

Effects of Dietary Cation-Anion Difference Levels on Serum Calcium Concentration and Relative Expression of Calcium-Binding Protein 9 kDa mRNA in Gastrointestinal Tissues of Mice: Postprint

Authors: Wenxuan Wu, Shen Hailiang, Jiahai Wu, Yang Yi, Zhu Lunqin

Date: 2017-10-10T00:00:00+00:00

Abstract

Based on the previously established detection system for gene expression levels related to calcium metabolism in the animal gastrointestinal tract and expression profile analysis, this study aimed to further determine the effects of dietary cation-anion difference (DCAD) levels on blood calcium concentration and the relative mRNA expression level of calcium-binding protein 9ku (CaBP-D9k) in gastrointestinal tissues, so as to provide a basis for revealing the mechanism by which low DCAD levels prevent and treat hypocalcemia in animals. One hundred and twenty Kunming mice were randomly divided into three groups (n=40 per group) and fed diets with DCAD levels of +300 (high DCAD level group, HD group), +150 (control group, CON group), and -50 (low DCAD level group, LD group) starting from 3 days before mating. Blood calcium concentration and relative mRNA expression level of CaBP-D9k in gastrointestinal tissues were measured in dams at 20 days prepartum (-20 d), 5 days prepartum (-5 d), the day of parturition (0 d), and 3 days postpartum (+3 d). The results showed that, compared with the HD group, the LD group significantly increased blood calcium concentration in mice at 0 d and +3 d during the peripartum period ($P<0.05$), and significantly upregulated the relative mRNA expression level of CaBP-D9k in small intestinal segments (duodenum, jejunum, ileum) and colon ($P<0.05$). This upregulatory effect was most pronounced at -5 d and 0 d ($P<0.05$), and at +3 d, the LD group also increased the relative mRNA expression level of CaBP-D9k in the jejunum and colon of mice ($P<0.05$). Statistical results showed that the relative mRNA expression levels of CaBP-D9k at the three sites of duodenum, jejunum, and colon were significantly correlated with DCAD levels and blood calcium concentration ($P<0.05$). Thus, reducing DCAD levels can upregulate the relative mRNA expression level of CaBP-D9k in

small intestinal and colonic segments of animals, accompanied by higher blood calcium concentration. This may be an important pathway through which low DCAD levels effectively maintain blood calcium homeostasis during the peripartum period and reduce the incidence of hypocalcemia.

Full Text

Effects of Dietary Cation-Anion Difference Level on Plasma Calcium Concentration and Gastrointestinal Tissue Calcium Binding Protein 9ku mRNA Relative Expression Level in Mice

WU Wenxuan^{1,2}, XIN Hailiang³, WU Jiahai *, YANG Yi¹, ZHU Lunqin^{1}

¹College of Animal Science, Guizhou University, Guiyang 550025, China

²Institute of New Rural Development, China Center for Western Development Capacity Research, Guizhou University, Guiyang 550025, China

³Qiandongnan National Polytechnic, Kaili 556000, China

Guizhou Institute of Prataculture, Guiyang 550006, China

Abstract

Building upon our previous establishment of a detection system for calcium metabolism-related gene expression profiles in the gastrointestinal tract, this study aimed to further determine the effects of dietary cation-anion difference (DCAD) level on plasma calcium concentration and gastrointestinal tissue calcium binding protein 9ku (CaBP-D9k) mRNA relative expression levels, thereby providing mechanistic insights into how low DCAD levels prevent hypocalcemia. One hundred twenty Kunming mice were randomly divided into three groups (n=40 each) and fed diets with DCAD levels of +300 (high DCAD, HD group), +150 (control, CON group), and -50 (low DCAD, LD group) beginning three days before mating. Plasma calcium concentration and gastrointestinal CaBP-D9k mRNA relative expression levels were measured at 20 days prepartum (-20 d), 5 days prepartum (-5 d), the day of parturition (0 d), and 3 days postpartum (+3 d).

The results demonstrated that compared with the HD group, the LD group significantly elevated plasma calcium concentrations at 0 d and +3 d during the peripartum period ($P<0.05$) and significantly upregulated CaBP-D9k mRNA relative expression in small intestinal segments (duodenum, jejunum, ileum) and colon ($P<0.05$). This effect was most pronounced at -5 d and 0 d ($P<0.05$), with elevated expression persisting in jejunum and colon at +3 d ($P<0.05$). Statistical analysis revealed significant associations between CaBP-D9k mRNA relative expression levels in the duodenum, jejunum, and colon with both DCAD level and plasma calcium concentration ($P<0.05$). These findings indicate that reducing DCAD level upregulates CaBP-D9k mRNA expression in the small intestine and colon while concurrently increasing plasma calcium concentration,

representing a likely mechanism through which low DCAD levels maintain peripartum calcium homeostasis and reduce hypocalcemia incidence.

Keywords: dietary cation-anion difference; plasma calcium concentration; calcium-binding protein; gastrointestinal tract tissues

Introduction

Prepartum low dietary cation-anion difference (DCAD) levels effectively maintain peripartum calcium homeostasis in mammals, accelerate postpartum calcium recovery, and reduce the incidence of clinical hypocalcemia and subsequent metabolic disorders, establishing it as an effective preventive measure against hypocalcemia. Research suggests that low DCAD promotes bone calcium mobilization into circulation, decreases urinary calcium excretion, and enhances gastrointestinal calcium absorption, thereby facilitating systemic calcium homeostasis and maintaining normal plasma calcium levels to prevent hypocalcemia. However, the detailed mechanisms remain unclear and represent an important research topic in animal nutrition with significant practical implications for improving animal performance.

Among the three major pathways of calcium homeostasis, renal resorption responds most rapidly to calcium deficiency, occurring within minutes, but its contribution to peripheral calcium replenishment is limited due to low urinary calcium content and finite reabsorptive capacity. Bone resorption responds most slowly, often requiring over one week and occurring only after inadequate dietary calcium absorption, making it ineffective for short-term calcium homeostasis. In contrast, dietary calcium absorbed by gastrointestinal epithelial cells enters circulation via capillaries, with observable responses to blood calcium balance within short timeframes, playing a crucial role in maintaining calcium homeostasis. Under normal physiological conditions, gastrointestinal absorption represents the most probable pathway for rapid supplementation of peripheral calcium and prompt restoration of calcium balance.

As a vitamin D-dependent calcium binding protein belonging to the EF-hand calcium binding protein family, CaBP-D9k serves as a critical component mediating intracellular calcium transport to the basolateral membrane and regulates kinetic parameters of calcium metabolism in mammalian bone, kidney, and gastrointestinal tract. CaBP-D9k is specifically expressed in calcium transport-related tissues including the gastrointestinal tract, kidney, and bone. Studies have demonstrated strong positive correlations between intestinal CaBP-D9k expression levels and site-specific calcium uptake capacity. Reduced intestinal calcium absorption in elderly individuals correlates highly with decreased CaBP-D9k expression, and the expression sites and relative levels of CaBP-D9k show close positive correlations with calcium absorptive capacity in respective organs/tissues. These findings provide valuable insights into the mechanisms underlying low DCAD prevention of hypocalcemia. However, research on the

relationship between different DCAD levels and gastrointestinal CaBP-D9k expression remains scarce, hindering deeper understanding of peripartum calcium nutrition interventions and their kinetic processes. Therefore, building upon our established detection system for gastrointestinal calcium metabolism-related gene expression, this study utilized mice as a model to investigate the effects of low DCAD on gastrointestinal CaBP-D9k mRNA expression, aiming to elucidate the molecular mechanisms through which low DCAD prevents hypocalcemia.

Materials and Methods

1.1 Reagents

Plasma calcium assay kits were obtained from Nanjing Jiancheng Bioengineering Institute. RNA storage solution (RNAstore) and total RNA extraction kits (RNAsimple) were purchased from Tiangen Biotech (Beijing). Reverse transcription kits (RT reagent Kit with gDNA eraser), conventional PCR kits (TaKaRa PCR amplification Kit), and real-time quantitative PCR kits (SYBR® Premix Ex Taq™ II) were products of Dalian Bao Biological Engineering Co., Ltd. All other reagents were analytically pure domestic products.

1.2 Animal Management and Grouping

One hundred twenty clean-grade healthy female Kunming mice aged 10 weeks were obtained from the Laboratory Animal Center of Guiyang Medical College. Using a randomized block design, mice were equally divided into three groups (n=40 each). Beginning three days before mating (preliminary period), groups were fed diets with DCAD levels of +300 (basal diet + NaHCO₃, high DCAD, HD group), +150 (basal diet, control, CON group), and -50 (basal diet + NH₄Cl, low DCAD, LD group). Sterilized mouse diets were provided by Suzhou Shuangshi Laboratory Animal Feed Technology Co., Ltd. DCAD levels were calculated as Na + K - Cl - S. Mice were housed in cages (4 per cage) with ad libitum access to feed and water. The basal diet consisted of corn, soybean meal, wheat bran, vitamins, and trace minerals with primary nutrient levels: crude protein (CP) 20.5%, crude ash 6.9%, neutral detergent fiber (NDF) 13.1%, acid detergent fiber (ADF) 7.5%, calcium 0.51%, and phosphorus 0.40%.

1.3 Sample Collection

At four time points—mating day +1 (-20 d), 5 days prepartum (-5 d), parturition day (0 d), and 3 days postpartum (+3 d)—10 mice per group were randomly selected, anesthetized with ether, and euthanized by enucleation. Orbital blood was collected, centrifuged at 3,000 rpm for 20 minutes to prepare plasma, and stored at -20°C. Gastrointestinal tissues (stomach, duodenum, jejunum, ileum, cecum, colon) were rapidly collected under RNase-free conditions, rinsed with precooled phosphate-buffered saline (PBS), and placed in cryovials prefilled with RNAstore. All samples were stored at -80°C until analysis.

1.4 Measurements

Plasma calcium concentration was determined according to kit instructions. CaBP-D9k mRNA relative expression levels in various tissues at different time points were measured and calculated using the real-time quantitative PCR detection system established by our research group.

1.5 Statistical Analysis

Data are expressed as mean \pm standard deviation. Statistical analysis was performed using SAS 9.3. Plasma calcium concentrations were analyzed using the Proc GLM module with Duncan's multiple range test. mRNA expression level normalization and relative expression calculations were performed according to established methods. To examine treatment effects, time effects, and their interactions on these parameters, LSMEANS, TDIFF, and PDIFF options were included in the GLM procedure. Proc REG and CORR modules were used for Pearson linear correlation analysis between DCAD level, plasma calcium concentration, and CaBP-D9k mRNA relative expression at different gastrointestinal sites. Significance was set at $P < 0.05$.

Results

2.1 Plasma Calcium Concentration

As shown in Figure 1 [Figure 1: see original paper], plasma calcium concentration increased gradually as parturition approached, peaking at -5 d before declining. Compared with -5 d, calcium concentrations at 0 d decreased by 17.68% (HD group), 25.83% (CON group), and 6.59% (LD group). Postpartum (+3 d), calcium concentrations showed recovery trends in all groups, with the LD group significantly higher than the other two groups ($P < 0.05$). Overall mean calcium concentrations were 1.052, 1.209, and 1.525 mmol/L for HD, CON, and LD groups, respectively, with the LD group showing the highest values significantly exceeding HD and CON groups at 0 d and +3 d ($P < 0.05$). No significant differences were observed between HD and CON groups ($P > 0.05$). Neither time effects nor interactions between time and DCAD level significantly affected plasma calcium concentration ($P > 0.05$).

2.2 Gastrointestinal CaBP-D9k mRNA Relative Expression Levels

Using baseline samples from each group as references, changes in gastrointestinal CaBP-D9k mRNA relative expression during the peripartum period are shown in Figure 2 [Figure 2: see original paper]. Gastric CaBP-D9k mRNA expression decreased gradually after trial initiation, reaching its lowest level at parturition (0 d). In contrast, duodenal CaBP-D9k mRNA expression peaked at -5 d and 0 d, significantly higher than at -20 d and +3 d ($P < 0.05$). Expression levels in jejunum, ileum, cecum, and colon decreased substantially at -5 d, then gradually

increased to peak at parturition (0 d). By +3 d postpartum, CaBP-D9k mRNA expression at all gastrointestinal sites gradually returned to -20 d levels.

2.3 Effect Analysis of DCAD Level on Gastrointestinal CaBP-D9k mRNA Expression

As shown in Figure 3 [Figure 3: see original paper], DCAD level effects on CaBP-D9k mRNA expression were observed only in duodenum, jejunum, ileum, and colon. After trial initiation, the LD group induced significantly higher CaBP-D9k mRNA expression in duodenum, jejunum, and colon compared with the HD group ($P < 0.05$). In duodenum and colon, this effect persisted through parturition (0 d) ($P < 0.05$). At +3 d postpartum, the LD group showed significantly elevated CaBP-D9k mRNA expression in duodenum, jejunum, and colon compared with the HD group ($P < 0.05$). Further statistical analysis (Table 1) revealed significant correlations between CaBP-D9k mRNA expression levels in duodenum, jejunum, and colon with both DCAD level and plasma calcium concentration ($P < 0.05$), indicating that decreased DCAD level and increased plasma calcium concentration were associated with increased CaBP-D9k mRNA expression.

Discussion

3.1 Effects of DCAD Level on Peripartum Calcium Homeostasis

Systemic calcium homeostasis is essential for nerve transmission, muscle contraction, and signal transduction, and forms the basis for meeting calcium requirements for skeletal integrity and animal products. Disruption of calcium homeostasis due to impaired vitamin D metabolism or excessive acute calcium loss triggers negative calcium balance symptoms. During homeostatic disturbances, organisms regulate parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D [1,25-(OH) D] secretion at the tissue level to enhance the efficiency of three calcium acquisition pathways—renal resorption, bone mobilization, and gastrointestinal absorption—thereby modulating the kinetic parameters of exchangeable calcium pool (TEP) release/storage to buffer plasma calcium fluctuations.

Some researchers propose that compromised gastrointestinal active calcium absorption may represent the molecular pathological basis for frequent hypocalcemia in peripartum animals with high calcium demand, and that low DCAD levels may prevent hypocalcemia by upregulating gastrointestinal active transmembrane calcium uptake to replenish TEP calcium sources. This hypothesis has garnered widespread attention in nutrition science, though supporting research remains limited. To test this hypothesis, we utilized peripartum mice as a model organism and examined CaBP-D9k—a key macromolecule associated with the rate-limiting step of calcium transmembrane absorption—measuring its mRNA expression across gastrointestinal segments while monitoring plasma calcium changes to evaluate nutritional intervention effects. Our results demonstrated a dose-dependent relationship between decreasing DCAD levels and in-

creasing plasma calcium concentration, with the LD group showing significantly higher calcium levels than the other groups. This finding aligns with previous research identifying increased plasma calcium as the classic response to low DCAD diets in peripartum dairy animals.

Regarding the peripartum timeline, initial calcium demands at trial onset imposed minimal stress on maternal calcium homeostasis, resulting in limited DCAD effects on plasma calcium and non-significant intergroup differences. As fetal development progressed, maternal blood volume expanded while calcium was actively transferred to the placenta. To counteract negative calcium balance, PTH and 1,25-(OH) D secretion increased, promoting bone calcium mobilization, upregulating intestinal calcium uptake, and reducing urinary calcium excretion to maintain rising plasma calcium concentrations, consistent with our observations at -5 d. Benefiting from activated gastrointestinal calcium absorption regulation by low DCAD, the LD group showed even greater plasma calcium increases compared with the previous timepoint. Although lactation volume is limited in mice compared with dairy cows, peripartum mice still lose calcium through colostrum synthesis, making rapid TEP replenishment challenging and causing substantial calcium declines at parturition across all groups. However, the LD group exhibited a smaller calcium decrease at 0 d than HD and CON groups, suggesting enhanced peripheral calcium capacity/transport efficiency, corroborated by the high plasma calcium concentrations observed in this group before -5 d. These results, consistent with previous findings, confirm that low DCAD levels mitigate the magnitude of peripartum calcium decline, increase plasma calcium concentration, and maintain calcium homeostasis, thereby preventing hypocalcemia.

3.2 Effects of DCAD Level on Gastrointestinal CaBP-D9k mRNA Expression

Acidification of the internal environment (pH reduction) represents another key characteristic of low DCAD levels. Classical theory suggests that decreased body fluid pH facilitates bone calcium dissolution and promotes gastrointestinal calcium absorption. Accordingly, we examined gastrointestinal CaBP-D9k mRNA responses to different DCAD levels. Our findings showed that gastric and cecal CaBP-D9k mRNA expression did not respond significantly to DCAD levels, likely because the stomach is not a primary calcium absorption organ and the cecum's short anatomical length limits its contribution to active/passive transmembrane calcium absorption. Overall, this suggests that stomach and cecum contribute minimally to low DCAD nutritional interventions for peripartum calcium homeostasis.

Following low DCAD intake, small intestinal and colonic CaBP-D9k mRNA expression was rapidly upregulated within 3 days postpartum, with this trend continuing after delivery. Specifically, low DCAD induced higher CaBP-D9k mRNA expression in all segments except stomach and cecum after pregnancy initiation, most prominently at parturition (0 d) and demonstrating clear dose-

dependency. We infer that low DCAD facilitated greater dietary calcium entry into epithelial cells in these tissues, indicating enhanced active transmembrane calcium absorption capacity that supports calcium homeostasis. This interpretation is supported by the plasma calcium changes observed in the LD group and aligns with reports that low DCAD activates calcium homeostasis responses within approximately 2 days. Furthermore, statistical analysis revealed strong associations between CaBP-D9k mRNA expression in duodenum, jejunum, and colon with DCAD level and plasma calcium concentration. Japanese researchers reported a statistically significant linear relationship between small intestinal CaBP-D9k mRNA expression and plasma calcium ($P=0.326$, $R^2=0.053$), with slightly higher association strength than our study, possibly due to different regression methodologies (Spearman rank vs. Pearson analysis).

Notably, although plasma calcium concentrations showed no statistical differences among groups at trial initiation (-20 d), low DCAD already significantly upregulated CaBP-D9k mRNA expression in duodenum, jejunum, and colon compared with high DCAD and control groups, most markedly in colon. This supports the theory that gastrointestinal calcium absorption responds rapidly, activating regulation before calcium metabolic disturbances occur to meet TEP replenishment/consumption demands. Interestingly, while prepartum mice (-5 d) showed increased plasma calcium compared with the previous timepoint, CaBP-D9k mRNA expression did not increase in most tissues except duodenum; instead, expression decreased markedly in other segments. Considering segment-specific calcium uptake patterns reported in literature, we hypothesize that maternal calcium demands had not yet peaked at this timepoint, and the downregulation of CaBP-D9k expression may relate to compensatory high expression of other transmembrane calcium uptake-related genes in these segments. Therefore, we propose that duodenum, jejunum, and ileum represent the primary target sites affecting maternal calcium homeostasis in peripartum mammals, warranting further investigation of protein expression patterns and net calcium transport rates.

In summary, based on reported effects of DCAD on acid-base balance and mineral homeostasis, we conclude that upregulation of CaBP-D9k mRNA expression in four gastrointestinal sites (duodenum, jejunum, ileum, colon) is closely related to the physiological regulatory effects of low DCAD. This suggests that low DCAD possesses the capacity to upregulate key rate-limiting macromolecules in the gastrointestinal active calcium absorption pathway, and that increased expression of these biomolecules may represent an important mechanism for low DCAD to effectively maintain normal plasma calcium concentrations.

This study demonstrates that low DCAD levels upregulate CaBP-D9k mRNA expression in duodenal, jejunal, ileal, and colonic tissues of peripartum mice, thereby increasing plasma calcium concentrations during the peripartum period.

References

- [1] GOFF J P, HORST R L, MUELLER F J, et al. Addition of chloride to a prepartal diet high in cations increases 1,25-dihydroxyvitamin D response to hypocalcemia preventing milk fever[J]. *Journal of Dairy Science*, 1991, 74(11): 3863-3871.
- [2] CAO Guofan. Synergistic effects and mechanisms of parathyroid hormone and calcium in promoting bone formation[D]. Ph.D. Thesis. Nanjing: Nanjing Medical University, 2008.
- [3] WU W X, LIU J X, XU G Z, et al. Calcium homeostasis, acid-base balance, and health status in periparturient Holstein cows fed diets with low cation-anion difference[J]. *Livestock Science*, 2008, 117(1): 7-14.
- [4] APPER-BOSSARD E, FAVERDIN P, MESCHY F, et al. Effects of dietary cation-anion difference on ruminal metabolism and blood acid-base regulation in dairy cows receiving 2 contrasting levels of concentrate in diets[J]. *Journal of Dairy Science*, 2010, 93(9): 4196-4210.
- [5] MARTÍN-TERESO J, VERSTEGEN M W A. A novel model to explain dietary factors affecting hypocalcaemia in dairy cattle[J]. *Nutrition Research Reviews*, 2011, 24(2): 228-243.
- [6] GOFF J P. Macromineral physiology and application to the feeding of the dairy cow for prevention of milk fever and other periparturient mineral disorders[J]. *Animal Feed Science and Technology*, 2006, 126(3/4): 237-257.
- [7] BERNE R M, LEVY M N, KOEPPEN B M, et al. Berne & levy physiology[M]. Philadelphia, PA: Mosby/Elsevier, 2008.
- [8] MARTÍN-TERESO LÓPEZ J. Rumen-protected rice bran to induce the adaptation of calcium metabolism in dairy cows[D]. Ph.D. Thesis. Wageningen: Wageningen University, 2010.
- [9] THILSING-HANSEN T, JØRGENSEN R J, ØSTERGAARD S. Milk fever control principles: a review[J]. *Acta Veterinaria Scandinavica*, 2002, 43(1): 1-19.
- [10] GROSS M, KUMAR R. Physiology and biochemistry of vitamin D-dependent calcium binding proteins[J]. *The American Journal of Physiology*, 1990, 259(2 Pt 2): F195-209.
- [11] MAO Meng, WANG Daocong. Roles of VD and CaBP-Ds in intestinal and renal calcium transport[J]. *Foreign Medical Sciences (Pediatrics)*, 1996, 23(6): 289-292.
- [12] YANG Yi, XIN Hailiang, XIA Xianlin, et al. Expression pattern analysis of genes related to calcium transmembrane absorption pathways in the gastrointestinal tract of Kunming mice[J]. *Chinese Journal of Animal Nutrition*, 2013, 25(2): 441-446.

- [13] LIU Hedi. Research progress on calcium binding proteins[J]. Foreign Medical Sciences (Physiology, Pathology and Clinical Medicine), 1995(4): 278-281.
- [14] REPLOGLE R A, LI Q, WANG L B, et al. Gene-by-diet interactions influence calcium absorption and bone mineral density in mice[J]. Journal of Bone and Mineral Research, 2014, 29(3): 657-665.
- [15] XIN Hailiang, WU Wenxuan, LI Shengli. Establishment of real-time fluorescent quantitative PCR method for detecting key genes involved in animal gastrointestinal calcium absorption[J]. Jiangsu Agricultural Sciences, 2013, 41(4): 31-34.
- [16] WILLEMS E, LEYNS L, VANDESOMPELE J. Standardization of real-time PCR gene expression data from independent biological replicates[J]. Analytical Biochemistry, 2008, 379(1): 127-129.
- [17] BOLT M J G, CAO L P, KONG J, et al. Vitamin D receptor is required for dietary calcium-induced repression of calbindin-D9k expression in mice[J]. The Journal of Nutritional Biochemistry, 2005, 16(5): 286-290.
- [18] MULLEN P A. Clinical and biochemical responses to treatment of milk fever[J]. Veterinary Record, 1975, 97(5): 87-92.
- [19] HORST R L, GOFF J P, REINHARDT T A. Adapting to the transition between gestation and lactation: differences between rat, human and dairy cow[J]. Journal of Mammary Gland Biology and Neoplasia, 2005, 10(2): 141-156.
- [20] WILKENS M R, OBERHEIDE I, SCHRÖDER B, et al. Influence of the combination of 25-hydroxyvitamin D and a diet negative in cation-anion difference on periparturient calcium homeostasis of dairy cows[J]. Journal of Dairy Science, 2012, 95(1): 151-164.
- [21] DONG Shujun, WU Xiaoqian, ZHAO Junhong. Changes in maternal blood calcium during pregnancy and analysis of blood calcium in 544 pregnant women[J]. Fudan University Journal (Medical Science), 2002, 29(5): 418-419.
- [22] LEAN I J, DEGARIS P J, MCNEIL D M, et al. Hypocalcemia in dairy cows: meta-analysis and dietary cation anion difference theory revisited[J]. Journal of Dairy Science, 2006, 89(2): 669-684.
- [23] LIESEGANG A. Influence of anionic salts on bone metabolism in periparturient dairy goats and sheep[J]. Journal of Dairy Science, 2008, 91(6): 2449-2460.
- [24] HIRATA Y, OKU Y. TRP channels are involved in mediating hypercapnic Ca^{2+} responses in pH-independent cultures of glia-rich medullary cells[J]. Cell Calcium, 2010, 48(2/3): 124-132.
- [25] PÉREZ A V, PICOTTO G, CARPENTIERI A R, et al. Minireview on regulation of intestinal calcium absorption. Emphasis on molecular mechanisms of transcellular pathway[J]. Digestion, 2008, 77(1): 22-34.

- [26] YAMAGISHI N, MIYAZAKI M, NATO Y. The expression of genes for transepithelial calcium-transporting proteins in bovine duodenum[J]. The Veterinary Journal, 2006, 171(2): 363-366.
- [27] SCHRÖDER B, BREVES G. Mechanisms and regulation of calcium absorption from the gastrointestinal tract in pigs and ruminants: comparative aspects with special emphasis on hypocalcemia in dairy cows[J]. Animal Health Research Reviews, 2006, 7(1/2): 31-41.
- [28] BRUN L R, BRANCE M L, RIGALLI A. Luminal calcium concentration controls intestinal calcium absorption by modification of intestinal alkaline phosphatase activity[J]. British Journal of Nutrition, 2012, 108(2): 229-233.
- [29] VANOEVERLEN J, JANSSENS A, HUITEMA L F A, et al. Trpv5/6 is vital for epithelial calcium uptake and bone formation[J]. The FASEB Journal, 2011, 25(9): 3197-3209.

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv – Machine translation. Verify with original.