Chapter 14
Calcium transporters and gene expression and absorption of calcium in pigs

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Abstract

Calcium (Ca) absorption occurs by two mechanisms, nonsaturable paracellular absorption and saturable transcellular absorption. The activity of these mechanisms depends on the concentration of Ca in the diet. At high dietary Ca concentration, the nonsaturable mechanism is more active, whereas at low dietary Ca concentration, the saturable mechanism is more involved. The saturable mechanism is vitamin-D dependent, because the expression of genes related to the transcellular transport may be influenced by the active form of vitamin D. Increasing dietary Ca decreases the mRNA expression of transient receptor potential cation channel, subfamily V, member 6 (TRPV6), which is used for absorption of Ca into the enterocytes in the jejunum. However, this does not result in less absorption of Ca, because as dietary Ca increases, an increased proportion of Ca is absorbed via the paracellular route in the small intestine and the total percentage of Ca that is absorbed is almost constant regardless of dietary Ca concentration. High concentrations of dietary Ca will also reduce the mRNA expression in the kidneys of TRPV6, TRPV5, S100 calcium binding protein G, and calbindin 1, but because there is no paracellular reabsorption of Ca in the kidneys excretion of Ca in the urine is increased as dietary Ca concentration increases. As a consequence, body Ca concentrations are primarily regulated in the kidneys.

Keywords: calbindin, calcium channels, paracellular absorption, transcellular absorption, vitamin D receptor

14.1 Introduction

Calcium (Ca) is important for bone health, muscle contraction, transmission of nerve pulses, and other important physiological functions (Crenshaw, 2001; Ewing and Charlton, 2007; Vitti *et al.*, 2010). Calcium is mainly absorbed in the small intestine by two mechanisms: paracellular absorption and transcellular absorption (Bronner, 1987). Paracellular absorption of Ca is nonsaturable and does not require energy, whereas transcellular absorption of Ca is a saturable process that not only requires energy, but also needs Ca channels and Ca transporters (Christakos, 2012).

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Vitamin D may influence the expression of genes involved in transcellular transport of Ca, which is mainly active if dietary Ca is relatively low (Kutuzova and DeLuca, 2004). Extensive research has been conducted in broiler chickens, rats, and mice to evaluate the effect of dietary Ca concentrations on expression of genes involved in transcellular transport of Ca in the small intestine and kidneys (Armbrecht *et al.*, 1980, 2003; Healy *et al.*, 2005; Hurwitz *et al.*, 1995; Ko *et al.*, 2009; Rosenberg *et al.*, 1986; Van de Graaf *et al.*, 2004). However, for pigs, only limited data have been reported.

14.2 Calcium absorption

Calcium absorption in pigs is similar to that in other species with the active energy-requiring transcellular route being the primary form of absorption at low concentrations of dietary Ca, whereas the paracellular route is used more extensively at greater dietary concentrations of Ca (Bronner, 2003). When combined, the two routes of absorption result in a near constant rate of absorption of Ca from the intestines regardless of the concentration of Ca in the diet (Stein *et al.*, 2011). However, increased dietary Ca has a negative impact on the digestibility and absorption of phosphorus (Stein *et al.*, 2011), which may be a result of formation of Ca-phosphorus complexes or possibly Ca-phosphorus-phytate complexes in the intestinal tract.

In the kidneys, Ca may also be absorbed into the bloodstream from the distal tubule, but in this case, only active transport is used, which means that Ca is absorbed only if there is a low concentration of plasma Ca. As a consequence, if plasma concentrations of Ca are adequate, less Ca will be absorbed from the kidney tubules, and more will be excreted in the urine (Blaine *et al.*, 2015).

14.2.1 Active absorption of calcium

Active absorption of Ca is also referred to as transcellular absorption because Ca is absorbed via active absorption passes through the cell. Active transport of Ca occurs primarily in the proximal small intestine (duodenum and proximal jejunum) and is a saturable process requiring energy and a Ca-binding protein for transport (Bouillon *et al.*, 2003; Gropper *et al.*, 2009). The low pH in the stomach causes dietary Ca to solubilize and Ca enters the lumen of the small intestine in the form of Ca salts. The free Ca enters the enterocyte by Ca channels located in the brush border membrane (Bronner, 2003; Gonzalez-Vega *et al.*, in press). The Ca channels are referred to as transient reception potential vanilloids (TRPV). In the small intestine TRPV6 is present, whereas TRPV5 is located in the kidneys (Christakos, 2012). Upon absorption into the enterocytes, Ca binds to the Ca-binding proteins (CaBP) which are upregulated or down regulated by Vitamin D. The Ca-binding proteins, also known as calbindin, move Ca across the enterocyte (Gropper *et al.*, 2009). The CaBP9k is mainly found in the intestines and CaBP28k in kidneys (Bouillon *et al.*, 2003). In mice, it has been demonstrated that Ca exits the cell on the basolateral membrane by active transport using a Ca-sodium (Na) pump or an ATPase (Bronner, 2003), an enzyme that releases ATP and allows Ca to exit from the cell (Gropper

et al., 2009), and it is believed that a similar mechanism is present in most other species (Bouillon *et al.*, 2003).

14.2.2 Passive absorption of calcium

Passive absorption is also referred to as passive diffusion or paracellular absorption. This process occurs primarily in the jejunum and ileum in the small intestine and is dependent on the concentration of Ca in the intestinal lumen and the electrochemical gradient across the epithelium (Gropper *et al.*, 2009). It is imperative that this pathway is regulated to allow selective permeability. Using the passive transport pathway, Ca is passively moved from the lumen of the small intestine through the tight junctions between the enterocytes (Gropper *et al.*, 2009). Passive absorption is increased with increased solubility of Ca in the distal small intestine and increased by the amount of time the chyme resides in these segments of the intestine (Gropper *et al.*, 2009).

14.3 Site of calcium absorption

Although Ca source and type of diet may influence the place of Ca absorption (González-Vega *et al.*, 2014; Partridge, 1978), Ca absorption mainly takes place in the small intestine (Liu *et al.*, 2000; Partridge, 1978), mostly in the duodenum at low Ca intake using active absorption, but at greater Ca intake, absorption occurs primarily via the paracellular route in the jejunum and ileum. There are however, differences among Ca sources in the exact place in the small intestine where Ca is absorbed, presumably due to differences in solubility, and for some sources of Ca, the main site of absorption appears to be the jejunum or ileum (Gonzalez-Vega *et al.*, 2014). Results of some experiments also indicated that absorption of Ca may take place in the colon (Liu *et al.*, 2000). However, results of recent studies have indicated that no absorption of Ca takes place in the large intestine (Bohlke *et al.*, 2005; González-Vega *et al.*, 2014).

14.4 Endocrine regulation of calcium homeostasis

The three primary hormones that are responsible for maintaining Ca homeostasis are parathyroid hormone (PTH), vitamin D, and calcitonin (Crenshaw, 2001; Eklou-Kalonji *et al.*, 1999; Gropper *et al.*, 2009). The PTH is produced and secreted by the chief cells in the parathyroid gland and secretion of PTH results in an increase in serum Ca levels, but a reduction in phosphorus levels. The primary regulation of PTH is a conformational change in the Ca sensing receptor, which indirectly inhibits PTH secretion from the parathyroid gland (Gropper *et al.*, 2009). Vitamin D in the active form is referred to as calcitriol. The overall effect of calcitriol is to increase levels of Ca in plasma. Calcitriol is synthesized in the kidneys from 25-OH vitamin D using the enzyme 1-hydroxylase and this process is stimulated by PTH. Calcitriol causes reabsorption of Ca in the kidneys using CaBP28k, and in the intestines calcitriol increases CaBP9k synthesis. Both CaBP28k and CaBP9k are used to transport Ca across the epithelial cells from the luminal site to the basolateral site and these proteins, therefore, are needed for the active absorption process of Ca.

Calcitonin is synthesized by the parafollicular cells in the thyroid gland and has the primary function of reducing serum Ca concentrations. This is primarily accomplished by inhibiting the action of osteoclasts in the bone and increasing Ca excretion in the urine. Calcitonin also inhibits bone resorption by osteoblasts (Gropper *et al.*, 2009). The primary function of calcitonin is to maintain the skeleton when Ca demands increase such as in growth and lactation (Wimalawansa, 1996).

14.5 Gene expression of calcium transporters

The release of 1-hydroxylase in the kidneys at low plasma Ca concentrations results in synthesis of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$), which is the active form of vitamin D (Crenshaw *et al.*, 2011; Hurwitz, 1996). Thus, the energy-requiring transcellular absorption of Ca is vitamin D-dependent (Healy *et al.*, 2005) and 1,25-$(OH)_2D_3$ increases the expression of CaBP9k and TRPV6 (Christakos, 2012). Expression of the Ca-ATPase that is needed to transport Ca out of the enterocyte via the basolateral membrane is also up-regulated by 1,25-$(OH)_2D_3$ (Kutuzova and DeLuca, 2004). Combined, the action of 1,25-$(OH)_2D_3$ results in increased transport of Ca from the small intestinal lumen into the enterocyte, transport inside the enterocyte to the basolateral membrane, and transport out of the enterocyte over the basolateral membrane. In addition, 1,25-$(OH)_2D_3$ increases expression of TRPV5 and CaBP28k in the kidneys which results in increased reabsorption of Ca from the distal kidney tubules to plasma (Bouillon *et al.*, 2003).

In broiler chickens, rats, and mice, the expression of TRPV6, TRPV5, the intestinal binding protein S100G, and/or the ATPase PMCA1 was increased in the small intestine and/or in the kidneys at low dietary Ca concentrations (Armbrecht *et al.*, 1980, 2003; Hurwitz *et al.*, 1995; Ko *et al.*, 2009; Rosenberg *et al.*, 1986; Van de Graaf *et al.*, 2004). In laying hens, expression of intestinal calbindin and vitamin D receptor (VDR) increased at low concentrations of non-phytate phosphorus, whereas at high concentration of non-phytate phosphorus, the mRNA expression of calbindin and VDR decreased (Nie *et al.*, 2013).

However, limited studies with pigs have been conducted to evaluate the effect of increasing concentrations of Ca on the gene expression of transporters of nutrients. In the jejunum, the concentration of Ca influenced the expression of some genes involved in nutrient absorption such as zonula occludens 1, occludin, toll-like receptor 2, and colonic GLUT2 (Metzler-Zebeli *et al.*, 2015). An experiment was also conducted to determine the effect of dietary Ca on the expression of genes involved in the transcellular transport of Ca in the jejunum and kidneys in pigs (González-Vega *et al.*, in press). Six diets were formulated to contain 0.36% standardized total tract digestible (STTD) phosphorus and 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca, by including increasing quantities of Ca carbonate in the diets at the expense of cornstarch. The three diets with the least concentrations of Ca contained less Ca than the requirement according to NRC (2012) and the three diets with the greatest concentrations of Ca met or exceeded the Ca requirement (NRC, 2012).

Two internal control genes, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and hydroxymethylbilane synthase (HMBS) were used to normalize the expression of tested

genes (Vigors *et al.*, 2014). The selected tested genes involved in the active transcellular transport of Ca were S100 calcium binding protein G (S100G), TRPV6, PMCA1, and VDR in jejunum samples; and S100G, calbindin 1, 28kDa (CALB1), TRPV6, TRPV5, ATP2B1, and VDR in kidney samples.

Results from this experiment indicated that the mRNA expression of TRPV6 and S100G in the jejunum (Figure 14.1) and the mRNA expression of TRPV5, TRPV6, S100G, and CALB1 in the kidneys (Figure 14.2) were down-regulated at high concentrations of dietary Ca. These results indicated that dietary Ca concentrations regulated the mRNA expression of Ca binding proteins and Ca channel proteins in the jejunum and the kidneys. These results are in close agreement with data obtained in mice, rats, and broiler chickens (Armbrecht *et al.*, 1980, 2003; Hurwitz *et al.*, 1995; Ko *et al.*, 2009; Rosenberg *et al.*, 1986; Van de Graaf *et al.*, 2004). In mice, high concentrations of dietary Ca increased mRNA expression of VDR in the kidney, but not in the duodenum (Healy *et al.*, 2005), which was in agreement with results observed by González-Vega *et al.* (in press). Thus, results of this experiment indicate that at high dietary concentrations of Ca, mRNA expression of Ca transporters involved in the transcellular route were down-regulated in the jejunum, however, most Ca may have been absorbed using the paracellular route. As a consequence, Ca that was not needed for bone tissue synthesis or was not retained in the body was subsequently excreted in the urine. Therefore, vitamin D-dependent transport may play a role in increasing Ca absorption from the small intestine and in Ca reabsorption in the

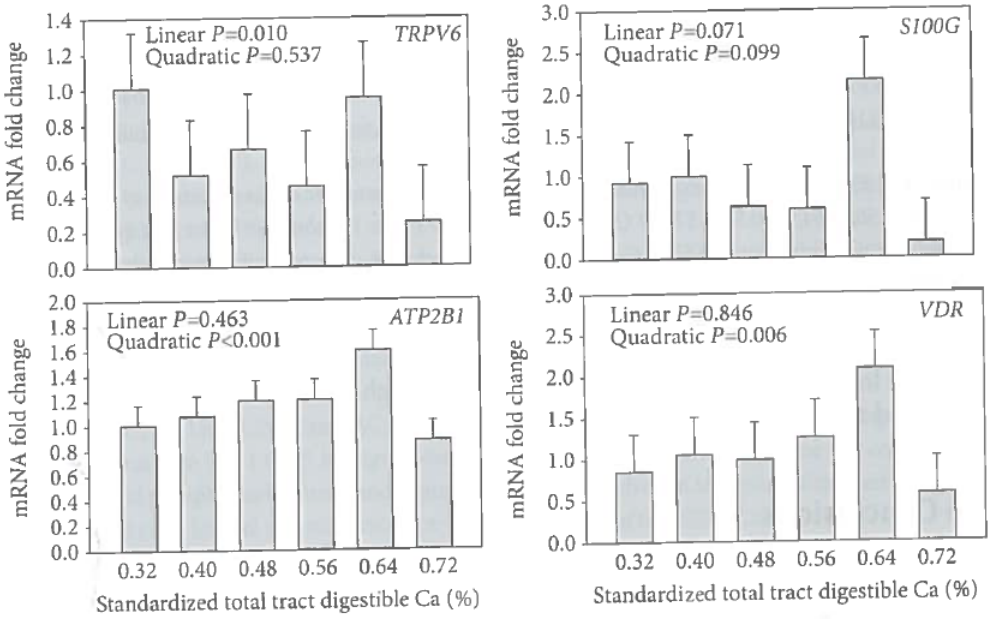

Figure 14.1. *Expression of genes related to transcellular transportation of Ca in jejunum of pigs fed 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca and 0.36% STTD P. The* P*-values for linear and quadratic effect of increasing concentration of STTD Ca, and means for each diet and standard errors (vertical bars) are indicated.*

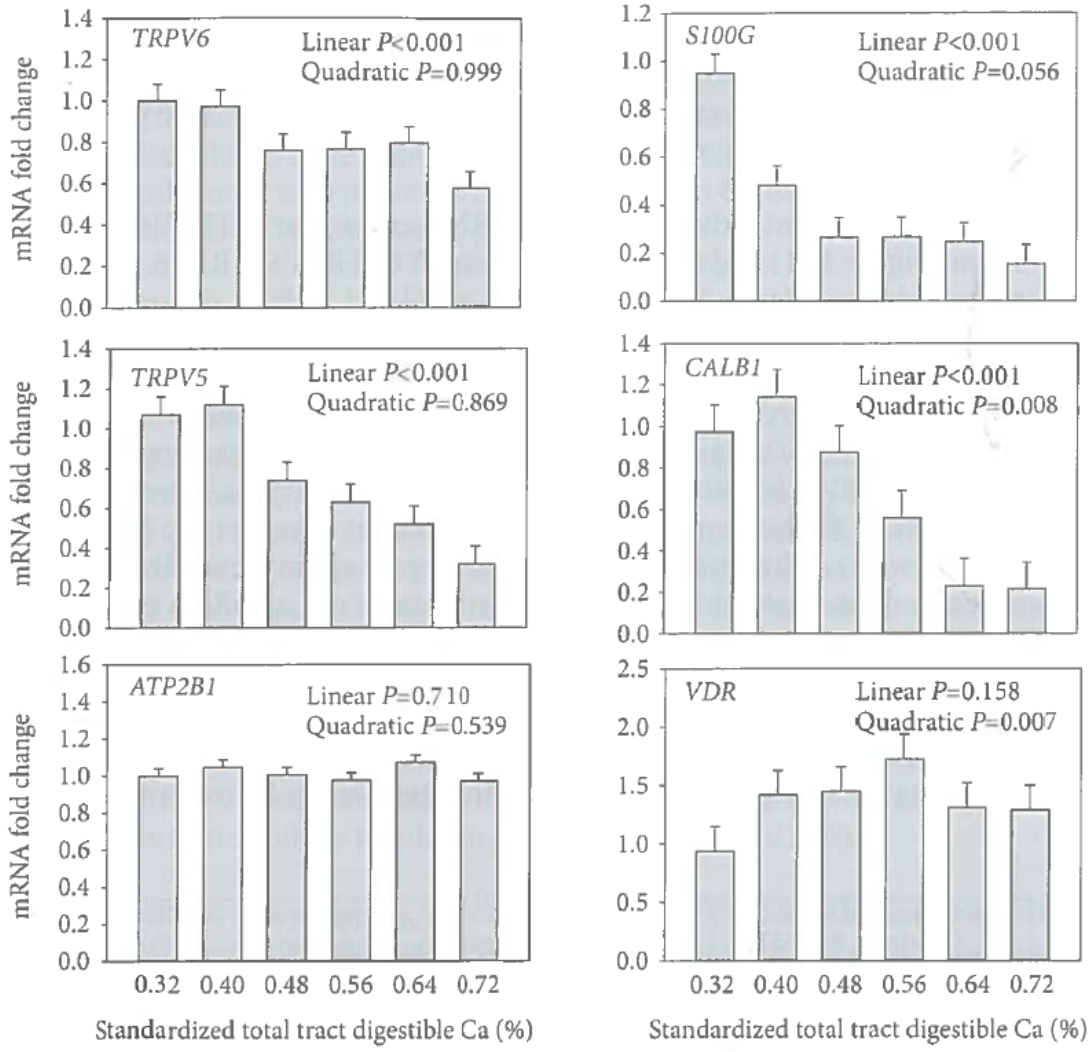

Figure 14.2. *Expression of genes related to transcellular transportation of Ca in kidney of pigs fed 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca and 0.36% STTD P. The P-values for linear and quadratic effect of increasing concentration of STTD Ca and means for each diet and standard errors (vertical bars) are indicated.*

kidneys at low concentrations of dietary Ca, but at high Ca concentrations, homeostasis is regulated mainly at the renal level.

14.6 Conclusions

1. Calcium is absorbed by paracellular and transcellular routes.
2. Expression of genes related to Ca transcellular transport in the jejunum and kidneys of pigs decrease if concentrations of dietary Ca increase. However, although absorption of Ca by the transcellular route decreases, more Ca is absorbed via the paracellular route if dietary Ca concentration increases, and there is, therefore, very limited regulation of Ca absorption from the intestinal tract.

3. However, expression of genes for proteins involved in reabsorption of Ca in the kidneys is upregulated if dietary Ca is low with a subsequent reduction in urinary excretion of Ca. In contrast, if dietary Ca concentrations are above the requirement, expression of genes for kidney proteins involved in reabsorption of Ca are downregulated with a subsequent increase in urinary excretion of Ca.
4. Thus the kidneys are the main regulators of body Ca concentrations in pigs.

References

Armbrecht, H.J., Boltz, M.A. and Bruns, M.E.H., 2003. Effect of age and dietary calcium on intestinal calbindin D-9k expression in the rat. Archives of Biochemistry and Biophysics 420:194-200.

Armbrecht, H.J., Zenser, T.V., Gross, C.J. and Davis, B.B., 1980. Adaptation to dietary calcium and phosphorus restriction changes with age in the rat. American Journal of Physiology 239:E322-327.

Blaine, J., Chonchol, M. and Levi, M., 2015. Renal control of calcium, phosphorus, and magnesium homeostasis. Clinical Journal of American Society of Nephrology 10:1257-1252.

Bohlke, R.A., Thaler, R.C. and Stein, H. H., 2005. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. Journal of Animal Science 83:2396-2403.

Bouillon, R., Van Cromphaut, S. and Carmeliet, G., 2003. Intestinal calcium absorption: Molecular vitamin D mediated mechanisms. Journal of Cellular Biochemistry 88:332-339.

Bronner, F. 2003. Mechanisms of intestinal calcium absorption. Journal of Cell Biochemistry 88:387-393.

Bronner, F., 1987. Intestinal calcium absorption: Mechanisms and implications. Journal of Nutrition 117:1347-1352.

Christakos, S., 2012. Recent advances in our understanding of 1,25-dihydroxyvitamin D3 regulation of intestinal calcium absorption. Archives of Biochemistry and Biophysics 523:73-76.

Crenshaw, T.D., 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In: Lewis, A.J. and Southern, L.L. (eds.) Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL, USA, pp. 187-212.

Crenshaw, T.D., Rortvedt, L.A. and Hassen, Z., 2011. Triennial growth symposium: a novel pathway for vitamin D-mediated phosphate homeostasis: implications for skeleton growth and mineralization. Journal of Animal Science 89:1957-1964.

Eklou-Kalonji, E., Zerath, E., Colin, C., Lacroix, C., Holy, X., Denis, I. and Pointllart, A., 1999. Calcium-regulating hormones, bone mineral content, breaking load and trabecular remodeling are altered in growing pigs fed calcium-deficient diets. Journal of Nutrition 129:188-193.

Ewing W. and Charlton, S.J., 2007. The minerals directory. 2nd ed. Context Products Ltd; Leicestershire, United Kingdom: Calcium; pp. 5a-5f, 19a.

González-Vega, J.C., Liu, Y., McCann, J.C., Walk, C.L., Loor, J.J. and Stein, H.H., in press. Requirement for digestible calcium by 11 to 25 kg pigs as determined by growth performance, bone ash concentration, calcium and phosphorus balances, and expression of genes involved in transport of calcium in intestinal and kidney cells. Journal of Animal Science. DOI: http://doi.org/10.2527/jas.2016-0444.

González-Vega, J.C., Walk, C.L., Liu, Y. and Stein, H.H., 2014. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level and Ca source on Ca digestibility. Archives of Animal Nutrition 68:126-142.

Gropper, S.S., Smith, J.L. and Groff, J.L., 2009. Macrominerals. In: Advanced nutrition and human metabolism. 5th ed. Wadsworth, Cengage Learning, Belmont, CA, USA, pp 429-457.

Healy, K.D., Frahm, M.A. and DeLuca, H.F., 2005. 1,25-Dihydroxyvitamin D3 up-regulates the renal vitamin D receptor through indirect gene activation and receptor stabilization. Archives of Biochemistry and Biophysics 433:466-473.

Hurwitz, S., 1996. Homeostatic control of plasma calcium concentration. Critical Reviews in Biochemistry and Molecular Biology 31:41-100.

Hurwitz, S., Plavnik, I., Shapiro, A., Wax, E., Talpaz, H. and Bar, A., 1995. Calcium metabolism and requirements of chickens are affected by growth. Journal of Nutrition 125:2679-2686.

Ko, S., Lee, G., Vo, T.T.B., Jung, E., Choi, K., Cheung, K., Kim, J.W., Park, J., Oh, G.T. and Jeung, E., 2009. Dietary Ca and 1,25-dihydroxyvitamin D3 regulate transcription of calcium transporter genes in calbindin-D9k knockout mice. Journal of Reproduction and Development 55:137-142.

Kutuzova, G.D. and DeLuca, H.F., 2004. Gene expression profiles in rat intestine identify pathways for 1,25-dihydroxyvitamin D3 stimulated calcium absorption and clarify its immunomodulatory properties. Archives of Biochemistry and Biophysics 432:152-166.

Liu, J., Bollinger, D.W., Ledoux, D.R. and Veum, T.L., 2000. Effects of dietary calcium:phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, cecum, and colon of pigs. Journal of Animal Science 78:106-109.

Metzler-Zebeli, B.U., Mann, E., Ertl, R., Schmitz-Esser, S., Wagner, M., Klein, D., Ritzmann, M. and Zebeli, Q., 2015. Dietary calcium concentration and cereals differentially affect mineral balance and tight junction proteins expression in jejunum of weaned pigs. British Journal of Nutrition 113:1019-1031.

Nie, W., Yang, Y., Yuan, J., Wang, Z. and Guo, Y., 2013. Effect of dietary nonphytate phosphorus on laying performance and small intestinal epithelial phosphate transporter expression in Dwarf pink-shell laying hens. Journal of Animal Science and Biotechnology 4:34.

NRC, 2012. Nutrient requirements of swine. 11th rev. ed. National Academic Press, Washington, DC, USA.

Partridge, I.G., 1978. Studies on digestion and absorption in the intestines of growing pigs: 3. Net movements of mineral nutrients in the digestive tract. British Journal of Nutrition 39:527-537.

Rosenberg, J., Hurwitz, S. and Bar. A., 1986. Regulation of kidney calcium-binding protein in the bird (*Gallus Domesticus*). Comparative Biochemistry and Physiology 83A:277-281.

Stein, H.H., Adeola, O., Cromwell, G.L., Kim, S.W., Mahan, D.C. and Miller, P.S., 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but reduces digestibility of phosphorus by growing pigs. Journal of Animal Science 89:2139-2144.

Van de Graaf, S.F.J., Boullart, I., Hoenderop, J.G.J. and Bindels, R.J.M., 2004. Regulation of the epithelial Ca2+ channels TRPV5 and TRPV6 by 1α,25-dihydroxy vitamin D3 and dietary Ca2+. Journal of Steroid Biochemistry 89-90:303-308.

Vigors, S., Sweeney, T., O'Shea, C.J., Browne, J.A. and O'Doherty, J.V., 2014. Improvements in growth performance, bone mineral status and nutrient digestibility in pigs following the dietary inclusion of phytase are accompanied by modifications in intestinal nutrient transporter gene expression. British Journal of Nutrition 112:688-697.

Vitti, D.M.S.S., Da Silva Filho, J.C., Louvandini, H., Dias, R.S., Bueno I.C.S. and Kebreab, E., 2010. Phosphorus and calcium utilization in ruminants using isotope dilution technique. In: Vitti, D.M.S.S. and Kebreab, E. (eds.) Phosphorus and calcium utilization and requirements in farm animals. CAB International, London, UK. p. 45-67.

Wimalawansa, S.J., 1996. Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology, and therapeutic potentials. Endocrine Reviews 17:533-585.