Effects of P and Ca depletion-repletion periods on intestinal and renal expression of genes associated with P, Ca and vitamin D metabolism in pigs

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Introduction

- Optimization of dietary phosphorus (P) and calcium (Ca) can reduce overfeeding and feeding costs and promote sustainable pig production
- Animals receiving P and Ca deficient diets (**depletion**) followed by non-deficient diets (**repletion**) improve digestive and metabolic utilization of both minerals (Létourneau-Montminy et al., 2014)
- However the underlying mechanisms are unknown

Material & Methods (animals and feeding strategies)

Table 1: Depletion and repletion sequences

Phases	1	2	3
Treatments	25-50 kg	50-75 kg	75-110 kg
CCC	C	C	C
CLC	C	L	C
LCC	L	C	C

- 60 castrated F1 males (initial BW 24±3.3 kg)
- 20 animals per treatment
- 3 growing phases of 28 days
- Depleted animals: L, CL (in phases 1 & 2)
  Repleted animals: C, LC, LCC and CCC (in phases 1, 2 & 3)

Table 2: Percentages of total Ca and digestible P of the depleted and repleted diets

Phases	1		2		3	
Chemical Composition	25-50 kg		50-75 kg		75-110 kg	
	C	L	C	L	C	L
Total Ca, %	0.91	0.51	0.67	0.39	0.56	0.33
Digestible P, %	0.30	0.18	0.24	0.14	0.19	0.11

- Control diet (C) → 100% P & Ca requirements (NRC, 2012)
  Low diet (L) → 60% P & Ca requirements.
- Ratio total Ca: Digestible P = 2.9

Objective

- Evaluate the effects of P and Ca depletion-repletion sequences on the relative mRNA abundance of genes related with P, Ca and vitamin D metabolism in mid-jejunum and kidney of growing pigs

Material & Methods (relative mRNA abundance)

- 30 pigs at the end of the 2nd phase (treatments CC, CL and LC) and 30 pigs at the end of the 3rd phase (treatments CCC, CLC and LCC) were slaughtered
- Samples of **mid-jejunum** and **kidney** were collected to study the relative mRNA abundance of genes related with P, Ca and vitamin D metabolism (Table 3). Tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C
- RNA extraction and gene expression measurements by quantitative PCR as previously reported[3]
- Statistical analyses: depletion and repletion results were analysed independently within each growing phase. A mixed procedure of SAS with a Tukey adjustment was used

Table 3: Selected genes for the quantification of the mRNA abundance in mid-jejunum and kidney

	Mid-jejunum	Kidney
P	*SLC20A1* *SLC20A2*	*FGFR1IIIC* *SLC20A2* *SLC34A1* *SLC34A3*
Ca	*ATP2B1* *S100G* *SLC8A1* *TRPV6* *TRPV5*	*S100G* *TRPV5* *CALB1*
Vit D	*CYP24A1* *CYP27B1* *KL*	*CYP24A1* *CYP27B1* *KL*

Results & Discussion

2nd feeding phase: DEPLETION (CC vs. CL)

Mid-jejunum (Figure 1)

- mRNA abundance of Ca related genes (*S100G* and *TRPV6*) were 36% and 42% higher in CL than CC pigs
  - Putative effect of Parathormone (PTH): higher intestinal Ca absorption

Kidney (Figure 2)

- mRNA abundance of Ca related genes (*S100G* and *CALB1*) were 400% and 51% higher in CL than CC pigs
  - Effect of PTH: higher Ca re-absorption in kidney

2nd feeding phase: REPLETION (CC vs. LC)

Mid-jejunum (Figure 3)

- No significant difference in mRNA abundance between treatments

Kidney (Figures 4-5)

- mRNA abundance of P related genes (*SLC20A2* and *SLC34A3*) were 29% and 25% lower in LC than CC pigs.
- mRNA abundance of Ca related genes (*S100G* and *CALB1*) were 8 times and 57% higher in CL than LC pigs.
  - Effect of PTH: higher P excretion and Ca re-absorption in kidney

3rd feeding phase: DEPLETION and REPLETION

There was no significant difference in relative mRNA abundance of studied genes between treatments

Figure 1: Depletion effects on mid-jejunum genes mRNA abundance

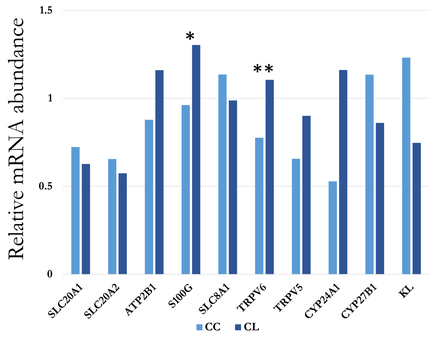


Figure 2 : Depletion effects on kidney genes mRNA abundance

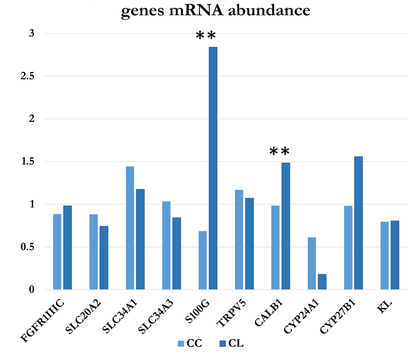


Figure 3: Repletion effects on mid-jejunum genes mRNA abundance

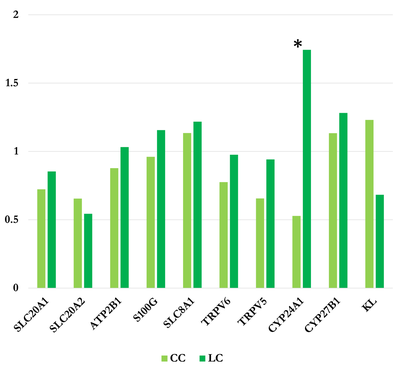


Figure 4: Repletion effects on kidney genes mRNA abundance

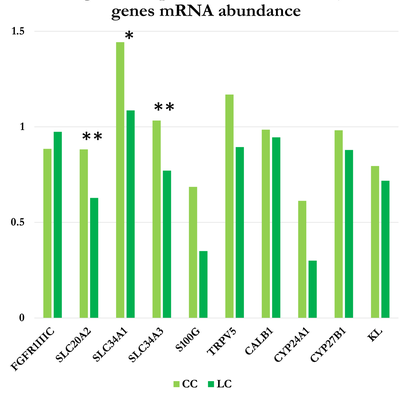
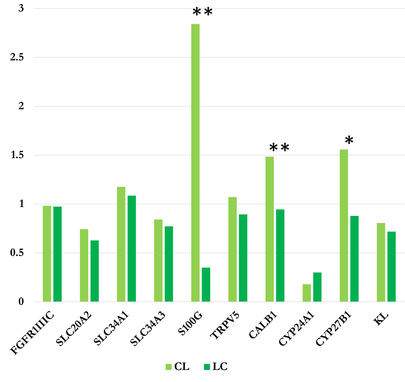


Figure 5: Repletion effects on kidney genes mRNA abundance



Conclusions

- **Depletion P & Ca:** Putative effect of PTH
  - → Increase mRNA abundance of Ca related genes and Ca intestinal absorption
- **Repletion P & Ca:** Putative effect of PTH
  - → mRNA abundance of P related genes: CC > LC pigs
  - → mRNA abundance of Ca related genes: CL > LC pigs
  - → Increase P excretion Ca re-absorption in kidney
- No mRNA abundance differences for studied genes at the end of the study (phase 3)

Acknowledgements

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References

1. Létourneau-Montminy, M.P., P. Lovatto and C. Pomar, 2014. Journal of Animal Sciences, 92:3914–3924
2. NRC, 2012. Nutrient requirements of swine. Washington, USA: National Academy Press
3. Palin, M-P., B. Labrecque, D. Beaudry, M. Mayhue, V. Bordignon and B. Murphy, 2008. Domestic Animal Endocrinology 35, 58–73