusals were collected and weighed each morning before the day's first feeding.

The BW, bone mineral content (**BMC**), fat and lean tissue mass were measured on day (**d**) 2, d14, and d26 (weaning), d70 (± 2; 40 days' gestation) and d110 (±2; 80 days' gestation) after farrowing via dual-energy x-ray absorptiometry (**DXA**, i-DXA, GE Medical Systems, Glattbrugg, Switzerland). The device's calibration was checked and passed the requirement before each session by scanning a calibration phantom, according to the manufacturer's instructions. To avoid any movement during image acquisition, the animals were subcutaneously injected with 1.5 mg of azaperone per kg BW (Stresnil, Streuli Pharma, Switzerland) and then sedated short-term via mask inhalation of isoflurane (max. 5% in oxygen, Isoflo, Abbott Laboratories, North Chicago, USA). The animals were placed on the absorptiometer in a prone position, with the front and back legs extended (Supplementary Material S1). The "Total Body – thick" acquisition mode was used in the Encore software (Version 18). Images were processed to remove artifacts (the mask and sedation device's tube) and regions of interest were delineated, as Kasper et al. (2021) described. Sows were not specifically fasted, and the mean duration between DXA scan and the last meal was 05h39 ± 02h16.

Blood was collected in serum tubes from the ear vein on d2, d14, d26, d70 and d110 after farrowing during sedation after the DXA scan (9 mL, Greiner Bio-One Vacuette GmbH, St. Gallen, Switzerland). The blood samples were centrifuged (3 000 g, 15 min) within 1 h after collection, and the serum was decanted and stored at −20 °C for mineral analysis and −80 °C for biomarker analysis. During the 3rd week of lactation, the sows were administered a subcutaneous injection of oxytocin (2 mL, Intertocine-S, MSD Animal Health GmbH, Luzern, Switzerland) to facilitate milk sample collection. Milk was collected from several nipples and stored at −20 °C. A spot urine sample was collected within 3 days of d2, d26, d70 and d110, and it was stored at −20 °C within 1 h after collection.

A fecal sample was collected from the rectum on three consecutive days during the 3rd week of lactation, and the sample pool was stored at −20 °C. The piglets were weighed on d1, d14, and d26.

Chemical analysis

Chemical analysis of the feed and lyophilized (during 72 h) fecal samples involved the following processes: after being ground to pass a 1-mm screen (Brabender rotary mill; Brabender GmbH & Co. KG, Duisburg, Germany), samples were analyzed for DM content by heating at 105 °C for 3 h followed by incineration at 550 °C until a stable mass was reached to determine the ash content according to ISO 5984_2002 (prepASH, Precisa Gravimetrics AG, Dietikon, Switzerland). The HCl insoluble ash content was determined gravimetrically after a 15-min treatment of the previously obtained ashes in 3 M of boiling HCl (ISO 5985). The CP content (total N × 6.25) was analyzed by the Dumas method (ISO 16634-1:2008) using a LECO TruMac (Leco, Mönchengladbach, Germany). The Ca and P content was analyzed, according to the European Standard EN 155510:2008, using an inductively coupled plasma optical emission spectrometer (ICP-OES 5800, Agilent Technologies, Switzerland) after microwave (Multiwave, Anton Paar) digestion with a solution of nitric acid. Dietary phytic P content was determined using the enzymatic kit (K-PHYT, Megazyme International, Bray, Ireland). In short, the released inorganic phosphate was quantified calorimetrically in reaction to added ammonium molybdate under acid condition, after a two-step enzymatic dephosphorylation (enzyme phytase followed by enzyme alkaline phosphatase). The initially present inorganic phosphate was quantified by the same method without enzyme addition. Phytic P content was determined via the subtraction of initially present inorganic phosphate from total released inorganic phosphate. Dietary phytase activity was measured according to ISO 30024 by colorimetry at 415 nm (UV/VIS Plate Reader Dynex) after the addition of sodium phytate as substrate and the formation of a vanadomolybdate complex with the released inorganic phosphate. The Ca and P content in milk was analyzed using a microwave plasma atomic emission spectrometry (Agilent 4200 MP-AES) after digestion with 65% nitric acid in a glycol solution. Blood serum Ca and P and urinary creatinine, Ca and P content in urine were analyzed using commercially available kits (for Ca: Arzenago III 115016 Greiner; for P: UV Method 179 016 Greiner; for creatinine: Biotechnica 164L Greiner), according to the manufacturer's instructions and a BT1500 autoanalyzer (Biotechnica Instruments S.p.A., Rome, Italy). Blood serum C-terminal propeptide of type I collagen (**CICP**; a marker of bone formation) and C-terminal telopeptide of type I collagen (**CTX**; a marker of bone resorption) were analyzed with an ELISA kit (MicroVue CICP EIA, Quidel, San Diego, USA, detection: 0.2 ng/ml, inter-assay variation: 5.0 – 7.2%; intra-essay variation: 5.5 – 6.8%) and an ELISA kit (Novus Biologicals, Centennial, USA, detection: 0.02 ng/ml, inter-assay variation: 2.5 – 10.9%; intra-essay variation: 1.8 – 3.0%) in all samples. Vitamin $1,25(OH)_2D_3$ was determined in serum samples from d2 and d26 after farrowing using an ELISA kit (Kit 2112, Immundiagnostik, Bensheim, Germany). All analyses except for $1,25(OH)_2D_3$ determination were performed at Agroscope laboratories accredited for feed chemistry and components groups, and all analyses were performed in duplicate. The determination of $1,25(OH)_2D_3$ was conducted by Herbonis Animal Health GmbH (Augst, Switzerland).

Calculations and data analysis

The ingested Ca and P during lactation were calculated as the amount of feed ingested multiplied by the analyzed dietary Ca and P contents. Body Ca and P content were calculated according to the method by Kasper et al. (2021), using the following equations: Ca (g) = 2.807 + 0.407 BMC (g); P (g) = 9.819 + 0.145 BMC (g) + 0.003 lean tissue mass (g). Body retention of Ca and P during lactation was calculated as the difference between their respective body content on d2 and d26 of lactation. The total amount of Ca and P excreted in feces and urine during lactation was determined via two approaches: total excretion (g) = total intake (g) − body retention (g) − milk secretion (g) or total excretion (g) = total intake (g) − body retention (g) − litter-body retention (g). The P and Ca secreted in milk (milk secretion) were determined by multiplying a sow's milk production following a Wood curve (Wood, 1967) according to Hansen et al. (2012), by the analyzed milk Ca and P concentration. Litter-body retention was determined by subtracting each piglet's body mineral content at birth from its corresponding content at weaning. Body mineral content was determined from the allometric equations of Ruiz-Ascacibar et al. (2019) considering each piglet's BW. The average daily gain per piglet was calculated by subtracting the birth BW from the BW at weaning, divided by lactation duration. The apparent total tract digestibility (**ATTD**) of Ca and P were calculated according to the indirect digestibility method using celite, as follows:

$$\text{ATTD}\,(\%) = 100 - (\text{Nutrient}_{\text{feaces}} \times \text{Celite}_{\text{diet}})/(\text{Celite}_{\text{faeces}} \times \text{Nutrient}_{\text{diet}})$$

where $Nutrient_{feaces}$ and $Nutrient_{diet}$ are the nutrient concentrations (g/kg DM) in feces and diets, respectively, and $Celite_{faeces}$ and $Celite_{diet}$ are the celite concentrations (g /kg DM) in feces and diets, respectively.

The experimental unit was the sow. All data were analyzed by contrast using the "PROC MIXED" SAS procedure (SAS Inst. Inc., Cary, NC, US; Supplementary Material S2). The phytase impact was studied using the linear effect between Lact100 and Lact50 + FTU. The Lact100, Lact75, and Lact50 were studied using a linear effect (Lact100 and Lact50; linear Ca and P effect) and a quadratic effect (Lact100, Lact75, and Lact50; quadratic Ca and P effect). When the data analysis was repeated, a linear time contrast (linear time effect) and a quadratic time contrast (quadratic time effect) were studied. The treatment was studied as a fixed effect. Differences were considered significant at $P < 0.05$, and a trend was noted when $P = 0.05–0.10$.

Results

Globally, the study could be conducted as planned: the Ca and P contents in lactation diets (Table 1) were considered as satisfactory (difference between analyzed and formulated, per kg: +0.9, +1.5, +1.5, +1.5 g Ca and 0.0, +0.2, +0.1, 0.0 g P in Lact100, Lact75, Lact50 and Lact50 + FTU, respectively); the phytase activity was similar between Lact100, Lact75, Lact50 diets and was increased by 641 FTU/kg in Lact50 + FTU; no health problems were observed during the experiment; all except two sows successfully weaned 13 piglets (one Lact100 sow weaned 12 piglets and one Lact75 sow weaned 11 piglets) and all sows were pregnant during the following gestation. However, due to a momentary technical failure of the DXA device, four scans (one Lact50 sow and one Lact75 sow on d2 and one Lact50 sow and one Lact50 + FTU sow on d14) were missed. Thus, a total of 116 out of the 120 planned scans were used for the study.

Animal performance

The sows' feed intake was similar among treatments during lactation (6.2 ± 0.59 kg/d) and during the following gestation (3.0 ± 0.79 kg/d). The sows' BW decreased during lactation and increased

during the following gestation (quadratic time effect, $P < 0.05$; Supplementary Table S1) independent of dietary treatment during lactation. The piglet BW on d1 (1.44 ± 0.16 kg), d14 (4.80 ± 0.72 kg) and d26 (6.97 ± 0.84 kg), as well as the litter average daily gain during lactation (2.69 ± 0.42 kg/d; Supplementary Table S1), were similar between treatments.

Dynamics of body composition

The BMC (g, Fig. 1) decreased during lactation and increased linearly during the next gestation, regardless of dietary treatment (linear and quadratic time effect, $P < 0.05$). The BMC (g/kg BW, Fig. 2a) was similar between the beginning of lactation and day 80 of gestation (linear time effect, $P < 0.05$). The BMC (g and g/kg BW) of the Lact50 sows tended to decrease more strongly over lactation (linear Ca and P effect × quadratic time effect, $P = 0.07$ and $P = 0.05$) than sows from other treatments, and they had a lower BMC (g/kg BW) compared to Lact100 on d14 and d26 (linear Ca and P effect, $P < 0.05$). The BMC (g/kg BW) of the Lact50 + FTU sows never differed from that of the Lact100 sows. Over the lactation, BMC (g) decreased by 10% in the Lact100 sows and by 22% in the Lact50 sows. During gestation, the Lact50 sows were able to recover the bone mineralization up to the level of the Lact100 sows. The effects recorded for body Ca (g, Supplementary Table S1, and g/kg BW, data not shown) and for body P (g, Supplementary Table S1 and g/kg BW, Fig. 2b), were identical to the results obtained for BMC, except in Lact50 where body P decreased during lactation to a higher extent than in Lact100 (linear Ca and P effect × quadratic time effect, $P < 0.05$).

Calcium and phosphorus balance

Feed intake and milk production were similar between treatments (Table 2). The Lact100 sows had a higher Ca and P intake during lactation than the Lact50 sows (linear Ca and P effect, $P < 0.05$) and the Lact50 + FTU sows (phytase effect, $P < 0.05$). Those intakes in Lact75 sows were closer to the ones of Lact100 sows than to Lact50 sows (quadratic Ca and P effect for Ca, $P < 0.05$ and P, $P = 0.06$). Body Ca and P retention was always negative during lactation. Compared to Lact100 sows, the values were similar in Lact50 + FTU but lower in Lact50 (linear Ca and P effect, $P < 0.05$).

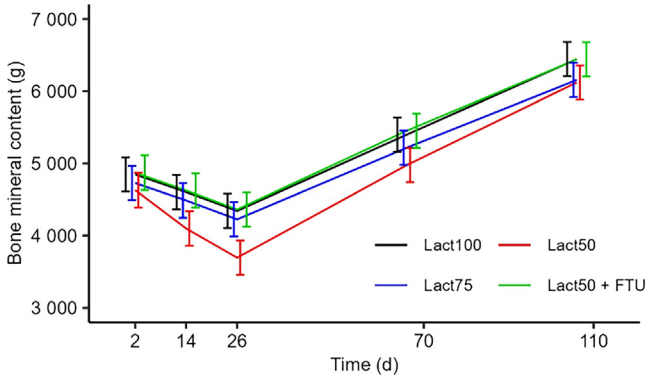

Fig. 1. Dynamics of bone mineral content of primiparous sows during lactation and subsequent gestation, supplied with lactation diets differing in Ca and P content. Abbreviations: Lact100 (control diet; 3.0 g digestible P and 9.9 g Ca /kg); Lact75 (75% of the digestible P and total Ca level of Lact100); Lact50 (50% of the digestible P and total Ca level of Lact100); Lact50 + FTU (Lact50 with 500 FTU of phytase) in g by diet. Time in day after farrowing. linear Ca and P effect × quadratic time effect, $P = 0.07$; linear time effect, $P < 0.05$; quadratic time effect, $P < 0.05$; other contrast not significant.

The values in Lact75 sows were closer to Lact100 (quadratic Ca and P effect for Ca, $P < 0.05$ and P, $P = 0.06$). Milk Ca concentration tended to be higher in Lact50 (linear Ca and P effect, $P = 0.06$), and milk P concentration was higher in Lact50 (linear Ca and P effect, $P < 0.05$) compared to the other treatments. Milk Ca and P secretion were similar between treatments except that the Lact50 sows secreted more P (linear Ca and P effect, $P < 0.05$) and tended to secrete more Ca (linear Ca and P effect, $P = 0.05$). The litter's body Ca and P retention was similar between treatments. The total Ca and P excretion through feces and urine, based on the milk-secretion approach or the litter-retention approach, was lower in Lact50 + FTU and Lact50 sows (phytase effect, $P < 0.05$; linear Ca and P effect, $P < 0.05$). The total P excretion of Lact75 sows was closer to Lact100 sows than to Lact50 sows, when based on the milk-secretion approach (quadratic Ca and P effect, $P < 0.05$) and the litter-retention approach (quadratic Ca and P effect, $P < 0.05$). The total Ca excretion of Lact75 sows tended to be closer to Lact100 sows than to Lact50 sows, when based on the litter-retention approach (quadratic Ca and P effect, $P = 0.08$). Based on the milk-secretion approach, Lact75 was similar to Lact100 and Lact50. The Ca ATTD was higher in Lact50 + FTU (phytase effect, $P < 0.05$) and Lact50 sows (linear Ca and P effect, $P < 0.05$). The Ca ATTD of Lact75 sows was closer to Lact50 than to Lact100 (quadratic Ca and P effect, $P < 0.05$). The P ATTD was higher in Lact50 + FTU sows (phytase effect, $P < 0.05$) and differed in Lact75 sows (quadratic Ca and P effect, $P < 0.05$). Fecal Ca and P excretion was higher in Lact100 than in Lact50 (linear Ca and P effect, $P < 0.05$) and Lact50 + FTU (phytase effect, $P < 0.05$). The fecal Ca excretion of Lact75 sows was comparable to Lact100 and Lact50. The fecal P excretion of Lact75 was closer to Lact50 than to Lact100 (quadratic Ca and P effect, $P = 0.05$).

Physiological response of phosphocalcic metabolism

Blood serum P increased during lactation and then stabilized during the following gestation (quadratic time effect, $P < 0.05$; linear time effect, $P < 0.05$; Fig. 3a). Blood serum P evolution during lactation was similar between treatments except that Lact50 + FTU sows showed a decrease between d2 and d14 before increasing to values similar to the other sows on d26 (phytase effect × quadratic time effect, $P < 0.05$). Blood serum Ca tended to increase quadratically during lactation (quadratic time effect, $P = 0.06$; Fig. 3b) and then decreased linearly during the following gestation (linear time effect, $P < 0.05$). Dietary treatments did not affect blood serum Ca. While time did not affect blood serum CTX (Fig. 3c) and 1,25 $(OH)_2D_3$ (Supplementary Table S1), blood serum CICP (Fig. 3d) concentration increased, peaked on d26, and then decreased during the following gestation (quadratic time effect, $P < 0.05$). Dietary treatments did not affect blood serum CTX, 1,25$(OH)_2D_3$, or CICP concentrations.

Urine P concentration remained relatively stable in Lact100 and Lact75 sows, but peaked on d26 in Lact50 + FTU sows (phytase effect, $P < 0.05$; quadratic time effect, $P = 0.07$; Fig. 4a) and tended to increase during gestation in Lact50 sows (linear Ca and P effect × quadratic time effect, $P = 0.09$). Urine Ca concentration was higher during lactation than during gestation (quadratic time effect, $P < 0.05$; Fig. 4b) and stabilized at a concentration similar to d2 during the following gestation (linear time effect, $P < 0.05$). For the Lact50 sows specifically, it peaked on d26 and then decreased to values similar to the other sows (linear Ca and P effect × quadratic time effect, $P < 0.05$; linear Ca and P effect, $P < 0.05$). Urine Ca:P ratio was similar between d2 and the following gestation (linear time effect, $P < 0.05$; Fig. 4c). This ratio tended to increase further during lactation in Lact50 before decreasing

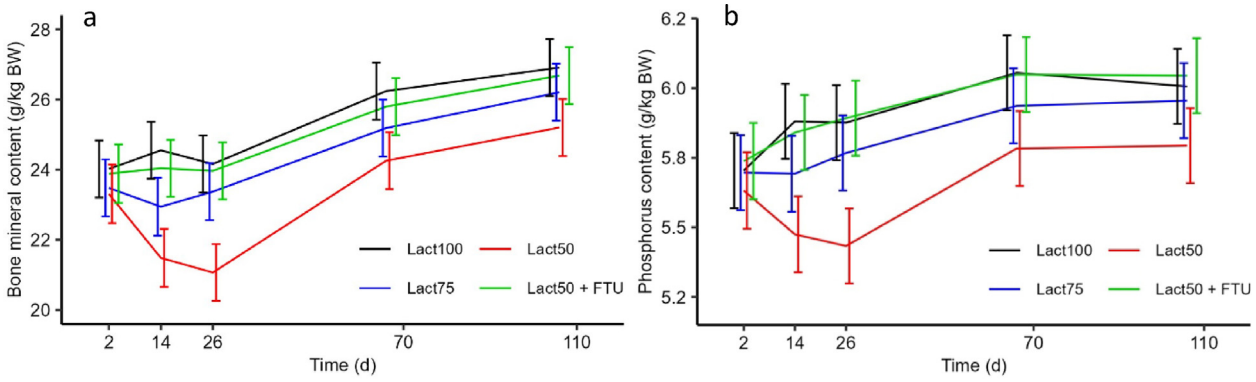

Fig. 2. Dynamics of bone mineral content (a) and phosphorus content (b) of primiparous sows during lactation and subsequent gestation, supplied with lactation diets differing in Ca and P content. Abbreviations: *Lact100* (control diet; 3.0 g digestible P and 9.9 g Ca /kg); *Lact75* (75% of the digestible P and total Ca level of Lact100); *Lact50* (50% of the digestible P and total Ca level of Lact100); *Lact50 + FTU* (Lact50 with 500 FTU of phytase) in g/kg BW by diet. Time in day after farrowing. linear Ca and P effect × quadratic time effect, $P = 0.05$ for 2a, $P < 0.05$ for 2b; linear Ca and P effect, $P < 0.05$ for 2a; linear time effect, $P < 0.05$ for 2a and 2b; quadratic time effect, $P < 0.05$ for 2b; other contrast not significant.

Table 2
Calcium and phosphorus balances of primiparous sows during lactation supplied with lactation diets differing in Ca and P content.

Response criteria	Treatment					Contrast		
	Lact100	Lact75	Lact50	Lact50 + FTU	SEM	Phyt	Lin	Quad
Number of sows	6	6	6	6				
Feed intake (kg)	167	176	176	172	13.0	0.53	0.25	0.45
Milk production (kg)[1]	284	274	300	294	23.4	0.47	0.23	0.13
Calcium balance								
Dietary Ca intake (g)	1 654	1 466	1 057	1 031	288.1	< 0.05	< 0.05	< 0.05
Body Ca retention (g)[2]	−205	−204	−388	−178	106.2	0.54	< 0.05	< 0.05
Milk Ca concentration (mg/kg)	1 980	2 218	2 250	1 955	260.1	0.58	0.06	0.39
Milk Ca secretion (g)[3]	563	613	674	573	91.3	0.83	0.03	0.90
Litter Ca retention (g)[4]	651	618	711	693	97.6	0.46	0.29	0.20
Total Ca excretion_milk (g)[5]	1 295	1 057	746	614	292.4	< 0.05	< 0.05	0.54
Total Ca excretion_litter (g)[5]	1 208	1 052	714	509	294.5	< 0.05	< 0.05	0.08
ATTD of Ca (%)	24.9	38.9	33.8	44.6	9.08	< 0.05	< 0.05	< 0.05
Fecal Ca (g)[6]	1 246	896	698	568	283.6	< 0.05	< 0.05	0.21
Phosphorus balance								
Dietary P intake (g)	985	936	757	722	130.4	< 0.05	< 0.05	0.06
Body P retention (g)[2]	−95	−105	−170	−85	41.7	0.54	< 0.05	0.06
Milk P concentration (mg/kg)	1 368	1 441	1 558	1 373	109.4	0.91	< 0.05	0.60
Milk P secretion (g)[3]	389	397	467	402	50.4	0.58	< 0.05	0.15
Litter P retention (g)[4]	430	409	470	458	64.4	0.46	0.29	0.20
Total P excretion_milk (g)[5]	692	644	386	447	143.5	< 0.05	< 0.05	< 0.05
Total P excretion_litter (g)[5]	650	632	443	343	146.2	< 0.05	< 0.05	< 0.05
ATTD of P (%)	34.4	45.2	35.7	57.3	10.17	< 0.05	0.64	< 0.05
Fecal P (g)[6]	646	512	487	307	132.8	< 0.05	< 0.05	0.05

Abbreviations: Lact100 (control diet; 3.0 g digestible P and 9.9 g Ca /kg); Lact75 (75% of the digestible P and total Ca level of Lact100); Lact50 (50% of the digestible P and total Ca level of Lact100); Lact50 + FTU (Lact50 with 500 FTU of phytase); ATTD (apparent total tract digestibility); phyt: phytase effect; lin: linear Ca and P effect; quad: quadratic Ca and P effect; FTU: phytase activity unit defined as the amount of phytase that liberates 1 mmol of inorganic phosphate per minute from 0.0051 mol/l sodium phytate at pH 5.5 and 37 °C.

[1] Milk production was determined by equations in the work of Hansen et al., 2012.

[2] Body P and Ca retention was calculated from the difference between final and initial Body P and Ca obtained from body bone mineral content and lean tissue mass, according to Kasper et al. (2021).

[3] The amount of P and Ca excreted in milk was determined by multiplying milk production and the P and Ca concentration of the milk.

[4] The body P and Ca retention of the litter was determined from the difference between the litter body's P and Ca content at weaning and birth. Body P and Ca contents were calculated according to equations by Ruiz-Ascacibar et al. (2019), considering litter BW.

[5] The total amount of P and Ca excreted in feces and urine was calculated as follows: ingested + body mobilization – milk secretion or litter retention.

[6] The P and Ca excreted in feces were determined by subtracting the amount of P and Ca ingested by the amount of P and Ca theoretically absorbed (dietary intake × ATTD).

during the following gestation (linear Ca and P effect × quadratic time effect, $P = 0.06$; linear Ca and P effect, $P = 0.07$). The amount of milk produced per sow over the 26 days of lactation (245 ± 71 kg) was similar between treatments (data not shown). The milk Ca concentration of Lact50 sows was higher (linear Ca and P effect, $P < 0.05$), while their milk P concentration tended to be higher (linear Ca and P effect, $P = 0.06$) than in milk of the other sows.

Discussion

Body calcium and phosphorus mobilization during lactation

This study investigated the ability of sows to mobilize body Ca and P reserves during lactation in order to achieve their high Ca and P requirements and to recover their body Ca and P reserves during their next gestation when their Ca and P requirements are

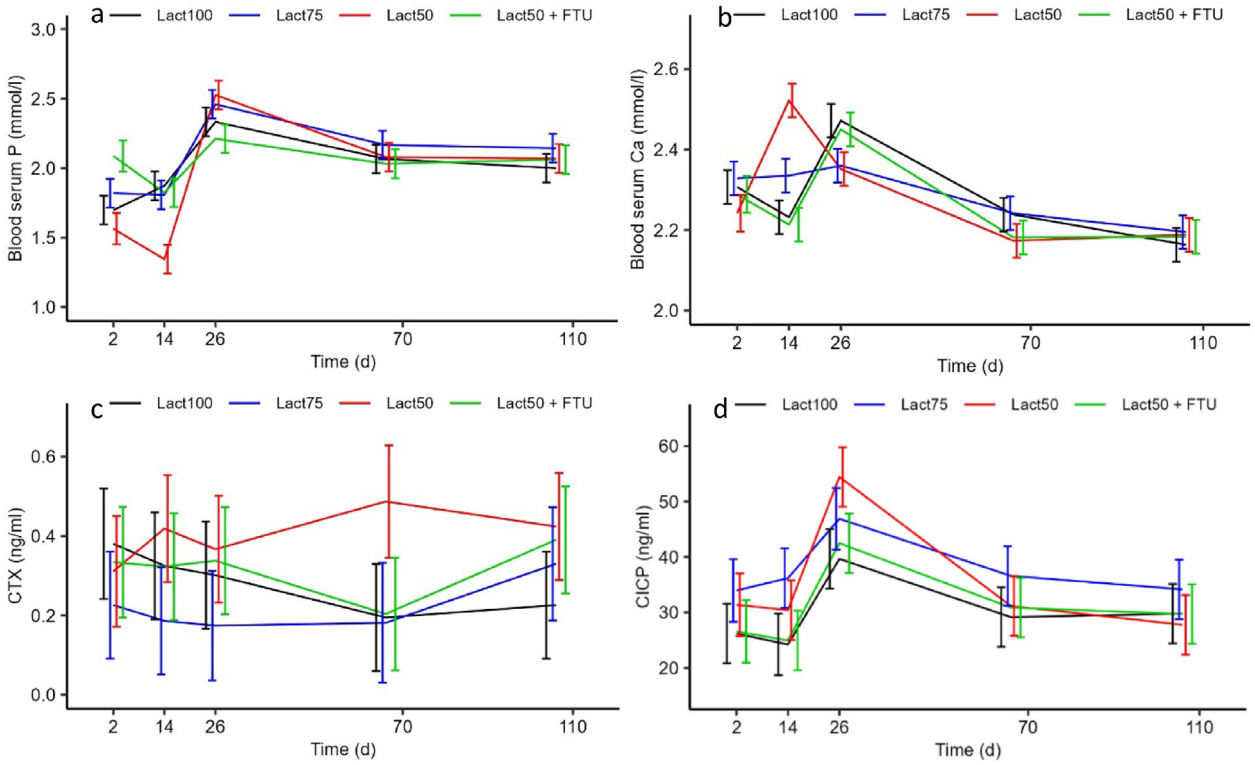

Fig. 3. Dynamics of blood serum P (a), Ca (b), CTX (c), and CICP (d) of primiparous sows during lactation and subsequent gestation, supplied with lactation diets differing in Ca and P content. Abbreviations: *Lact100* (control diet; 3.0 g digestible P and 9.9 g Ca /kg); *Lact75* (75% of the digestible P and total Ca level of Lact100); *Lact50* (50% of the digestible P and total Ca level of Lact100); *Lact50 + FTU* (Lact50 with 500 FTU of phytase); CTX: C-terminal telopeptide of type I collagen; CICP: C-terminal propeptide of type I collagen. Time in day after farrowing. phytase effect × quadratic time effect, $P < 0.05$ for 3a; linear time effect, $P < 0.05$ for 3a and 3b; quadratic time effect, $P < 0.05$ for 3a and 3d, $P = 0.06$ for 3b; other contrast not significant.

low. During lactation, the BMC (and body Ca and P) decreased in each individual sow, but the extent of the decline was dependent from the dietary Ca and digestible P contents. Expressed in g/kg BW, this result is all the more apparent if the BMC is independent of the sows' initial BW and its change during lactation is mainly expressed by a loss of fat tissue mass (Supplementary Table S1).

Mammals' bone resorption to support a high mineral requirement during lactation is well known (Kovacs, 2016). Bone ash content in ribs, vertebrae and humerus of sows (Mahan and Fetter, 1982) and bone bending strength of sows (Giesemann et al., 1998) declined over lactation, suggesting that the bone resorption of lactating animals occurred. The amount of dietary Ca and digestible P provided during lactation in the current study influenced bone resorption since the Lact50 sows mobilized twice as much as did the Lact100 sows, which lost 10% of their BMC (in g). Therefore, it can be hypothesized that a diet providing more than the recommended digestible P and Ca (Bikker and Blok, 2017) could limit or even suppress body Ca and P mobilization during lactation. An initial insight into this open question may be provided by Gutzwiller and Schlegel (2015), who compared recommended Ca and digestible P levels (9.4 and 3.3 g/kg, respectively) to increased levels (both + 15%) in lactating primiparous and multiparous sows with either 12 or 6 weaned piglets. These authors showed that the higher dietary supply of Ca and digestible P did not modify bone ash (in rib and metacarpus) when analyzed three days after weaning. This indicates that an oversupply of digestible P during lactation may not be of any benefit to reduce or even suppress body Ca and P mobilization during lactation. Such a strategy is also not in line with an aim for an efficient use of dietary P.

Bone resorption during lactation can be explained by homeostatic regulation in response to increased physiological P and Ca requirements. Phosphocalcic regulation is controlled by several hormones. A decrease in extracellular Ca leads to an increase in parathyroid hormone secretion, which then leads to an increase in $1,25(OH)_2D_3$ synthesis; meanwhile, a decrease in extracellular P results in reduced production of FGF-23 by osteocytes and osteoblasts, thereby promoting the expression of 1α-hydroxylase, which is essential for $1,25(OH)_2D_3$ formation (Crenshaw et al., 2011; Blaine et al., 2015; Wubuli et al., 2020). Therefore, the observed numerical increase in serum $1,25(OH)_2D_3$ concentration during lactation may correspond to the decrease in BMC. Additionally, $1,25(OH)_2D_3$ is thought to increase P absorption (Bergwitz and Jüppner, 2010) and Ca absorption (DiMeglio and Imel, 2019). During bone resorption, osteoclasts cleave the ends of type 1 collagen, a cross-linked fragment from the C-terminus of type 1 collagen that can be measured indirectly by CTX concentration in blood serum. An increase in CTX was however not observed during lactation in the present study, which is likely to be due to high individual variation. However, the numerical increase of blood serum Ca on d14 in Lact50 sows may be partly explained by the increased release of bone Ca. Indeed, Ca and P are stored as hydroxyapatite molecules in bone and the Ca:P mass ratio is 2.16 (Crenshaw, 2001). Thus, a dietary P deficiency would induce the mobilization of bone P and Ca, leading to increased blood serum Ca. Elevated blood serum Ca induces calcitonin secretion (González-Vega and Stein, 2014). This hormone helps to lower circulating $1,25(OH)_2D_3$ (Gao et al., 2004), inhibits osteoclast activity, reduces the mobilization of Ca and P from bone and reduces the kidneys' Ca reabsorption (Crenshaw, 2001); in turn, it increases urinary Ca and P losses. Accordingly, urinary Ca concentration in the Lact50 sows increased on d26. Previous studies with growing pigs have associated increased urinary Ca with dietary P deficiency (Vipperman et al., 1974; Pointillart et al., 1986; Fernández, 1995). Interestingly, the urinary Ca:P ratio in Lact50 sows

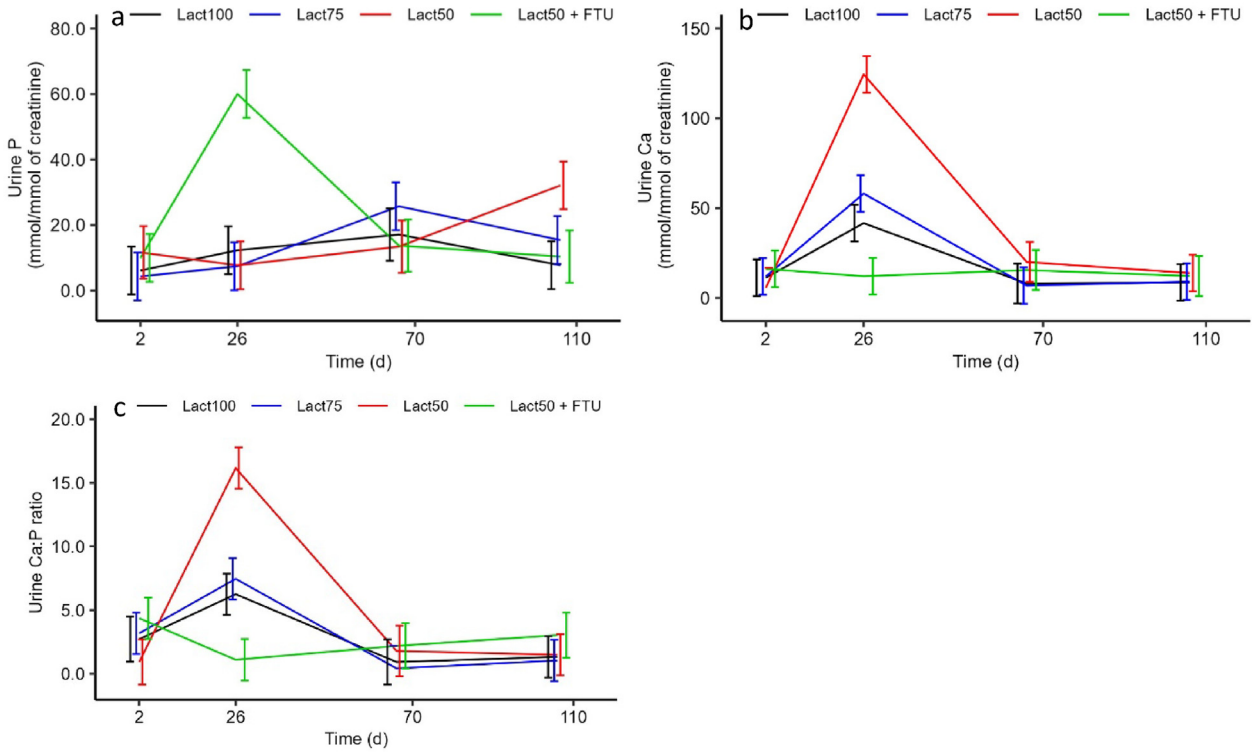

Fig. 4. Dynamics of urinary P (a), Ca (b), and the Ca:P ratio (c) of primiparous sows during lactation and subsequent gestation, supplied with lactation diets differing in Ca and P content. Abbreviations: *Lact100* (control diet; 3.0 g digestible P and 9.9 g Ca /kg); *Lact75* (75% of the digestible P and total Ca level of Lact100); *Lact50* (50% of the digestible P and total Ca level of Lact100); *Lact50 + FTU* (Lact50 with 500 FTU of phytase). Time in day after farrowing. 4a: linear Ca and P effect × quadratic time effect, $P = 0.09$ for 4a, $P < 0.05$ for 4b, $P = 0.06$ for 4c; linear Ca and P effect, $P < 0.05$ for 4b, $P = 0.07$ for 4c; phytase effect, $P < 0.05$ for 4a; linear time effect, $P < 0.05$ for 4b and 4c; quadratic time effect, $P = 0.07$ for 4a, $P < 0.05$ for 4b; other contrast not significant.

increased at the end of lactation which indicates, according to Grez-Capdeville and Crenshaw (2021a), a P deficiency.

The effect of phytase on Ca and P digestibility is well-documented in growing pigs and has recently been studied in sows (Zhai et al., 2021). Nonetheless, as data on the effect of phytase on sow diets remain scarce, the digestible P and Ca equivalency of phytase coming from growing pigs is generally used to formulate sow diets. In the present study, the dietary addition of phytase was effective enough to compensate for the difference in dietary Ca and digestible P between the Lact50 and Lact100 diets since BMC of the Lact50 + FTU sows was equal to that of the Lact100 sows, while P excretion was reduced by 43%. The calculated phytase equivalency of the supplemented Escherichia coli phytase (Quantum® Blue) was 1.3 g/kg of digestible P for 500 FTU/kg. This value is close to the one reported by the supplier (1.5 g/kg of digestible P for 500 FTU) and therefore does not require to be adjusted at this stage of knowledge.

Maintaining milk production is essential for optimal litter growth (Tokach et al., 2019). The nutrient requirements for milk production are not only met by dietary intake but also by body nutrient mobilization, such as milk fatty acid and protein through body fat and protein tissues (Tokach et al., 2019), or as demonstrated in the present study, milk Ca and P, through body bone tissues. It is supposed, that milk Ca and P are independent of dietary Ca and P (based on minimal dietary P of 5.0 g/kg with a Ca:P ratio of 1:1.3; Mahan and Fetter, 1982; Maxson and Mahan, 1986), but findings in the present study indicate the contrary as Ca and P concentrations in milk from Lact50 sows were 12% higher than in milk from Lact100 sows. This finding should however be considered with caution as based on a single sampling per sow during the same lactation week. Collecting more than one milk sample during lactation may consolidate such findings and improve the quality of the calculated Ca and P secretion quantities over milk since their concentration may increase during lactation (Harmon et al., 1974; Hu et al., 2019). Moreover, an eventual modified milk Ca and P concentration due to dietary Ca and P may influence the P status and the body P retention of suckling piglets which can be measured in a future study. Although the number of sows is limited in the present study to allow any conclusion on the growth performance of offspring, the piglets' growth performance was independent of the lactation diets, in line with findings of previous studies (Maxson and Mahan, 1986; Grez-Capdeville and Crenshaw, 2021b).

Body calcium and phosphorus recovery during subsequent gestation

As discussed previously, the sows mobilized their bone mineral reserves a quantity which seemed higher when a diet low in Ca and P was fed. During the subsequent gestation, the primiparous sows continued to grow and, thus, increased their BW and BMC. As the Lact50 sows were able to recover their bone mineralization deficit during gestation, the P and Ca requirements currently defined for the first third of gestation appear sufficient even for strongly depleted sows.

As in lactation, bone formation during gestation can be explained by homeostatic regulation in response to a decreased physiological P and Ca requirement. Bone homeostasis depends on bone resorption via osteoclasts and bone formation via osteoblasts, which are tightly coupled processes (Chen et al., 2018). The observed peak in blood serum CICP, a biomarker of bone formation, at the end of lactation corresponds with the findings of Grez-Capdeville and Crenshaw (2021b). The carboxy-terminal propeptide of type 1 collagen (CICP) is increasingly released at the end of lactation into the extracellular environment during the synthesis of bone tissue. The decreased BMC (in g) measured in each individual sow during lactation and, the subsequent bone

remineralization could explain the observed increase in CICP which was also higher in the sows which mobilized most BMC during lactation (Lact50 and Lact75). As blood serum P remained stable during gestation, the P and Ca requirements currently defined for the first third of gestation appear sufficient also from a physiological aspect.

Phytase to replace inorganic phosphates and reduce phosphorus excretion

The sustainability of an agricultural system on national level can be improved by seeking a balance between the amount of imported and exported P into that system (Spiess and Liebisch, 2022). The most efficient way to promote this in animal production is probably by reducing the use of dietary P originating from mineral phosphates as such a source is not produced by the agricultural system per se. The Lact50 + FTU diet only contained 2.71 g/kg of monocalcium phosphate, representing 0.59 g P per kg of diet or 14% of total dietary P. This content is 75% less than in the Lact100 diet. Applying globally such a feeding strategy, would in the case of Switzerland, represent a potential saving of 40 t from the, according to Spiess and Liebisch (2022), yearly 2400 t of P imported as mineral phosphates for animal nutrition. The sustainability of an agricultural system on farm level can be improved by seeking a balanced P cycle (for eg. P requirement for fertilization vs P supply through fertilization). A primiparous Lact100 sow excreted 0.75 kg of P per lactation, which is slightly lower than the Swiss reference value of 1.0 kg P per lactation of primi- and multiparous sows (Menzi et al., 2016). A change to Lact50 + FTU strategy would reduce the P excretion down to 0.5 kg P per lactation, a difference which would greatly reduce the ev. amounts of slurry to be exported by a pig farm with limited P requirements for plant production.

Determination of phosphorus and calcium requirements of sows

The P and Ca requirements of lactating sows are largely determined by milk production and the amount of P and Ca exported in milk predicted from litter size and average daily gain (NRC, 2012). Additionally, lean and fat tissue mobilization is calculated, and the P present in lean tissue is assumed to be available for milk production (Bikker and Blok, 2017). To date, however, body P and Ca mobilization from bone tissue has not been considered, probably due to a lack of data. In the present study, the lactating sows mobilized 205 and 95 g of body Ca and P, respectively (Lact100; Table S1). Per day, this amount is 8 and 4 g of Ca and digestible P, respectively, or 12 and 10% of the respective dietary intakes for the Lact100 sows. It should be noted that the regressions used to determine body Ca and P from DXA data by Kasper et al. (2021) are based on growing pigs from 20 to 100 kg BW, thus not validated on heavier animals, such as sows. The Ca and P concentration in BMC and in lean mass may potentially change with heavier BW although it is not the case between 20–100 kg BW as solely linear effects are included in the used equations. Therefore, the quantified values of Ca and P in the body could theoretically deviate from reality, but the statistical effects of time and dietary treatment in this study remain. The P in bone was assumed at a fixed Ca:P ratio of 2.16, representing 43% of BMC (Crenshaw, 2001), the P mobilized from bone represented 95%, and the remainder originated from lean tissue. The Ca and P requirements of gestating sows are based on the need for maintenance, growth, and conceptus development (estimated at 446 g of Ca and 141 g of digestible P for the first 40 days of gestation for a primiparous sow; Bikker and Blok, 2017). Considering bone Ca and P mobilization during lactation would encourage the addition of respective quantities during gestation in order to recover bone reserves. The total Ca and digestible P requirements during the first 40 days of gestation of primiparous sows would be 651 g of Ca and 236 g of digestible P. The diet containing 8.2 g/kg of Ca and 2.6 g/kg of digestible P led to respective intakes of 708 g and 224 g for the first 40 days of gestation; thus, it was sufficient for Ca but limiting for P. Note that this acquired data could potentially redefine the P and Ca requirements for sows; however, for the purpose of considering individual variations, further investigations will be necessary to confirm the suggested quantities of body Ca and P mobilization, to provide data for multiparous sows, and to better quantify the timing of bone recovery during gestation.

Conclusion

This study provides new data on body Ca and P changes during lactation and the following 80 days of gestation, which can help to fine-tune mineral requirement. It confirms the hypothesis that sows have the capacity to mobilize important quantities of Ca and P from body tissues during lactation and that these were dependent from the ingested amounts of digestible P. It also confirms that sows seem to be able to recover their bone reserves during the following gestation. Finally, this study confirms the possibility of replacing inorganic phosphates with microbial phytase during lactation, reducing P excretion without inducing adverse effects on sows' bone mineralization and litter performance. The same result was possible without supplying phytase which, however, led to a reduced bone mineralization at weaning, which was compensated throughout the subsequent gestation. Nonetheless, further investigations are needed to confirm the present data and to extend this knowledge for multiparous sows.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2024.101130.

Ethics approval

This study's experimental procedure was approved by the Office for Food Safety and Veterinary Affairs (2020_42_FR/2020_49_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 this study's findings are available upon request from the corresponding author.

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.

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Declaration of interest

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

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