INTRODUCTION

Phosphorus (P), as an element necessary for all forms of life, has the peculiarity of being a finite resource. Modern animal husbandry systems rely on supplements of mineral P, which is usually extracted from mining rock (Campbell et al., 2017; Cordell & White, 2013). The massive agricultural use of P leads to an increased input into the environment with negative effects mainly on aquatic ecosystems (Campbell et al., 2017; Diaz & Rosenberg, 2008). For this reason, the new fertilizer ordinance regulates the use of P (Ministerium für Landwirtschaft und Umwelt Mecklenburg Vorpommern, 2018). In animal husbandry, a better P efficiency can be reached either by an improvement of the feeding system or by a better selection of animals used for breeding. Currently, there is a number of promising approaches to improve P efficiency via precision phase feeding (Pomar, Hauschild, Zhang, Pomar, & Lovatto, 2009), liquid feeding, phytase supplementation (Brady, Callan, Cowan, McGrane, & O’Doherty, 2002; Huber, Hempel, & Rodehutscord, 2006) and
putative additivity of phytase/phytate content in feed components (Poulsen, Voergaard, Strathe, & Blaabjerg, 2019) in order to ensure an appropriate P supply during growth.

The P requirement of pigs depends on the age of the animals, their body composition and on their genetics (AFBN, 2006; Frederick & Stahly, 1999; Hitte, Grapes, Lensing, Rothschild, & Stahl, 2006). It was shown that a low dietary mineral content affects bone mineral density (Nico de medo, Scott, Buchan, Duncan, & Robins, 1998). There seems to be an optimum range of the P content (0.52% available P for animals between 15 kg and 30 kg), which should not be undercut or exceeded to reach an optimal bone ash content (Alebrante et al., 2011). Likewise, an optimum Ca content was determined for piglets between 11 and 25 kg, at which an optimum bone ash content is achieved (González-Vega et al., 2016). An insufficient supply of the animals with P and Ca manifests in a deficient bone mineralization or in a loss of performance in body weight gain (BWG) (Adeola et al., 2015; Euclydes Drews et al., 2016; Rieger, 2017). Previous work has shown this might also apply to an oversupply (Gerlinger et al., 2019). Lameness and an increased incidence of irregular bone morphology and fractures can be attributed to P shortage (Doige, Owen, & Mills, 1975; Harper, Kornegay, & Schell, 1997; Rieger, 2017).

The balance of bone formation and resorption can be shifted in case of P and Ca over- or undersupply due to its storage function for minerals towards maintenance of mineral homeostasis (Berndt & Kumar, 2009; Sapir-Koren & Livshits, 2011; Talmage & Mobley, 2008). A number of serum markers are known to provide information on the balance between bone formation and bone resorption. Osteoblasts and osteoclasts mediate these processes in a very complex system in which hormones like parathyroid hormone (PTH) or calcitriol (Vitamin D), receptors (receptor activator of NFκB, RANK), signalling molecules (receptor activator of NFκB ligand, RANKL) and other molecules (e.g. osteoprotegerin, OPG) are involved using feedback mechanisms and which is strongly linked to other parts of the very complex regulatory network of Ca and P homeostasis (Berndt & Kumar, 2009; Dusso, Brown, & Slatopolsky, 2005; Fukumoto, 2014; Takayanagi, 2015; Talmage & Mobley, 2008). Analysis of bone homeostasis biomarkers is a way to elucidate the processes of bone metabolism affected by P and Ca supply more precisely (Sørensen, Kruger, Hansen-Møller, & Poulsen, 2018).

Apart from the optimal dietary P content, it is extremely important to select an optimal Ca:P ratio for the design of the diets used in order to avoid negative effects on growth performance while at the same time safeguarding animal welfare (Alebrante et al., 2011; Reinhart & Mahan, 1986). The aim of this study was to identify benefits and limitations of high and low dietary P supply at constant or variable Ca:P ratios, respectively, in order to provide a better knowledge basis for optimizing the feeding system without compromising the health of the animals. By combining data on performance traits and bone architecture from previous work (Gerlinger et al., 2019; Oster et al., 2016, 2018) with newly generated data (for further details see Table S1), the effects of different dietary Ca and P levels and ratios were integrated with the endocrine determinants critical for P homeostasis. Therefore, this study has a meta-analytical character in which data from a total of 61 piglets were analysed for dietary induced systemic processes of bone formation and resorption in growing pigs.

2 | MATERIAL AND METHODS

2.1 | Animals, housing and sampling

In two trials, a total of 61 German Landrace piglets (Sus scrofa domesticus, Erxleben) were assigned to wheat/barley/soybean-based diets differing in P content with constant Ca content (trial 1) or with a variable Ca and P content but a constant Ca:P ratio (trial 2), for a period of five weeks (28- to 64-day post-natum [dpn]). The botanical and analysed compositions of the diets are shown in Table 1. The animals of trial 1 were obtained from six litters, and the animals of trial 2 were obtained from four litters. The piglets were kept individually on a flat-deck in both trials. Each group comprised at least six females and six castrated males (trial 1) or three females and three castrated males (trial 2). In trial 1, the piglets received diets with lower (L1 diet; P: 0.6% of dry matter [DM]; Ca:P ratio = 2.2; n = 14), medium (M1 diet; P: 0.9% of DM; Ca:P ratio = 1.4; n = 12) (AFBN, 2006) and higher P supply (H1 diet; P: 1.1% of DM; Ca:P ratio = 1.2; n = 14). There was no variation in Ca content of the feed (Ca: 1.3% of DM). In trial 2, the pigs received diets with lower (L2 diet; P: 0.6%; Ca: 0.8% of DM; Ca:P ratio = 1.4; n = 7), corresponding (medium; M2 diet; P: 0.8%; Ca: 1.3% of DM; Ca:P ratio = 1.5; n = 7) (AFBN, 2006) and higher mineral supply (H2 diet; P: 1.0%; Ca: 1.7% of DM; Ca:P ratio = 1.7; n = 7) than recommended. The animals fed the M diets served as control groups with a P supplementation fitting the current recommendation for weaning piglets (AFBN, 2006). Neither phytase nor other phosphatases were supplemented to any of the diets. Pigs had ad libitum access to water and feed. Zootechnical data, chemical bone composition of animals of trial 2 and microstructural bone measurements of animals of trials 1 and 2 have already been partially described (Gerlinger et al., 2019; Oster et al., 2018, 2016; Table S1). Here, the data were complemented by zootechnical parameters and bone chemical composition for trial 1 as well as serum parameters for trials 1 and 2. Feed intake and body weight gain (BWG) of the individual animals were measured weekly (days 28, 35, 42, 49, 56 and 63). Zootechnical parameters such as average daily weight gain (ADG), P
intake and average daily feed intake (ADFI) were calculated as mean values for each week. On day 63, blood samples were obtained from Vena cava cranialis for serum and heparin plasma preparation. Serum and plasma were stored at −80°C. At slaughter (day 64), the animals were anesthetized by electric stunning and killed by exsanguination at the Institute's experimental slaughter facility. Thereafter, the left femurs were sampled and stored for micro-CT analysis. For analysis of the chemical bone composition, the right femurs were removed from the carcasses and stored at −20°C until further analyses.

2.2 Analysis of feed composition

The crude nutrients (DM, crude ash (CA), crude protein, crude fibre, crude fat, sugar and starch) in the diets were analysed according to the standard methods (Weende analysis) recommended by the VDLUFA for the chemical analysis of feedstuffs (VDLUFA, 1976). For mineral analyses, 0.5 g of the feed samples was solubilized with 7 ml 65% nitric acid, 3 ml of 30% hydrogen peroxide solution and 3 ml of ultrapure water and ashed at 180°C. The total P

| TABLE 1 | Botanical composition of the experimental diets, calculated and analysed nutrient composition of the experimental diets |
|----------|--------------------------------------------------|
| Ingredient | Unit | Trial 1 Low | Medium | High | Trial 2 Low | Medium | High |
| Botanical composition | | | | | | | | |
| Wheat | % | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Barley | % | 27.4 | 26.9 | 26.3 | 28.4 | 26.8 | 25.1 |
| Barley flakes | % | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 | 12.0 |
| Soybean meal | % | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 | 21.0 |
| Soybean oil | % | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| Premix a | % | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 | 7.4 |
| Monocalcium phosphate | % | 0.5 | 1.4 | 2.3 | 0.5 | 1.4 | 2.4 |
| Calcium carbonate | % | 1.1 | 0.8 | 0.4 | 0.1 | 0.8 | 1.6 |

Calculated composition

| Metabolizable energy | MJ/kg | 13.5 | 13.4 | 13.4 | 13.6 | 13.4 | 13.2 |
| Crude protein | % | 18.7 | 18.7 | 18.6 | 18.6 | 18.6 | 18.4 |
| Digestible lysine | % | 1.0 | 1.0 | 1.0 | 1.0 | 0.9 | 0.9 |
| Phosphorus | % | 0.5 | 0.7 | 0.9 | 0.5 | 0.7 | 0.9 |
| Digestible phosphorus | % | 0.3 | 0.3 | 0.5 | 0.7 | 0.3 | 0.5 |
| Calcium | % | 1.1 | 1.1 | 1.1 | 0.7 | 1.1 | 1.5 |

Analysed composition

| Dry matter | g/kg FM | 882.0 | 883.0 | 884.0 | 898.0 | 897.0 | 905.0 |
| Crude ash | g/kg DM | 59.0 | 63.0 | 69.0 | 49.7 | 61.5 | 79.0 |
| Crude protein | g/kg DM | 205.0 | 203.0 | 201.0 | 196.0 | 196.0 | 210.0 |
| Crude fibre | g/kg DM | 66.0 | 63.0 | 65.0 | 52.0 | 48.9 | 49.7 |
| Crude fat | g/kg DM | 32.0 | 31.0 | 29.0 | 35.6 | 33.7 | 31.7 |
| Sugar | g/kg DM | 67.0 | 68.0 | 67.0 | 67.6 | 71.0 | 69.8 |
| Starch | g/kg DM | 474.0 | 463.0 | 453.0 | 393.0 | 399.0 | 383.0 |
| Metabolizable energy | MJ/kg DM | 12.5 | 12.5 | 12.3 | 14.7 | 14.6 | 14.3 |
| Calcium | g/kg DM | 13.0 | 13.0 | 13.0 | 7.9 | 12.7 | 16.9 |
| Phosphorus | g/kg DM | 6.0 | 9.0 | 11.0 | 5.7 | 8.4 | 10.2 |
| Phosphorus, soluble | g/kg DM | 2.8 | 4.3 | 6.7 | 3.1 | 4.9 | 6.0 |
| Ca:P ratio | | 2.2 | 1.4 | 1.2 | 1.4 | 1.5 | 1.7 |
| Ca:P soluble ratio | | 4.6 | 3.0 | 1.9 | 2.6 | 2.6 | 2.8 |
| Phytase activity | U/kg | 252.0 | 258.0 | 253.0 | 252.0 | 251.0 | 253.0 |

Abbreviations: DM, dry matter; FM, fresh matter.

aComposition of the Premix (per kg diet): lysine, 0.31%; methionine, 0.17%; threonine, 0.16%; tryptophan, 0.06%; calcium, 0.40%; sodium, 0.19%; vitamin A, 10,000 IU; vitamin D3, 1,000 IU; vitamin E, 60 mg; vitamin K3, 2.0 mg; vitamin B1, 2.5 mg; vitamin B2, 6.7 mg; vitamin B6, 4.8 mg; vitamin B12, 37.0 mg; nicotinic acid, 29.6 mg; calcium pantothenate, 16.0 mg; folic acid, 0.95 mg; biotine; 160.0 mg; choline chloride, 200.0 mg; ferrous sulphate, 240.0 mg; copper sulphate, 6.0 mg; 82.5 mg; manganese (oxide), 55.0 mg; iodate, 1.2 mg; selenite, 0.4 mg; and cobalt carbonate, 0.8 mg.
concentration was measured colorimetrically by comparing the extinction at a wavelength of 365 nm with a standard series using the ammonium vanadate-molybdate yellow method using Ammonium vanadate and Ammonium molybdate tetrahydrate (Gericke and Kurmies, 1952), UV cuvette, 2.5 ml, Brand GmbH + Co.KG, Wertheim and a Thermo Spectronic Genesys 5 (336001) UV-Visible Spectrophotometer, Thermo Fisher Scientific. For soluble P, analyses were performed with the resulting solution in the same way after boiling the feed samples in 0.5% hydrochloric acid and water for 30 min each to allow P to dissolve. The Ca level was determined using a 0.5% lanthanum (III) chloride heptahydrate solution by an atomic absorption spectrometer (Solaar AA Spectrometer iCE 3000, Thermo Fisher Scientific). Phytase activity was determined by incubation with sodium phytate which was stopped by the addition of a molybdic vanadate solution and a subsequent photometric measurement (415 nm) according to VDLUFA (1976).

2.3 | Chemical analysis of bone components

The proximal 30% of the femurs were used for the analyses. The DM (g/kg) was determined by lyophilization (Delta 1-24 LSCplus, Fa. Martin Christ Gefriertrocknungsanlagen GmbH). Subsequently, the samples were degreased and ground with a centrifugal mill (ZM1, Retsch GmbH, mesh size: 0.5 mm). The determination of CA and minerals was performed following the recommendations of the VDLUFA and as previously described (Miesorski et al., 2018; Oster et al., 2018; Rieger, 2017; VDLUFA, 1976).

2.4 | Microstructural bone properties

The left femurs of a subset of the animals of trial 1 (n = 18) and of all animals of trial 2 (n = 21) were analysed by a high-resolution micro-CT Imaging System (SkyScan 1076, Bruker-MICRO-CT, Kontich, Belgium) and have been presented previously (Gerlinger et al., 2019; Oster et al., 2016). Scans were performed as described before (Gerlinger et al., 2019). Prior to the scans, the samples were stored in 0.9% saline solution at 4°C for at least 24 hr. For the femurs obtained in trial 1, the volume of interest (VOI) comprised 500 slices proximal and distal of the middle of each bone (1,000 slices in total, Figure 1, (a)). For trial 2, the VOI was selected distal to the hip joint and proportionally fixed at 30% of the total femur length to account for the individual size (Figure 1, (a)). The VOI covered 400 slices in both directions (800 slices in total). Two regions of interest (ROI) were determined within each VOI for both trials to determine the properties of the corticalis and the spongiosa separately (Figure 1, (b) and (c)). Phantom rods, containing 0.25 and 0.75 g calcium-hydroxyapatite/cm³, were used for calibration. Trabecular bone mineral density (BMD) and cortical tissue mineral density (TMD) were determined and a three-dimensional analysis was performed (Figure 1, (b) and (c)). Microstructural parameters, including the trabecular number (Tb.N), trabecular separation (Tb.Sp), trabecular thickness (Tb.Th), trabecular bone volume/tissue volume ratio (BV/TV) and structure model index (SMI), were acquired (Hildebrand & Rüegsegger, 1997).

2.5 | Analysis of serum components

Serum minerals (inorganic P, Ca and Mg) and other physiological parameters (alkaline phosphatase (ALP), creatinine, lactate dehydrogenase (LDH) and creatine phosphokinase (CPK)) were analysed in samples obtained at 63 dpn with commercial assays using Fuji DriChem 4000i (FujiFilm). Dickkopf-related protein1 (DKK1) was measured in heparin plasma sampled at 63 dpn using commercial assays available for the MAGPIX system (magnetic bead-based quantitative immunoassay) according to the manufacturer’s specifications (Merck KGaA, EMD Millipore). Gastric inhibitory polypeptide (GIP) (E6055:-K-RGIP, Merck KGaA, EMD Millipore, Darmstadt, Germany), β-CTX (SerumCrossLaps (CTX-I) ELISA, Immunodiagnostic Systems GmbH (IDS GmbH), Germany), calcidiol

FIGURE 1 Images illustrating the micro-CT analyses of the porcine femurs. (a) For trial 1, the volume of interest (VOI) was set at the median of the total femur length (blue line) and covered 1,000 slices (light blue area), and for trial 2, the VOI was set distal to the hip joint at 30% of the total femur length (green line) and covered 800 slices (light green area). The area proximal to the green line was used for chemical bone analysis. (b) Region of interest (ROI) (red) represents the trabecular bone for measurements of bone mineral density (BMD). (c) ROI (red) to approximate the tissue mineral density (TMD) in cortical bone.
(IBL), calcitriol (trial 2; IDS GmbH) were analysed in duplicate using commercial ELISA assays according to the manufacturer’s instructions. Calcitriol for trial 1 was analysed via chemiluminescent immunoassay (DiaSorin) according to the manufacturer’s instructions.

2.6 | Data analysis

Data obtained by micro-CT imaging were analysed by the use of CTaganalyzer (SkyScan) and NRecon (SkyScan). The R software (v3.5.2, R Foundation for Statistical Computing) was used to calculate a mixed model, where dietary group and sex were considered as fixed effects and the litter was included as a random effect. Weaning weight was considered as covariate. Differences between the dietary groups of each trial were examined using the Tukey’s post hoc test. Significance level was set at \( p < .05 \); a numerical tendency was taken into account for \( p \)-values ranging between .05 and .1. In Figures 2–4, the relative values of each parameter are displayed as mean value of each group ± standard errors in relation to the mean values of the M groups for each trial separately.

3 | RESULTS

The study was conducted with piglets fed wheat/barley-based diets with variable P (trial 1) or a constant Ca:P ratio (trial 2) during five weeks after weaning to determine effects on growth, bone development and serum parameters.

3.1 | Feed composition

The P and \( P_{\text{soluble}} \) contents differed between the experimental groups in both trials (L1 < M1 < H1 and L2 < M2 < H2), whereby the Ca content was constant (L1 = M1 = H1) or rising (L2 < M2 < H2). The resulting Ca:P ratios in trial 2 were within a narrow range. The level of phytase activity averaged 250 U/kg in all dietary groups. Table 1 summarizes the results of the analysed feed compositions.

3.2 | Zootechnical data

With regard to the intake of P and \( P_{\text{soluble}} \), significant differences could be observed between the three groups in both studies (L < M < H). Concerning Ca intake, a significant difference between all three groups could be observed in trial 2 (L2 < M2 < H2), but not in trial 1. In trial 1, there was a significant difference in FCR (L1 < M1), whereas in trial 2 the FCR was different when comparing L2 and H2 (L2 < H2). At the end of trial 2, the body weight (BW) of the H2 animals was significantly lower than the BW of the animals of L2 and M2 (H2 < M2; H2 < L2), while there were no observable differences in trial 1 (Figure 2, Table S2).

3.3 | Chemical bone composition

The analysis of the chemical bone composition (Figure 3, Table S3) showed a lower DM, CA in DM and Ca content in DM among the

![Figure 2](image)
animals of the L-groups than for the two compared groups (L < H; L < M). The same applied to P in DM in trial 1 (L1 < H1; L1 < M1). In trial 2, the levels for P in DM show a tendency (L2 < H2; p = .094). In both trials, no differences in the Ca:P ratio were observed.

3.4 | Microstructural bone properties

The dietary effects of a variable mineral supply on bone structure observed in both studies are shown in Figure 3 (Table S4). A significantly lower trabecular BMD, BV/TV, SMI and Tb.N was recognized in the L1 group compared with H1 (L1 < H1). In trial 1, Tb.Sp showed the highest value in the L1 group with a significant difference to M1 and H1 (L1 > H1; L1 > M1). A significantly lower trabecular BMD and BV/TV (L2 < H2; L2 < M2) but increased Tb.Sp was observed in the L2 group in contrast to M2 and H2 (L2 > H2; L2 > M2). A lower Tb.N was found in L2 compared with M2 (L2 < M2). The SMI showed no dietary effect in trial 2. Values for TMD were unaffected by mineral intake.

3.5 | Blood hormone and mineral concentrations

The blood parameters are visualized in Figure 4 (Table S5). In blood samples obtained from 63 dpn for trial 1, the Ca, calcitriol and ALP levels found in the L1 group were significantly higher than those observed in the M1 and H1 groups (L1 > H1; L1 > M1).
same was seen for the Ca:P ratio in blood. In contrast, inorganic P, DKK1 and GIP were numerically lowered in L1 than in M1 and H1 (L1 < H1; L1 < M1; GIP: L1 < H1, p = .055; L1 < M1, p = .073). A significantly elevated LDH was determined in H1 compared with L1 and M1 (L1 < H1; M1 < H1). Serum calcidiol level was found to be lower in L1 than in H1 (L1 < H1). No significant difference was found for Mg and CPK in trial 1, as well as for creatinine and β-CTX in both trials.

In samples obtained from 63 dpn for trial 2, significant lower levels of Ca were found in H2 compared with M2 and L2 (H2 < L2; H2 < M2). Conversely, lower levels of CPK were measured in the L2 group compared with M2 and H2 (L2 < H2; M2 < H2). The calcitriol levels were significantly elevated in L2 compared with the other groups involved in trial 2 (L2 > M2 > H2). Lowered DKK1 was found in L2 compared with H2 (L2 < H2) while in L2, lower levels of serum calcidiol and LDH were determined compared with M2 (L2 < M2). For the Ca:P ratio and GIP, no effect of the dietary intervention in trial 2 could be detected.
DISCUSSION

After weaning, piglets experience a phase of rapid growth and the accumulation of bone minerals, which is critical for lifelong skeletal health (Colaiani et al., 2019). In this study, the effects on bone caused by differences in P supplementation in the first five weeks post-weaning were evaluated.

Although the ADFI in trial 1 shows no significant differences, FCR differed significantly (L1 > M1), possibly caused by the elevated Ca:P ratio in L1 (Brady et al., 2002). In trial 2, which deals with constant Ca:P ratios, the effects on feed efficiency traits were more pronounced. However, effects of dietary Ca or P on FCR (Hittmeier et al., 2006), ADFI (Nimmo, Peo, Moser, & Lewis, 1981) or both (Alebrante et al., 2011) have been described controversially. The weight difference between M2 and H2 animals in conjunction with an unaffected bone structure might be an indicator for the independence of bone mineralization and BW, which has been previously suggested by Tanck, Homminga, Van Lenthe, and Huiskes, (2001). Interestingly, bone mineralization is not necessarily increased due to an elevated dietary supply of minerals (M vs. H), which is supported by the lack of significant differences in CA, Ca and P concentrations of bones as observed in both trials. Moreover, the measurements for PTH (Oster et al., 2016, 2018), Tb.Sp and Tb.N comparing M and H within both trials point to the possibility that bone mineralization may no longer be prioritized once required bone stability has been established. However, the difference in bone minerals in trial 1 shows that a lower level of dietary P may prevent an improvement in bone mineralization, even if Ca is sufficient.

Bone mass and microstructural architecture are known to impact on bone stability. Consequently, the mechanical fracture load was considerably lower due to the L diet in trial 2 (Gerlinger et al., 2019). In general, the low dietary P supply caused a detrimental effect on bone mass and architecture and thus on the stability of the bones. Indeed, bone characteristics such as BMD, BV/TV and Tb.N were lower while values for Tb.Sp were increased in both trials irrespective of the Ca:P ratios (Gerlinger et al., 2019; Oster et al., 2016). These aspects indicate an important contribution of the trabecular architecture to bone stability. In this context, the additional intake of dietary P beyond the recommendations provided no evidence that a high supply of P generates additional positive effects. The reduced trabecular BMD following L diets and the lack of differences in cortical TMD among all dietary groups are indicative of a modification of the spongiosa but not of the corticalis. Notably, these effects seem to be completely independent of the dietary Ca:P ratios used in these trials. Consistently, negative implications on BMD by dietetic Ca or P level reduction have already been demonstrated by Liesegang et al. (2002) and Bai et al. (2017). Regarding the BV/TV values, Eklou-Kalonji et al. (1999) showed the responsiveness to a lowered dietary Ca supply. The results of the current study suggest that also a poor P diet may prompt the same effect on BV/TV, even with a sufficient Ca supply. Regarding the trabecular characteristics, Tb.N was lowered in L diets, while Tb.Th appeared to be unaffected by dietary Ca and P supply. The latter corresponds to results obtained from Ca deficient diets in piglets (Eklou-Kalonji et al., 1999). In fact, it has been shown that Tb.N is of greater importance for bone strength than Tb.Th (Seeman & Delmas, 2006). The trabecular marker SMI was sensitive to a low P diet with high Ca:P ratio (trial 1) but remained unaltered due to a constant Ca:P ratio (trial 2). However, SMI has been described as a rather rough indicator for trabecular plate-rod-like shape and might be of limited value according to current research (Salmon, Ohlsson, Shefelbine, & Doube, 2015).

Bone remodelling takes place on respective surfaces. Consequently, divergent P and Ca diets may lead to stronger effects on trabecular bone, which exhibits a larger surface than the cortical bone (Seeman & Delmas, 2006). Calcitriol and PTH represent major determinants of bone remodelling (Berndt & Kumar, 2009; Dusso et al., 2005). Consequently, the respective calcitriol and PTH levels in trial 1 and 2 were responsive to the experimental L and H diets (Oster et al., 2016, 2018). According to current knowledge, a low dietary P level leads to the synthesis of calcitriol, while a high dietary P level leads to a reduced calcitriol level (Penido & Alon, 2012; Moe, 2008; Tanaka & Deluca, 1973). This suggests that maintaining a constant Ca:P ratio in the blood requires an adjustment of calcitriol production. The secretion of PTH due to low Ca levels in blood subsequently promotes differentiation and activity of osteoclasts, resulting in bone resorption and release of Ca and P (Rauner, Sipos, & Pietzschmann, 2007; Teitelbaum, 2000). Therefore, a possible shift in the balance between bone mineralization and resorption towards an osteoclast-controlled decrease in bone resorption in the L compared with H animals may be assumed. Furthermore, low levels of PTH could have a negative effect on the bone properties of the L-groups by reducing collagen production (Talmage & Mobley, 2008). In this context, β-CTX, which is a marker for bone resorption, is of interest as it correlates negatively with the content of raw ash, Ca, P and BMD (Sørensen et al., 2018). Higher levels of blood β-CTX point to a higher bone resorption; however, no significant differences for β-CTX could be observed in the current pig trials. Further bone markers such as GIP and DKK1 can help elucidate potential shifts in the relationship between bone formation and bone resorption. The activity of osteoclasts is reduced by GIP which simultaneously increases the activity of osteoblasts, thus reducing bone resorption and strengthening bone formation (Hansen, Tencerova, Frølich, Kassem, & Frost, 2018; Xie et al., 2007). The observed trend of lower GIP in L1 compared with M1 and H1 may suggest an increased bone resorption rate due to variable Ca:P ratios. A decrease in bone formation is mediated by DKK1, which is primarily expressed by osteoblasts (Pinzone et al., 2009). According to that, lower DKK1 levels in the L-groups might indicate lower activity and number of osteoblasts (Li et al., 2006) thus presumably lowering bone formation and mineralization. In general, bone turnover variations could be superimposed by organismal developmental processes during the early growth of the animals.
The bone serves as a reservoir for approx. 85% of the body P and the vast majority of the body Ca reserves (Moe, 2008). The availability of these minerals in serum reflects the underlying mechanisms of the P homeostasis. In addition to the bones, a number of other organs such as the intestines, kidneys and parathyroid glands act on P homeostasis, which is of relevance for the mineral values in serum (Berndt & Kumar, 2009). In the overall view, the various regulatory mechanisms could not sufficiently respond to the dietary challenges. Hence, serum P differed between the divergent groups L and H. Trial 1 shows that efforts to increase the availability of P in serum simultaneously lead to a significant increase in serum Ca levels and increased Ca:P ratio. Accordingly, shifts in the Ca:P ratio in the blood are susceptible to dietary mineral status, while the Ca:P ratio in the bone reflects the stoichiometry of hydroxyapatite. With regard to serum ALP, the influence of P and Ca levels is discussed controversially (Sapir-Koren & Livshits, 2011; Euclydes Drews et al., 2016; Doige et al., 1975). The ALP with osteoblastic origin provides P for the mineralization of bones by the hydrolysis of pyrophosphate (Sapir-Koren & Livshits, 2011). Therefore, the altered ALP values in L1 and H2 groups might reflect P recruitments from other sources than bone.

5 | CONCLUSION

Feeding of phosphorus beyond the level of the groups fed the medium phosphorus level had no additional benefits. However, the growth performance of the animals tended to be impaired if the diets containing lower levels of phosphorus. Considering mineralization of the bones, it can be concluded that a reduced mineral supply possibly impacts on the degree of mineralization. Furthermore, it can be stated that a phosphorus deficit has an influence on mineralization even in the case of sufficient calcium. At the expense of further storage in the bone, the excretion of calcium and phosphorus appears to be preferred at a nutrient content higher than recommended. Both bone mineral density and bone volume–total volume ratio suggest that if calcium and phosphorus are simultaneously reduced in the diet, mineralization is weaker than if the calcium to phosphorus ratio is shifted. The conclusion that a high calcium to phosphorus ratio contributed to the architecture of cancellous bone and thus to bone stability is supported by the variation of the structure model index in trial 1. However, trabecular number and trabecular separation are independent from the calcium to phosphorus ratio. The results of the serum measurements of dickkopf-related protein1 (DKK1) and gastric inhibitory polypeptide (GIP) indicate a lowered bone formation in the L-groups.

The current feeding recommendations for the phosphorus requirement are sufficient, without the addition of microbial phytase. Based on the results, the addition of extra phosphorus has no further positive effects. Balancing the supply of phosphorus and calcium along with further resource-saving applications such as precision phase feeding, liquid feeding and phytase supplementation will help to lower phosphorus intake and subsequently reduce phosphorus losses to the environment.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ANIMAL WELFARE STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to. The study was approved by the Scientific Committee of the Leibniz Institute for Farm Animal Biology. The experimental setup was generally licensed by the Ethics Committee of the federal state of Mecklenburg-Western Pomerania, Germany (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei; LALLF M-V7221.3-1-053-15). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and feed legislation.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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