Does Low Dietary Phosphate Cause Kyphosis?

Soh Wei Yan*, Dr Andreas Werner (Supervisor), Wilhelmina Lucinescu, Hany Zinad, Prof. Georg Lietz, Adam Clark.
Institute of Cell and Molecular Biosciences, in collaboration with Human Nutrition Research Centre, Newcastle University
MBBS (NUMed Malaysia) • 160743083 • W.Y.Soh2@newcastle.edu.my

Introduction

10% of livestock pigs develop kyphosis, which is a backbone disorder affecting normal growth. It is hypothesised that these kyphosis pigs are deficient of phosphate (Pi), an important mineral in bone structure, due to insoluble precipitates formed with zinc (Zn) supplemented for growth enhancement replacing antibiotics. Evidence showed that 100% of pigs fed without phytase, an enzyme that releases phosphate in organic materials, developed kyphosis.

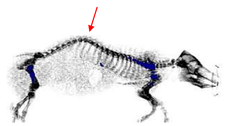

Figure 1: Kyphosis pig.

Aim

The project aims to study the phosphate levels in kyphosis pigs by investigating the expression levels of phosphate and zinc transporters in pig kidney and intestinal tissues. High phosphate or zinc transporter expression indicates possible deficiency of the mineral.

Methodology

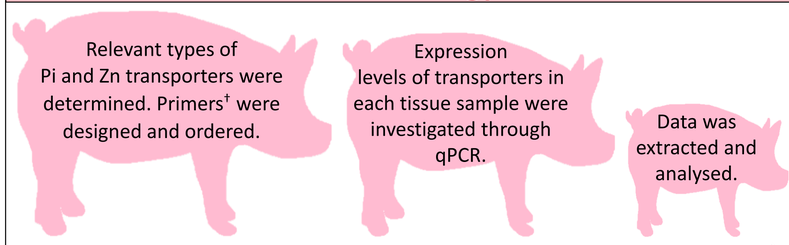

Figure 2: Project overview. qPCR = real-time Polymerase Chain Reaction (procedure used to determine amount of expressed genes). † Primers are short gene sequences, used in PCRs to amplify specific stretches of genes.

- Online databases (NCBI, Ensemble, IDT) were used to identify suitable transporters and their properties, as well as primer design and sequence analysis. Polymerase chain reaction (PCR) was used to test for primer functionability.
- 13-week-old kyphosis piglets and their controls were investigated using kidney (K) and small intestine (SI) tissues. Three samples of each tissue, taken from different pigs, were analysed for the expression levels of Pi and Zn transporters using qPCR. Three replicates of each sample were made. The whole experiment was repeated twice.

Category	Kyphosis		Controls	
Tissue	K	SI	K	SI
Number of pigs	3	3	3	3

Table 1: Samples used.

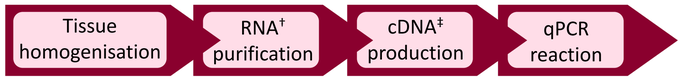

Figure 3: Preparation process for qPCR. Solid tissue was shredded to enable RNA extraction. † RNA is a macromolecule which aids in synthesising proteins. ‡ cDNA is a genetic material synthesised from RNAs, which is used to carry out qPCR.

- Analysis was done using Roche qPCR Software. Graphs were plotted using the difference between raw data and the reference gene ACTB, which is constantly expressed in all tissues.

Results

Gene of Primers		SLC39A4	SLC39A10	SLC30A1	SLC34A1	SLC34A2	SLC34A3	SLC20A2	TBP	ACTB
Transporter		Zinc			Phosphate				Reference Gene	
PCR Product Length (Bp)		148	178	180	111	142	138	169	160	179
Expression Distribution	K	-	+	++	++++	-	+++	+	+	++++
	SI	+++	+	+++	-	++	++	++	++	++++

Table 2: Selected genes for designed primers and their expression distribution in kidney and intestinal tissues under normal conditions. Bp = base pair (measurement of PCR product length for the primers designed). Highlighted: transporters that are highly expressed.

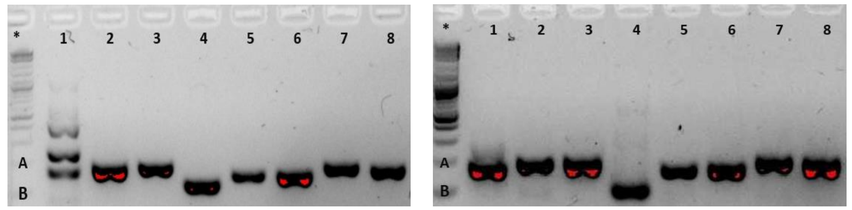

Figure 4: PCR results for primer testing. Left panel: Kidney samples. Right panel: Intestinal samples. Numbering (primers): 1 = SLC39A4; 2 = SLC39A10; 3 = SLC30A1; 4 = SLC34A1; 5 = SLC34A2; 6 = SLC34A3; 7 = SLC20A2; 8 = TBP. * DNA Ladder (standard reference to determine the length of PCR product). A = 200Bp; B = 100Bp.

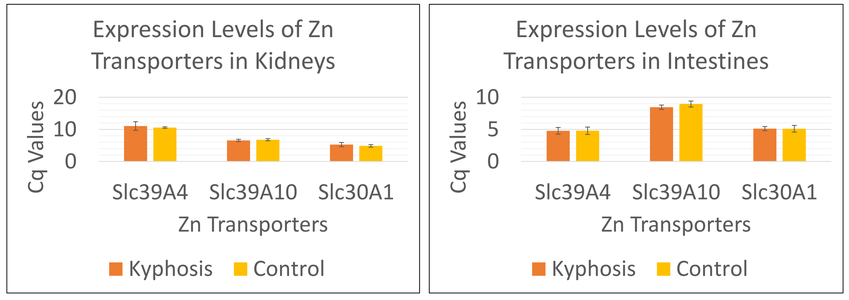

Figure 5: qPCR results for Zn transporters. Left panel: K samples. Right panel: SI samples. Cq = quantitative cycle (used to determine expression levels – low Cq indicates high expression).

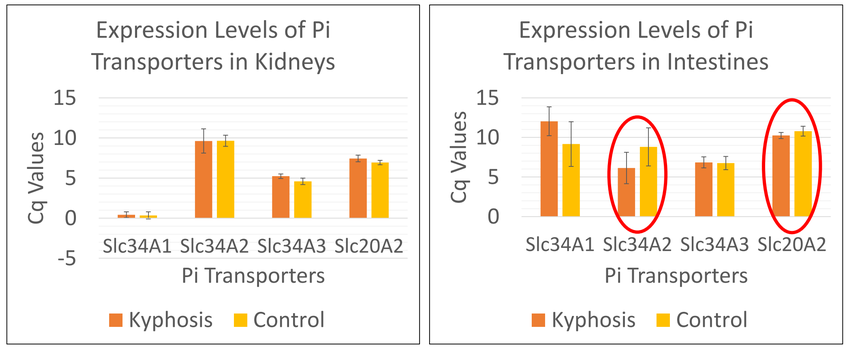

Figure 6: qPCR results for Pi transporters. Left panel: K samples. Right panel: SI samples.

Discussion

- For Zn transporters, SLC39A4 is mainly expressed in intestines while SLC30A1 can be found in both tissues. SLC34A1 and SLC34A3 Pi transporters are highly expressed in kidneys, while intestines express SLC34A2, SLC34A3, and SLC20A2. Reference genes TBP and ACTB act as controls which enables comparison with the genes of interest (Table 2).
- Length of PCR products (Figure 4) are coherent with the expected length as designed (Table 2). Thus the primers are specific to the genes of interest.
- Expression of Zn transporters do not show significant difference between kyphosis pigs and controls in both tissue types (Figure 5). The expression pattern reflects the normal distribution (Table 2). This shows that there is no relationship between kyphosis and Zn transporters.
- Comparing the data for kyphosis pigs with controls (Figure 6), similar expression levels for Pi transporters are seen in the kidneys, while kyphosis pigs showed higher expression of SLC34A2 and SLC20A2 in intestinal samples (marked). The difference seen in intestinal samples supports the hypothesis that kyphosis pigs are deficient in phosphate, hence there is upregulation of Pi transporters. It may be possible that the lack of significant expression difference in kidney samples is due to highly saturated expression levels of Pi transporters in young growing piglets, where phosphate uptake is at its maximum point.
- To minimise errors and possible bias, replicates were used for each sample tested, and the average value was taken. Possible contamination was controlled by physical separation, where each procedure (RNA purification, cDNA preparation, qPCR) were carried out in different laboratories.
- Due to limited time and materials, sample size used was relatively small. All samples used in the study were also obtained from the same batch of farm pigs, thus it may not be able to represent the whole population. Future studies may replicate the study using larger sample sizes from multiple sources.

Conclusion

There is a relationship between low phosphate levels and kyphosis in livestock pigs, due to possible interaction between phosphate and zinc in the diet. Pig farmers should supplement adequate amounts of phosphate to avoid loss due to kyphosis. Nevertheless, over-supplementation of phosphate is unpractical as phosphate is costly and excess phosphate in the feed will be excreted into the environment causing pollution, hence demanding a delicate balance in supplementing phosphate.

References

1. Pokharel BB. Effect of Phosphorus on Growth Performance, Skeletal Integrity and Phosphorus Utilization in Growing Pigs [master's thesis]. Winnipeg: University of Manitoba; 2014.
2. Amundson LA, Hernandez LL, Laporta J, Crenshaw TD. Maternal Dietary Vitamin D Carry-over Alters Offspring Growth, Skeletal Mineralisation and Tissue mRNA Expressions of Genes Related to Vitamin D, Calcium and Phosphorus Homoeostasis in Swine. British Journal of Nutrition. 2016;116(5):774–787.
3. Crenshaw T. Nutritionally Induced Cellular Signals that Affect Skeletal Integrity in Swine. IPVS 2014- XXIII International Pig Veterinary Society Congress. 2014 [Online] https://en.engormix.com/pig-industry/articles/nutritionally-induced-cellular-signals-t36231.htm.

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