SCIENCE CAMPUS
PHOSPHORUS RESEARCH
ROSTOCK

Responses to various dietary P levels in growing pigs

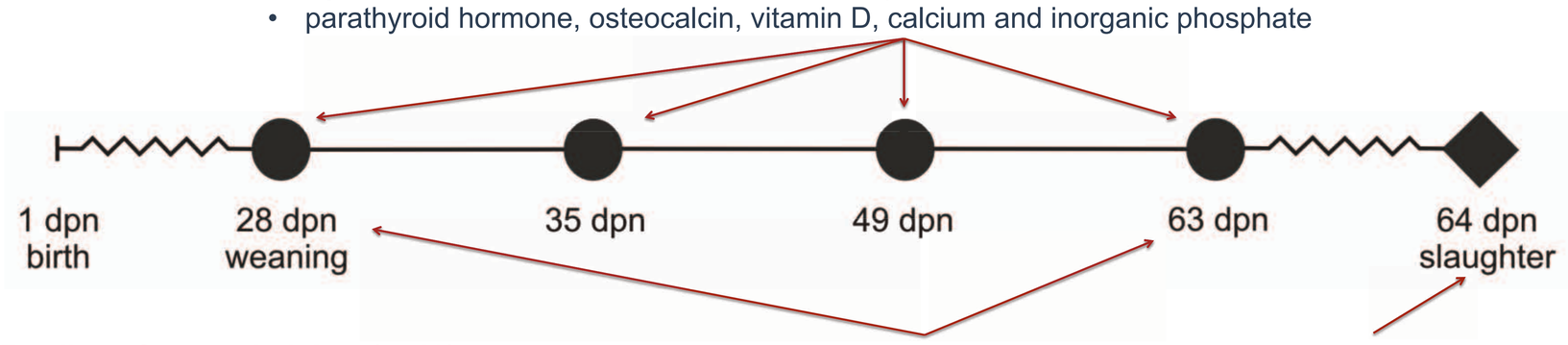
New solutions regarding housing conditions, feeding and breeding of pigs with high P efficiency

Experimental design

Excerpt from experimental diets

% of dry matter	L	M	H
Calcium	1.0	1.0	1.2
Phosphorus	0.6	0.8	1.1
Digestible Phosphorus	0.29	0.48	0.66

Hormones, physiological and blood cell parameters
Analyses of blood cell counts and bioactive components of P metabolism:
- parathyroid hormone, osteocalcin, vitamin D, calcium and inorganic phosphate



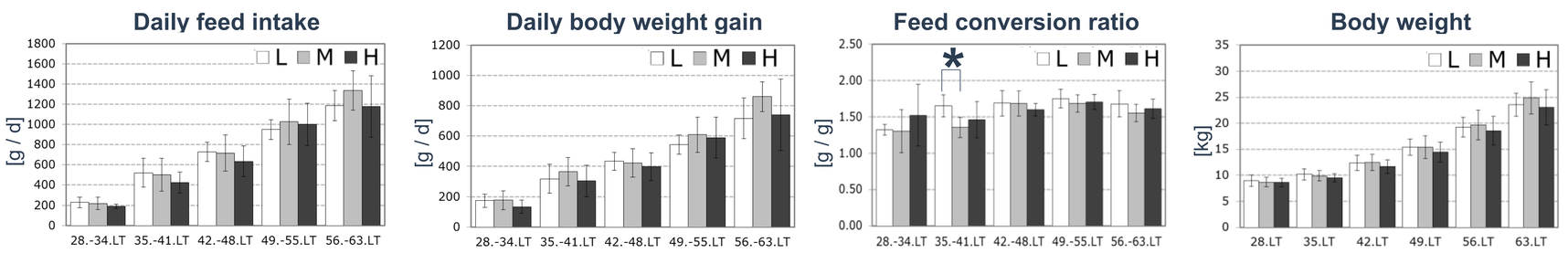
Feeding of weaned piglets (n=19)
Reference (R): varying P supply started on 28 dpn (days post-natum)
- medium (M), low (L) und high (H) P-supply

Analysis of expression profiles
RNA isolation from PBMCs
- Transcriptome profiling and qRT-PCR

Bone characteristics
Analysis of bone mineral density (BMD) and tissue mineral density (TMD)

Feeding & fermentation

Growth & feed conversion



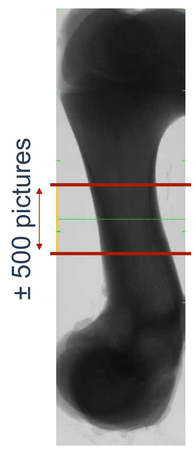
LT is representative for days of lifetime

Diarrhea and fermentation acids in feces

	L	M	H	SEM	*P*
Days of diarrhea [d]					
LT 28-63	13[b]	55[a]	42[a]		*0.050*
Acetate [mmol/L]					
LT 35	87.9[a]	67.0[b]	62.0[b]	*1.18*	*0.021*
LT 49	80.2	79.8	93.7	*1.21*	*0.571*
LT 63	57.6	87.6	82.3	*1.21*	*0.093*
Ammoniac [mmol/L]					
LT 35	64.4[a]	46.2[b]	48.1[b]	*1.17*	*0.045*
LT 49	71.4	61.2	59.1	*1.20*	*0.664*
LT 63	51.0	63.8	60.6	*1.19*	*0.549*

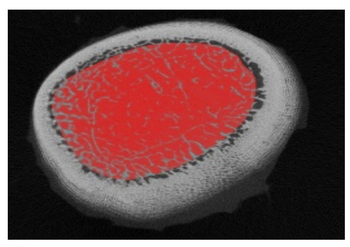
Bone characteristics

Workflow of radiological bone analysis



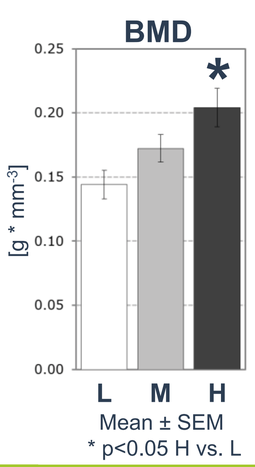
- **µCT-Scan**
  - Digital Raw (19 µm Voxel)
- **NRecon**
  - Reconstruction of cross-sectional slices
  - Limiting dataset (1000 slices)
- **Data-Viewer**
  - Alignment of the bone
  - 2nd dataset reduction
- **CTAnalyzer**
  - Select Region of Interest (ROI)
  - Binarisation of the grey-value image and 3D-analysis
  - Evaluation of BMD and TMD
- **BATMAN**
  - Additional specific 3D-analysis
  - Microstructural results

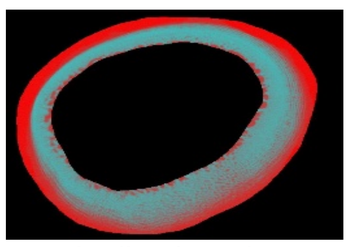
Bone Mineral Density (BMD) and Tissue mineral density (TMD)



ROI (red) selected in the area of trabecular bone

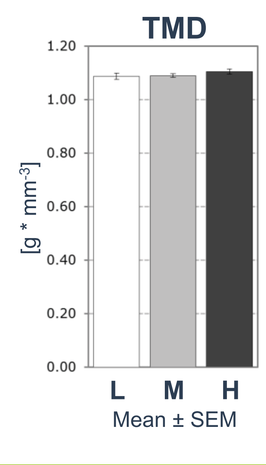
BMD = the combined density of mineralized trabecular bone and tissue



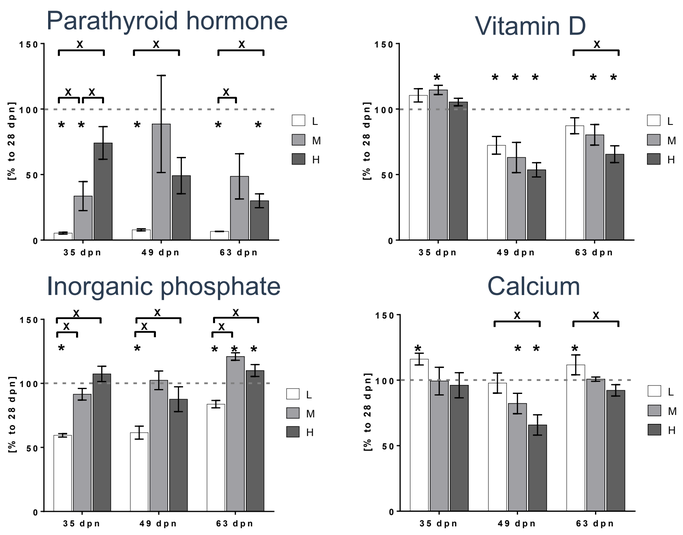


ROI (red) selected in the area of cortical bone

TMD = the density of mineralized cortical bone which is an adequate material property with a significant impact on bony strength



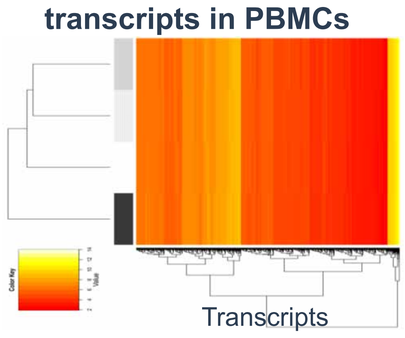
Hormone & serum minerals



* – Difference to R (p ≤ 0.05); X – Difference between P-supply (p ≤ 0.05)

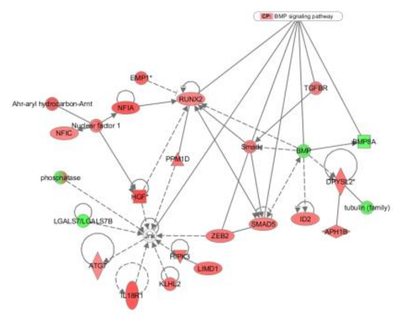
Transcriptome analyses

Hierarchical cluster of transcripts in PBMCs



Microarray experiment:
- Diet-specific gene expression pattern: clustering according to dietary group
- 687 transcripts differentially expressed
- Among these are candidate genes for P-efficiency

Selected gene network



Networks:
Differentially expressed genes belong to the BMP signaling pathway regulating development of osteoblasts & osteocytes and expression of osteocalcin.

qRT-PCR of selected transcripts (H-63 vs. R; p<0.05)

Gene name	Fold change Microarray	Fold change qRT-PCR	Correlation (p ≤ 0.001)	Associated functions
ATP2B1	1.42	1.70	0.64	Intracellular calcium homeostasis
CALCRL	2.32	3.98	0.82	Stimulation of bone reabsorption & calcium excretion in kidney
MMP2	1.38	1.31	0.64	Regulation of vascularization & inflammatory response
OSTM1	1.30	1.33	0.62	Maturation of osteoclasts & melanocytes
SMAD5	1.47	1.64	0.53	Involved in BMP signaling

qRT-PCR validation:
Confirmed changes of transcript abundance of genes related to bone morphology and immune response.

Conclusion

1. Bone characteristics, hormone, and serum mineral levels indicate that the P diets were effective.
2. Transient diet-specific effects on Feed conversion ratio (FCR) and fermentation acids appeared.
3. P sensitive transcripts relevant for P utilization and improved P efficiency were identified.

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