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Transcriptional and physiological responses due to diets varying in phosphorus supply in weaned piglets

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Inhaltsverzeichnis

1 Summary and Conclusions

Phosphorus (P) is an important element of various metabolic and signalling processes, including bone metabolism and immune function. Sufficient supply of pigs with calcium (Ca) and phosphorus (P) is essential for animal health and welfare during the growth period but frequently exceeds age specific requirements in practice. However, the P content in animal manure is considered as a cause of massive environmental problems in soil and aquatic ecosystems. The objective of this study is the investigation of effects of a reduced and increased dietary P supplementation in combination with constant or variable Ca levels on bone mineralization and bone structure compared to the current dietary recommendation. Further, possible serum markers that would allow the assessment of adequacy of P supply for bone health during growth should be found. To elucidate the routes of P homeostasis and utilization, a five-week feeding study was conducted with weaned piglets receiving a diet with recommended amounts of P and Ca (M), or a diet with lower (L) or higher (H) P values and a constant Ca:P-ratio. Furthermore the effect of a variation in dietary P and Ca was compared to a variable Ca:P-ratio with constant dietary Ca levels. Dietary responses were deduced via performance parameters, bone characteristics (MicroCT), genome-wide transcriptomic profiles of peripheral blood mononuclear cells (PBMCs) and molecular data retrieved from serum, intestinal mucosa, and kidney cortex.

Animals of the L group showed attempts to maintain mineral homoeostasis via intrinsic mechanisms, whereas the high-fed animals adapted at the expense of growth and development. MicroCT revealed significantly lower bone mineral density, trabecular number, and mechanical fracture load in (L). Gene expression analyses showed transcripts of 276 and 115 annotated genes with higher or lower abundance in the (H) than (L) that were related to basic cellular and metabolic processes as well as response to stimuli, developmental processes and immune system processes. Transcripts associated with vitamin D hydroxylation (Cyp24A1, Cyp27A1, Cyp27B1) were regulated by diet at local tissue sites.

The result validated that the recommended Ca and P supply is sufficient, without the addition of microbial phytases. However, addition of P has no further beneficial effects on bone stability, while P supplementation below the recommended level affects bone development and growth performance.

This study shows the many molecular routes involved in P homeostasis that should be considered to improve endogenous mechanisms of P utilization. Further fine-tuning of the P supply in conjunction with an appropriate Ca supply will contribute to a reduction in P losses along the agricultural value chain and associated environmental impact while maintaining animal health and welfare.

2 Introduction and Objectives

P is used as fertilizer for the production of fodder plants, and as an additive to the livestock feed to cover the mineral requirements of monogastric animals and to standardize feed as

well.[1] 50% to 80% of the P components present in feed are excreted in the manure.[2,3] Besides these leaching losses and their impact on aquatic ecosystems, a negative impact of a high P level on soil quality is observable.[4]

P is present in every cell since it is part of DNA, ATP and cell membranes.[5,6] Furthermore, P is involved in many metabolic processes (e.g. energy metabolism,[7,8] cellular signaling,[7,8] or nucleic acid metabolism[9]) and is a component of the urinary and the blood buffer system.[8] Storage of Ca and P in the bones actually occurs by mineralization in the form of hydroxyapatite making up 80% to 90% of the P present in the body.[7] Regarding the fracture risk, an optimal bone mineralization contributes to animal health, inadequate bone mineralization is as harmful as excessive bone mineralization.[10,11] The balance and the regulation of Ca and P homeostasis are strongly interlinked.[5] German livestock farmers are guided by the Gesellschaft für Ernährung (GfE) recommending dietary P and Ca levels.[12]

Nutrients themselves may be strong signals which impact homeostasis regulation. The trifecta of intestinal absorption, renal excretion/resorption and osseous mobilization/storage substantially contributes to the maintenance of P and Ca homeostasis.[5] The maintenance system for P and Ca homeostasis includes (among many other factors) hormones such as parathyroid hormone (PTH),[5] activated vitamin D (1,25-dihydroxyvitamin D (1,25$(OH)_2$D) also called calcitriol)[13] and fibroblast growth factor 23 (FGF23)[13] Systemic effects due to an altered P and Ca supply indicate dynamic influxes and effluxes of P, Ca, and superior endocrine metabolites in the blood.[14] A variation of renal transport proteins is observed which modulates renal and intestinal mineral uptake or excretion.[15,16]

The third important factor for the maintenance of P and Ca homeostasis, beneath intestinal absorption and renal reabsorption of P and Ca in balance with excretion, are processes involved in bone mineralization and in resorption of minerals from bones.[13,17] The stability of the bones and their compensation for the rapid increase in weight and size contribute to animal welfare and health in today's rapidly growing high-performance pig breeds. A fundamental distinction can be made between two differently structured areas of long bones. The cancellous part of the bone, the *Substantia spongiosa* and the tube like part of the bone, the *Substantia corticalis* which surrounds the cancellous bone.[13] The trabeculae in the spongiosa considerably contribute to stability and their structure and shape can be used to estimate the stability of the bones and the status of P and Ca supply.[18]

Peripheral blood mononuclear cells (PBMCs) are a valuable surrogate tissue for *in vivo* analyses of processes in other tissues, which are easily accessible.[19,20] On the basis of known differences in the efficiency of P utilization between different pig breeds distinguished by different genetics, a hereditary basis or at least a genetic contribution to P metabolism is conceivable.[21] Thus, differences in P efficiency or excretion within individual breeds could also be based on genetics.[19,22] It is necessary to identify genetic markers for mechanisms influenced by a P divergent diet and the impact on pathways of P metabolism by means of holistic or targeted analyses. Identified genetic markers might have a high prognostic value,

thus enabling the deduction of the nutritive impact on individual animals *in vivo* by blood- or transcript analyses and a reactive adjustment of feeding regimen if necessary.[23]

Objectives of the dissertation:

- Analysis of potential consequences for performance, serum and bone parameters and genetic expression following dietary P and Ca levels and Ca:P-ratios differing from recommendations.
- Determination of possibilities to reduce the environmental impact of animal production in the form of P, while ensuring animal welfare and performance.
- Identification of potential candidate genes by means of expression analyses to provide a method for in vivo monitoring of the health and performance status of livestock.

3 Material and Methods

Animal trial

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]). 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)[12] 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)12 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.12 Neither phytase nor other phosphatases were supplemented to any of the diets. Pigs had ad libitum access to water and feed. Zootechnical parameters such as body weight (BW), average daily weight gain (ADG), and average daily feed intake (ADFI) were recorded and the mean presented for each week (days 28, 35, 42, 49, 56, 63). Blood samples were taken on day 63 from the Vena cava cranialis. On day 64, animals were anaesthetized by electrical stunning and killed by exsanguination at the Institute's experimental slaughter facility. The left femurs were excised and stored at -20 °C.

Bone analyses

For micro-CT analyses of femurs, a high-resolution Micro-CT Imaging System was used (SkyScan 1076, Bruker-MICROCT, Kontich, Belgium). Cortical tissue mineral density (TMD) and trabecular bone mineral density (BMD) were measured, and a three-dimensional analysis was performed (Figure 1B,C). Microstructural parameters, including the trabecular

bone volume/tissue volume ratio (BV/TV), structure model index (SMI), trabecular number (TbN), trabecular separation (TbSp), and trabecular thickness (TbTh) were obtained. Chemical analyses of the bone composition were performed according to the instructions of the VDLUFA.[24]

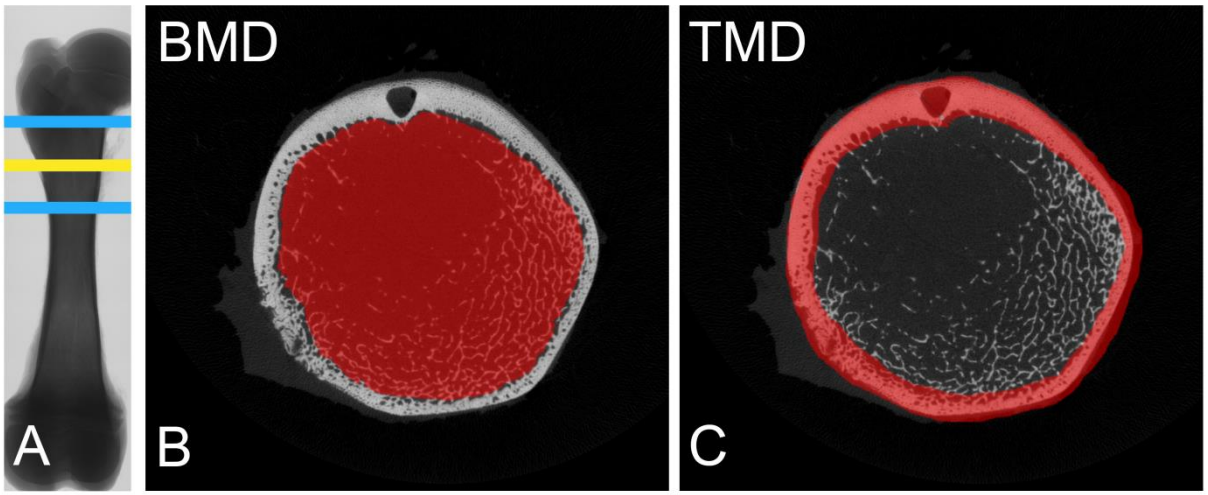

Figure 1. *Micro-CT analyses of porcine femurs. (**A**). The volume of interest (VOI) was set distal to the hip joint at 30% of femur length (yellow line) and covered 800 slices (light blue lines). (**B**). Region of interest (ROI) (red) represents the trabecular bone to approximate bone mineral density (BMD). (**C**). ROI (red) for measurements of the tissue mineral density (TMD) in cortical bone.*

Serum analyses

Serum minerals (inorganic P, Ca, Mg) and other physiological parameters (alkaline phosphatase (ALP), creatinine, lactate dehydrogenase (LDH), creatine phosphokinase (CPK)) were analyzed in samples obtained at 63 dpn with commercial assays using Fuji DriChem 4000i (FujiFilm, Minato, Japan). 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, Darmstadt, Germany). Gastric inhibitory polypeptide (GIP) (E6055:-K-RGIP, Merck KGaA, EMD Millipore, Darmstadt, Germany), β-CTX (SerumCrossLaps (CTX-I) ELISA, Immunodiagnostic Systems GmbH (IDS GmbH), Frankfurt am Main, Germany), calcidiol (IBL, Hamburg, Germany), calcitriol (trial 2; IDS GmbH) were analyzed in duplicate using commercial ELISA assays according to the manufacturer`s instructions. Calcitriol for trial 1 was analyzed via chemiluminescent immunoassay (DiaSorin, Saluggia, Italy) according to the manufacturer`s instructions.

Analyses of genetic expression

For genome-wide analyses of the pig transcriptome, single stranded cDNA was synthesized, biotin-labelled, and fragmented using the WT Plus Expression kit according to the manufacturer's instructions (Affymetrix, Santa Clara, CA, USA).[25] The samples were hybridized on snowball arrays comprising 47,845 probe-sets.[25] The arrays were processed following the manufacturer's instructions using the GeneChip Hybridization, Wash and Stain

Kit (Affymetrix, Santa Clara, CA, USA). Raw data were generated with Affymetrix GCOS 1.1.1 software (Affymetrix, Santa Clara, CA, USA).

Data analyses

Data obtained by microCT imaging was analyzed by the use of CTanalyzer (SkyScan, Kontich, Belgium) and NRecon (SkyScan, Kontich, Belgium). The R software (v3.5.2, R Foundation for Statistical Computing, Vienna, Austria) 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 $p<0.05$, a numerical tendency was taken into account for p-values ranging between 0.05 and 0.1. In figures 2 to 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.

Expression data were processed by Expression Console (Affymetrix, version 1.4, Santa Clara, CA, USA) and R software (v3.2.3) (R Foundation). In line with phenotypic data measurements, differential expression was established by variance analysis (SAS Institute, Cary, NC, USA). To correct for multiple testing in large datasets, q-values were calculated considering the p-value distribution. The cut-off for q-values was set to $q < 0.21$, which is equivalent to $p < 0.01$. To reveal differences in mRNA abundances, fold changes (FC) were calculated between dietary groups. Additionally, the lists of differentially abundant transcripts were evaluated via Ingenuity Pathway Analysis to visualize canonical pathways and bio-functions (IPA; Ingenuity Systems, Redwood City, CA, USA). The significance of the association between pathway and data was set at $p < 0.05$ (corrected by Benjamini-Hochberg). Pathways that explicitly refer to human diseases were excluded from the results.

The study was approved by the Scientific Committee of the FBN, and 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-V/TSD/7221.3-1-053-15).

4 Results

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. The analysis of the chemical bone composition showed a lower dry matter (DM), crude ash (CA) in DM and Ca content in DM among the 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). The dietary effects of a variable mineral supply on bone structure observed in both studies are shown in figure 2.

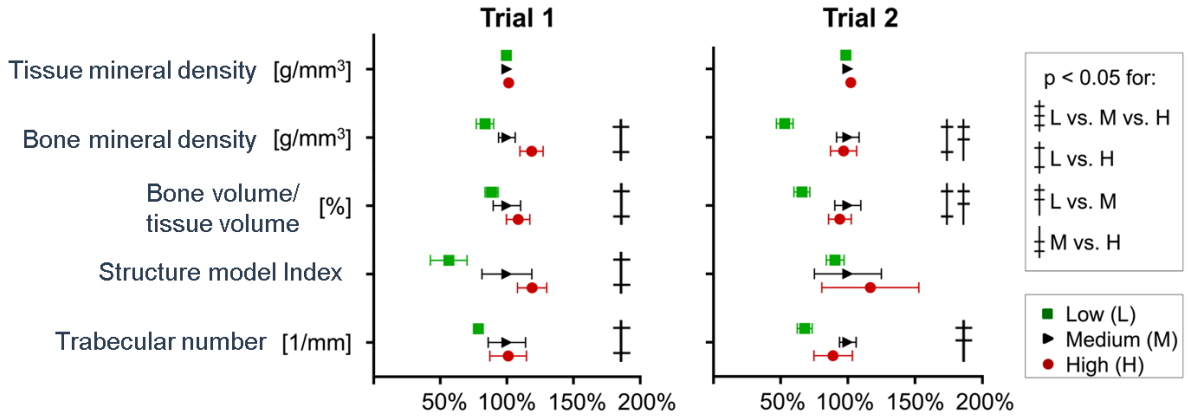

Figure 2. *Relative values of the microstructural parameters of the bones. Values are displayed in relation to the mean values of the M group (mean ± SE). Crosses are indicating significant differences between groups within each trial as explained in the legend on the right.*

A significantly lower trabecular BMD, BV/TV, SMI and Tb.N was recognized in the L1 group compared to 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). No differences were found for cortical bone (tissue mineral density (TMD)) in either trial. 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 to M2 (L2 < M2). The SMI showed no dietary effect in trial 2. The results of the bone marker analyses are visualized in figure 2.

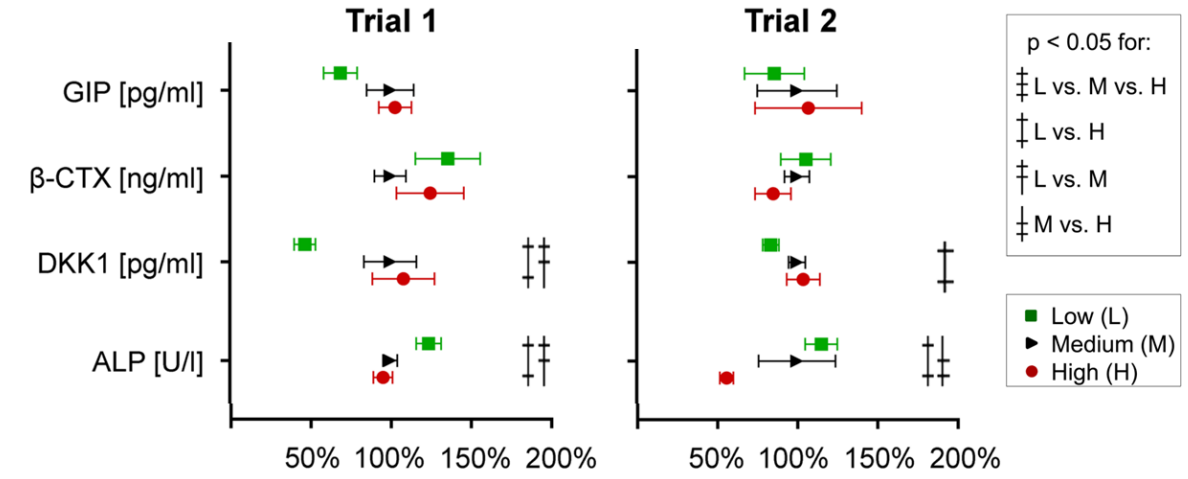

Figure 3. *Relative values of the bone marker concentrations at 63 dpn. Values are displayed in relation to the mean values of the M group (mean ± SE). Crosses are indicating significant differences between groups within each trial as explained in the legend on the right. ALP – Alkaline phosphatase; β-CTX – Carboxy-terminal collagen crosslinks (Crosslaps); DKK1 – Dickkopf-related protein1; GIP – Gastric inhibitory polypeptide*

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). The 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=0.055; L1 < M1, p=0.073). Serum calcidiol level was found to be lower in L1 than in H1 (L1 < H1). No significant difference was found for β-CTX in both trials (figure 3).

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

The comparison of microarray data between the animals of the L- and the H-fed groups of trial 2 revealed 391 differentially expressed annotated genes (276 L < H; 115 L > H) involved in 33 pathways that passed the threshold criteria ($p < 0.01$; $q < 0.21$). Due to multiple testing corrections, no significant differences in gene expression were found when compared L to M or M to H groups (figure 4). Therefore, these comparisons were not considered in the downstream pathway analyses.

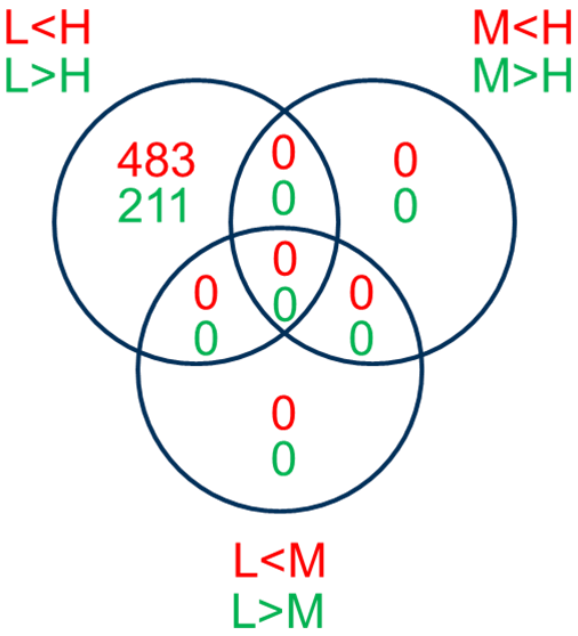

Figure 4. *Numbers of probeset measurements with a different abundance for the animals of trial 2. Comparing the L group with the H group, a total of 694 probesets showed an altered abundanca. 483 probesets (annotated to 276 differentially expressed genes) were measured as upregulated in the H group, whereas 211 probesets (annotated to 115 differentially expressed genes) were determined as downregulated in H. No differences in genetic expression could be found comparing M with H and L with M.*

Comparison of L and H data revealed that pathways involved in the modulation of humoral and cellular immunity were affected. Moreover, analyses revealed molecular patterns that are involved in the differentiation and functions of T cells. Furthermore, mRNA expression patterns associated with genes involved in monocyte and macrophage function. Besides the prominence of immune pathways, genes exhibiting altered mRNA abundances were associated with pathways related to signal transduction, biosynthesis, P metabolism and gene expression machinery. Moreover, “p38 MAPK signaling”, “PPAR signaling”, “TREM1 signaling”, “interferon signaling”, “RhoA signaling”, and “iNOS signaling” differed in L and H animals. However, the prominence of immune-related pathways is moderated by the fact that only 4.3% of enriched GO-terms mapped to “immune system process”.

Transcripts associated with vitamin D hydroxylation (Cyp24A1, Cyp27A1, Cyp27B1) were regulated by diet at local tissue sites.

5 Discussion

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.[26] 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[21], ADFI[27] or both[28] 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[29]. Moreover, the measurements for PTH[30,31], 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.

In general, the low dietary P supply caused a detrimental effect on bone mass and architecture and thus on the stability of the bones.[32] Indeed, bone characteristics such as BMD, BV/TV and Tb.N were lower while values for Tb.Sp were increased in both trials.[30,32] 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.[3334] The current study implicates that a poor P diet may prompt an 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.[35] In fact, it has been shown that Tb.N is of greater importance for bone strength than Tb.Th.[18] 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.[36]

Divergent P and Ca diets may lead to stronger effects on trabecular bone which exhibits a larger surface than the cortical bone.[18] Calcitriol and PTH represent major determinants of bone remodeling.[14,37] Consequently, the respective calcitriol and PTH levels in trial 1 and 2 were responsive to the experimental L and H diets.[30,31] 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.[38,39] Therefore, a possible shift in the balance between bone mineralization and resorption towards an osteoclast-controlled decrease in

bone resorption in the L compared to 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.[40] No significant differences for β-CTX pointing at collagen resorption could be observed in the current pig trials.[41] 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.[42,43] The observed trend of lower GIP in L1 compared to 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.[44] According to that, lower DKK1 levels in the L groups might indicate lower activity and number of osteoblasts thus presumably lowering bone formation and mineralization.[45] In general, bone turnover variations could be superimposed by organismal developmental processes during the early growth of the animals.

With regard to serum ALP, the influence of P and Ca levels is discussed controversially.[17,46,47] The ALP with osteoblastic origin provides P for the mineralization of bones by the hydrolysis of pyrophosphate.[17] Therefore, the altered ALP values in L1 and H2 groups might reflect P recruitments from other sources than bone.

With respect to the expression analysis in trial 2, IL1R2 might affect osteoclastogenesis via the putative effects of IL1 on the expression of M-CSF by marrow stromal cells.[39] Perhaps this decoy receptor (IL1R2) acts via some mechanism to counteract excessive weakening of bone structure and for the parallel maintenance of P and Ca homeostasis. Downregulation of pro-osteoblastic activity via the Wnt pathway by PLPP3 potentially leads to reduced bone formation and supports the results of reduced bone parameters in the L group.[48] The effects on NF-κB signaling potentially have an effect on bone turnover.[38]

6 Literature

1. Huyghebaert, et al. in *World Poultry Science Association (WPSA), 17th European Symposium on Poultry Nutrition, Edinburgh, UK, 23-27 August, 2009* 21–23, ref. 29 (2009).
2. Cordell, D. & White, S. *agronomy* **3**, 86–116 (2013).
3. Kornegay, E. T. & Harper, A. F. *Prof. Anim. Sci.* **13**, 99–111 (1997).
4. Sharpley, A. et al. **51**, 160–166 (1996).
5. Fukumoto, S. *Bonekey Rep.* **3**, 1–5 (2014).
6. Berndt, T. J., Schiavi, S. & Kumar, R. *Am. J. Physiol. - Ren. Physiol.* **289**, F1170–F1182 (2005).
7. Penido, M. G. M. G. & Alon, U. S. *Pediatr. Nephrol.* **27**, 2039–2048 (2012).
8. Proszkowiec-Weglarz, M. & Angel, R. *J. Appl. Poult. Res.* **22**, 609–627 (2013).
9. Shaikh, A., Berndt, T. & Kumar, R. *Pediatr. Nephrol.* **23**, 1203–1210 (2008).
10. Loveridge, N. et al. *Bone* **35**, 929–941 (2004).
11. S Viguet-Carrin, S., Garnero, P. & Delmas, P. D. *Int.* **17**, 319–336 (2006).
12. AfBN (ed.). *Empfehlungen zur Energie- und Nährstoffversorgung von Schweinen*. (DLG-Verlag, 2006).
13. Moe, S. M. *Prim. Care Clin. Off. Pract.* **35**, 215–237 (2008).
14. Berndt, T. & Kumar, R. *Physiology* **24**, 17–25 (2009).
15. Silver, J. & Naveh-Many, T. *Kidney Int.* **75**, 898–905 (2009).
16. Crenshaw, T. D., Rortvedt, L. A. & Hassen, Z. *J. Anim. Sci.* **89**, 1957–1964 (2011).
17. Sapir-Koren, R. & Livshits, G. *IBMS Bonekey* **8**, 286–300 (2011).

18. Seeman, E. & Delmas, P. D. *N. Engl. J. Med.* **354**, 2250–2261 (2006).
19. de Mello, V. D. F. et al. *Mol. Nutr. Food Res.* **56**, 1160–1172 (2012).
20. Liew, C. C. et al. *J. Lab. Clin. Med.* **147**, 126–132 (2006).
21. Hittmeier, L. J. et al. *J. Nutr. Biochem.* **17**, 385–395 (2006).
22. Just, F. *et al. BMC Genomics* **19**, 1–11 (2018).
23. Müller, M. & Kersten, S. *Nat. Rev. Genet.* **4**, 315–322 (2003).
24. VDLUFA, *VDLUFA-Methodenbuch Band III* (VDLUFA-Verlag, 1976).
25. Freeman, T. C. *et al. BMC Biol.* **10**, 1–21 (2012).
26. Brady, S. M. et al. *J. Sci. Food Agric.* **82**, 1780–1790 (2002).
27. Nimmo, R. D. et al. *J. Anim. Sci.* **52**, 1330–1342 (1981).
28. Alebrante, L. *et al. Rev. Bras. Zootec.* **40**, 323–330 (2011).
29. Tanck, E. et al. *Bone* **28**, 650–654 (2001).
30. Oster, M. *et al. Am. J. Physiol. - Regul. Integr. Comp. Physiol.* **310**, R917–R925 (2016).
31. Oster, M. *et al. Ambio* **47**, 20–29 (2018).
32. Gerlinger, C. *et al. Nutrients* **11**, 436–450 (2019).
33. Liesegang, A. L. *et al. J. Anim. Physiol. a. Anim. Nutr.* **86**, 1–16 (2002).
34. Bai, L. L. *et al. Anim. Feed Sci. Technol.* **224**, 59–65 (2017).
35. Eklou-Kalonji, E. *et al. J. Nutr.* **129**, 188–193 (1999).
36. Salmon, P. L. et al. *Front. Endocrinol. (Lausanne).* **6**, 1–10 (2015).
37. Dusso, A. S., Brown, A. J. & Slatopolsky, E. *Am J Physiol Ren. Physiol* **289**, F8--F28 (2005).
38. Rauner, M., Sipos, W. & Pietschmann, P. *Int. Arch. Allergy Immunol.* **143**, 31–48 (2007).
39. Teitelbaum, S. L. *Science (80-.).* **289**, 1504–1508 (2000).
40. Talmage, R. V. & Mobley, H. T. *Gen. Comp. Endocrinol.* **156**, 1–8 (2008).
41. Sørensen, K. U. et al. *J. Anim. Sci.* **96**, 4693–4703 (2018).
42. Hansen, M. S. S. et al. *Basic Clin. Pharmacol. Toxicol.* **122**, 25–37 (2018).
43. Xie, D. *et al. Bone* **40**, 1352–1360 (2007).
44. Pinzone, J. J. *et al. Blood* **113**, 517–525 (2009).
45. Li, J. *et al. Bone* **39**, 754–766 (2006).
46. Euclydes Drews, J. *et al. Ciência Rural. St. Maria* **46**, 1076–1081 (2016).
47. Doige, C. E., Owen, B. D. & Mills, J. H. L. *Can. J. Anim. Sci.* **55**, 147–164 (1975).
48. Shockley, K. R. *et al. J. Cell. Biochem.* **106**, 232–246 (2009).

Annex:

- Presentations at the symposia of the P-Campus 2016 to 2018
- Contribution to the research camp of the University of Rostock 2016 to 2018
- Presentation at the lecture conference of the Deutsche Gesellschaft für Züchtungskunde e.V. 2017 in Hohenheim
- Poster contribution and Abstract at the 22nd Congress of the European Society of Veterinary and Comparative Nutrition, 2018, Munich
- Contribution to the supervision of the Bachelor thesis by Wiebke Braatz, Uni Rostock, AUF

Literatur:

- Oster, M. *et al.* Lower dietary phosphorus supply in pigs match both animal welfare aspects and resource efficiency. *Ambio* **47**, 20–29 (2018).
- Gerlinger, C. *et al.* Physiological and transcriptional responses in weaned piglets fed diets with varying phosphorus and calcium levels. *Nutrients* **11**, 436–450 (2019).
- Gerlinger, C. et al. Effects of excessive or restricted phosphorus and calcium intake during early life on markers of bone architecture and composition in pigs. *Journal of animal Physiology and Nutrition* in press.