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Research article

Changes in duodenal and nephritic Ca and P absorption in hens during different egg-laying periods

Jishuang San [1], Zaixiang Zhang [1], Shuyang Bu, Mingxi Zhang, Jianmin Hu, Jiancheng Yang [*], Gaofeng Wu [**]

College of Animal Science & Veterinary Medicine, Shenyang Agricultural University, Shenyang, Liaoning, 110866, PR China

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ABSTRACT

Ca and P metabolic disorders during the egg-laying period can reduce egg production, impair eggshell quality, and even cause bone problems in hens; however, little is known regarding the capacity of duodenal and nephritic Ca and P absorption. Here, the levels of serum Ca and P metabolic indices and the expression of duodenal and renal Ca and P transporter genes were measured in hens at different egg-laying stages. The Ca, 25-(OH)-VD_3, and 1,25-$(OH)_2$-VD_3 content increased during the peak (43 weeks of age) and late (72 weeks of age) egg-laying periods compared to that during the early (23 weeks of age) egg-laying period; however, there were no differences in Pi levels among the three egg-laying periods. Moreover, duodenal *VDR* and *CaBP-D28k* mRNA expression was markedly higher but *NPt2b* mRNA expression was markedly lower during the peak and late egg-laying periods than during the early egg-laying period. Furthermore, nephritic *CaBP-D28k*, *PMCA1b*, and *FGFR1* mRNA expression was markedly higher but *NPt2a* and *Cyp24a1* mRNA expression was markedly lower during the peak and late egg-laying periods than during the early egg-laying period. In conclusion, the present study indicated that the increased duodenal and nephritic Ca absorption during the peak and late egg-laying periods may be associated with the VD–VDR pathway, while the decreased P absorption despite relatively stable serum P levels in all three egg-laying stages may associated with osteolysis.

1. Introduction

Ca and P are essential nutrients for humans and animals, which are involved in numerous biochemical and physiological functions, such as constituting the main component of the skeleton and teeth in the form of calcium phosphate, participating in blood coagulation, maintaining the acid–base balance, partaking neurotransmitter transmission and muscle contraction, and participating in energy and nucleic acid metabolism [1, 2, 3, 4]. For hens, Ca and P are paramount for productive performance and eggshell quality during the egg-laying period [5]. Ca constitutes approximately 1.5% of the total hen body weight and 40% of the eggshell weight; calcium carbonate accounts for 94% of total Ca and is closely related to eggshell quality [6, 7]. P plays important roles during eggshell formation in maintaining bicarbonate levels by decreasing blood acidosis through promoting excess hydrogen ion excretion [8], although there is little P in eggshell. During eggshell formation, Ca derived from food and medullary bone dissolution is excreted from the eggshell gland; thus, Ca and P metabolism homoeostasis associated with small intestinal absorption, nephritic reabsorption/excretion, and bone remodelling is particularly important for egg-laying hens.

Ca and P are obtained from food, specifically through absorption in the small intestine [9]. In poultry, the duodenum is the major site of Ca and P uptake, and active transport and regulation through the vitamin D receptor (VDR) signalling pathway are implicated in this uptake [10]. Ca absorption in the duodenum includes three steps [11]. Ca is first taken up into the epithelium through transient receptor potential cation channel subfamily V member 5 and 6 (TRPV5 and TRPV6) [12]. Next, it is combined with calbindin-D28k (CaBP-D28K) and transferred to the basolateral membrane [13]. Finally, it is released into to the extracellular fluid by sodium-calcium exchanger member 1 (NCX1) and plasma membrane Ca ATPase 1b (PMCA1b) [14]. In birds, duodenal P absorption is primarily controlled by sodium-dependent phosphate transport

* Corresponding author.
** Corresponding author.
E-mail addresses: syndyjc@syau.edu.cn (J. Yang), gaofengwu@syau.edu.cn (G. Wu).
[1] These authors contributed equally to this work.

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protein 2b (NPt2b) [15], which is located at the brush-border membranes of the epithelium [16].

The kidney is a remarkable organ responsible for maintaining Ca and P homoeostasis through reabsorption and excretion in the renal and connecting tubules. Majority of the Ca reabsorption in the proximal tubule, thick ascending limb of the loop of Henle, distal convoluted tubule, and connecting segment occurs via passive paracellular and active transcellular transport [17, 18]. Similar to intestinal Ca absorption, TRPV5/6, CaBP-D28K, NCX1, and PMCA1b are implicated in nephritic Ca transport [19], and these factors are primarily controlled by parathyroid hormone (PTH), calcitonin (CT), and 1,25-dihydroxyvitamin D3 ($1,25\text{-}(OH)_2\text{-}VD_3$) [20]. Approximately 80%–90% of the filtered phosphate (Pi) is absorbed in the proximal tubule, which is primarily controlled by type 2a sodium-phosphate cotransporter (NPt2a) via an active transcellular mechanism [21, 22]. PTH and fibroblast growth factor 23 (FGF23) stimulate Pi excretion by inhibiting *NPt2a* expression [23, 24].

Inadequate Ca and P intake or supplementation can reduce egg production and quality and even lead to osteoporosis in egg-laying chickens [25, 26, 27]. Moreover, many studies have shown that dietary Ca or P deficiency upregulated the expression of Ca and P transporters, including *CaBP-D28K*, *PMCA1b*, and *NPt2*, in the small intestine, kidney, and eggshell glands of laying hens [12, 28, 29]. In addition, decreased intestinal Ca absorption substantially reduced eggshell quality in aged layers [30]. Furthermore, duodenal calbindin concentration was lower in young laying hens than in aged laying hens [31]. To date, however, changes in Ca and P absorption in the small intestine and kidneys of hens during different egg-laying periods have not been elucidated. Therefore, the objective of the present study was to investigate changes in duodenal and renal Ca and P absorption based on the activity of various transporters in hens at different egg-laying stages.

2. Materials and methods

2.1. Hens

A total of 36 Hy-line Brown laying hens with approximately the same egg-laying status were obtained from a laying hen farm in Liaoning. The hens were housed individually in cages and provided a complete formula feed twice a day (at 8:00 and 16:30) and water *ad libitum*. A corn-soybean meal diet, formulated according to the recommended nutrient levels for Hy-line Brown laying hens in the 1994 National Research Council Nutrient Requirements of Poultry, was used. The composition of the corn-soybean meal diet for Hy-line Brown laying hens is presented in Table 1. The laying hens were housed under a 16-8 h light-dark cycle at controlled temperature (18–21 °C) and humidity (40%–70%) in a specific pathogen-free facility. The hens were divided into three groups according to the laying period, namely early (23 weeks of age), peak (43 weeks of age), and late (72 weeks of age), with 12 birds in each group. After 1 week of acclimation, the hens were sacrificed 6 h after the first oviposition, and the laying rate was calculated. Their blood, duodenum, and left kidney were collected for biochemical and gene expression analyses. The study protocol was approved by the Laboratory Animal Welfare and Ethical Committee of the Shenyang Agricultural University (ethical approval code: 201606007).

Table 1. Formulation (%) and nutrient levels ($MJ{\cdot}kg^{-1}$) of diet for Hy-line Brown laying hens.

Material	Content	Nutrient	Content
Corn	64.71	Metabolic energy	11.30
Soybean	19.20	Crude protein	16.30
Cotton pulp	1.70	Lysine	0.65
Rapeseed	2.00	Methionine	0.41
Fish meal	1.50	Calcium	3.60
Stone powder	8.00	Available phosphorus	0.30
Salt	0.30		
Choline chloride	0.09		
Dicalcium phosphate	1.50		
1% Premix	1.00		

Note: Metabolic energy was calculated, and the other nutritional indicators were measured.

2.2. Serum biochemical analysis

Blood was collected from the jugular vein and centrifuged at 3,500 rpm for 15 min. The serum was separated and stored in a refrigerator at -20 °C to measure blood biochemical indices. The levels of serum Ca and Pi were measured according to the protocols of the respective reagent kits (Nanjing Jiancheng Bioengineering Institute, China). Serum PTH, CT, $25\text{-}(OH)\text{-}VD_3$, and $1,25\text{-}(OH)_2\text{-}VD_3$ content was measured using radioimmunoassay kits (Diagnostic Systems Laboratories, INC, USA) according to the manufacturer's instructions.

2.3. mRNA expression analysis by real-time RT-PCR

After the laying hens were sacrificed, the abdominal cavity was cut open and the duodenum and left kidney were immediately collected, frozen in liquid nitrogen, stored at -80 °C in a refrigerator, and subjected to fluorescence quantitative RT-PCR. Total RNA from the duodenum and kidney was extracted using an animal tissue total RNA extraction kit (Sangon, China). Then, cDNA was synthesised using the PrimeScript RT Reagent Kit according to the manufacturer's instructions (Takara Bio, Dalian, China). The expression levels of Ca and P metabolism-related genes were quantified by real-time RT-PCR using SYBR Premix Ex Taq II (Takara Bio). Sequences of all primers used for RT-PCR are shown in Table 2. Relative gene expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method [32]. Results are presented as relative fold changes in the value of the control group after normalising to the endogenous control β-actin.

2.4. Statistic analysis

Data were analysed using SPSS 17.0 and presented as mean and standard deviation (SD). Significant differences were subjected to one-way and least significant difference test, and significance was declared at $P < 0.05$ and $P < 0.01$.

3. Results

3.1. Ca and P metabolism-related serum biochemical indices

We first determined the serum Ca and P metabolism-related biochemical indices of hens during different egg-laying periods. The Ca, $25\text{-}(OH)VD_3$, $1,25\text{-}(OH)_2D_3$, and CT content during the peak and late egg-laying periods was significantly higher than that during the early egg-laying period ($P < 0.05$), but there were no significant differences in their content between the peak and late egg-laying periods ($P > 0.05$) (Figure 1). Moreover, serum Pi and PTH levels were not significantly different among the three egg-laying periods ($P > 0.05$).

3.2. Expression of Ca- and P-Absorption related genes in the duodenum

Ca and P in the body are absorbed from food in the intestinal tract, specifically the duodenum. Therefore, we measured the mRNA levels of duodenal Ca and P absorption-related factors using real-time RT-PCR. *VDR* and *CaBP-D28k* expression was significant higher ($P < 0.05$) but *NCX1* expression was significantly lower ($P < 0.01$) during the peak and late egg-laying periods than during the early egg-laying period (Figure 2). Moreover, the duodenal *PMCA1b* expression during the peak egg-laying period was significantly higher than that during the early and late egg-

Table 2. List of primers used in this study.

Gene	Primer sequence
Klotho	Forward: 5′-TCCAGGAACGACCAAGAAGT-3′
	Reverse: 5′-CAACGCTGTTTCTCTGGTGA-3′
FGFR1	Forward: 5′-GGGAGCGAGACCACCTACTT-3′
	Reverse: 5′-CTGGTTCGGCTTGGTGTTAT-3′
FGFR3	Forward: 5′-CTGCCAATCAGACTGTGGTG-3′
	Reverse: 5′-ACTTGCTGCCGTTGACTTCT-3′
FGFR4	Forward: 5′-TCTGAGGTGGAGGTGCTGTA-3′
	Reverse: 5′-ACGCTGACTGGTAGGAGAGG-3′
TRPV5	Forward: 5′-CATCTTCCAGACGGAGAACC-3′
	Reverse: 5′-GGCAGGTCCACATCGTAGTT-3′
TRPV6	Forward: 5′-TGGAACGGACTAAGTCAGAAGTTG-3′
	Reverse: 5′-CGTTATGGCTGGGATGTTGTT-3′
NCX1	Forward: 5′-AGCTTGGTGGCTTCACAATC-3′
	Reverse: 5′-CTTCGTTCTCCTCTCGGATG-3′
PTHR1	Forward: 5′-AGGAGATGGAGCGGATTTCT-3′
	Reverse: 5′-TGGTTGCCAGGAAGTAGAGG-3′
PMCA1b	Forward: 5′-TTCAGGTACTCATGTGATGGAAGG-3′
	Reverse: 5′-CAGCCCCAAGCAAGGTAAAG-3′
NaPi-IIa	Forward: 5′-CGCTGGAGGTAGTGAGTGG-3′
	Reverse: 5′-GATGAGGCTGCGGTTGC-3′
NaPi-IIb	Forward: 5′-TTGCTCCTGTTTGGTGATGA-3′
	Reverse: 5′-GGTATCCAGCCAGCCAAGTA-3′
CaBP-D28k	Forward: 5′-TTAAATCTGCGTTGCTTCCATACA-3′
	Reverse: 5′-GGCCCATCCTGCACTCCATAAC-3′
VDR	Forward: 5′-CGTGGACATTGGGATGATG-3′
	Reverse: 5′-AGTTTGGGCTTCAGGCTCTC-3′
CYP24a1	Forward: 5′-AAACAGCCAAGCACTCCATT-3′
	Reverse: 5′-GTGGCGTACAGCTCCTTCTT-3′
β-Actin	Forward: 5′-GAGAAATTGTGCGTGACATCA-3′
	Reverse: 5′-CCTGAACCTCTCATTGCCA-3′

laying periods ($P < 0.01$), and the level during the late egg-laying period was significantly lower than that during the early egg-laying period ($P < 0.05$). Although there were no significant differences in *NPt2b* expression between the peak and late egg-laying periods ($P > 0.05$), the level was obviously lower than that during the early egg-laying period ($P < 0.01$). Unfortunately, although *TRPV5* and *TRPV6* expression in the duodenum and jejunum of the laying hens was also measured, these levels could not be detected, perhaps because of the low TRPV5/6 expression in the small intestine.

3.3. Expression of Ca and P reabsorption-related genes in the kidney

Blood Ca and Pi levels are regulated by the kidneys as these elements are filtered, reabsorbed, and excreted when blood flows through the renal system. We assayed the mRNA levels of nephritic Ca and P reabsorption-related genes during the three egg-laying periods. *TRPV5* expression presented a decreasing trend, and its expression was significantly lower during the peak and late egg-laying periods than during the early egg-laying period ($P < 0.05$) (Figure 3). *CaBP-D28k* and fibroblast growth factor receptor 1 (*FGFR1*) expression during the peak and late egg-laying periods was markedly higher than that during the early egg-laying period ($P < 0.01$). Meanwhile, renal *PMCA1b*, *FGFR3*, and *FGFR4* expression during the peak egg-laying period was markedly higher than that during the early and late egg-laying periods ($P < 0.05$). Furthermore, there were no significant differences in *TRPV6*, *NCX1*, *Klotho*, and *PTHR* expression among the three egg-laying periods ($P > 0.05$). Of note, similar to duodenal *NPt2b* expression, nephritic *NPt2a* expression during the peak and late egg-laying periods was significantly lower than that during the early egg-laying period ($P < 0.01$). In addition, 1,25-$(OH)_2$-VD_3 and cytochrome P450 family 24 subfamily A member 1 (*Cyp24a1*) showed significant decreasing trends in all egg-laying periods ($P < 0.05$).

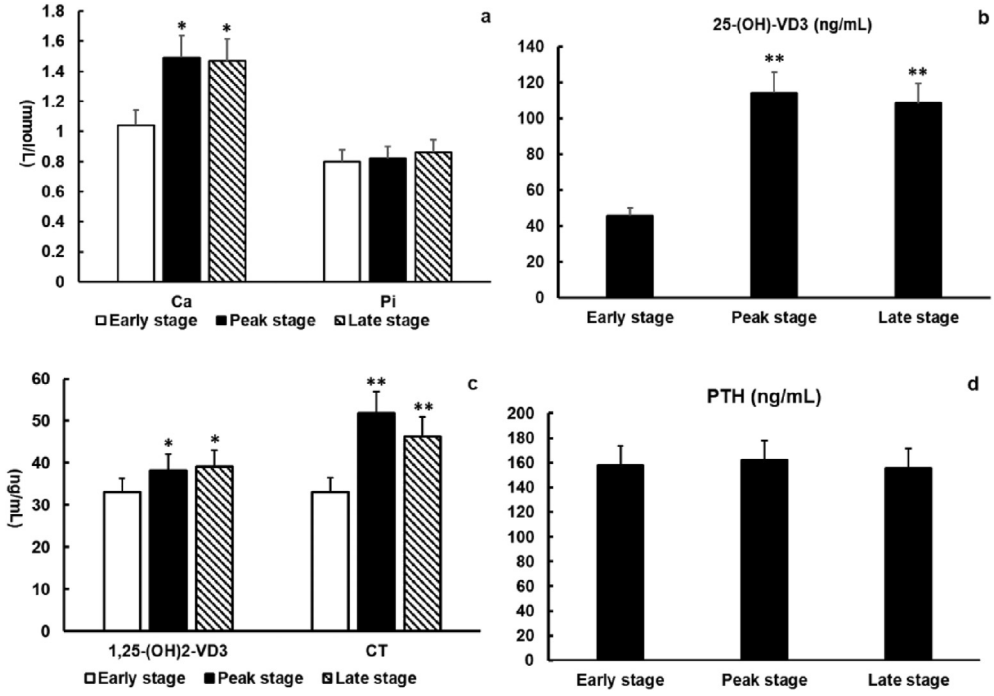

Figure 1. Ca and P metabolism-related serum biochemical indices during different egg-laying periods. Results are presented as mean ± SD ($n = 6$). *$P < 0.05$ and **$P < 0.01$, compared with the early egg-laying period.

4. Discussion

Ca and P are the two most abundant mineral elements in animals, accounting for approximately 70% of the total ash in the body, and these elements are mainly found in bones and teeth in the form of inorganic salts. Moreover, Ca and P serve a wide range of physiological functions. For instance, Ca is involved in blood coagulation, muscle contraction, and nerve impulse conduction [33], and P is involved in biomembrane formation, genetic information transmission, and energy supply [34]. In laying hens, in addition to the abovementioned functions, Ca and P constitute important components of the eggshell and are closely related to its quality; specifically, Ca is associated with the brittleness of the eggshell, while P determines its toughness and elasticity [35]. In particular, laying hens require large amounts of Ca and adequate amounts of P to meet the demands of normal metabolism and egg production; therefore, it is crucial to maintain high haemocircular Ca and P levels [36]. Insufficient Ca and P supplementation or Ca and P metabolism disorders during the egg-laying period may reduce productive performance, impair eggshell quality, and in severe cases, may cause osteoporosis [37]. Therefore, unravelling the changes in Ca and P metabolism during different egg-laying periods in hens would provide a theoretical foundation for appropriate dietary supplementation of Ca and P, improvement of eggshell quality, and prevention of osteoporosis.

In the present study, serum levels of Ca and P during different egg-laying periods in hens were examined. Serum Ca levels during the peak and late egg-laying periods were remarkably higher than those during the early egg-laying period, but there were no differences in serum P levels among the three periods. The egg-laying rates during the three periods are presented in Table 3. The egg-laying rate was high during the peak egg-laying period and slightly decreased during the early and late egg-laying period, suggesting that hens require more Ca than P during the peak and late egg-laying periods to meet the demand for egg production, specifically the eggshell formation. These results are consistent with previous observations by Harms and colleagues that older hens (60 weeks of age) exhibited markedly higher plasma Ca levels than younger hens (30 weeks of age), but the phosphate levels were comparable between the two age groups [38]. Similarly, Bar and Hurwitz demonstrated that plasma Ca levels of peak-laying chickens (8 months of age) were similar to those of late-laying chickens (20 months of age) [39]. As early as 1980, Ousterhout demonstrated that low Ca levels in hens during the early months of egg production may maximise early egg weight, while increasing Ca levels with aging may improve shell weight; meanwhile, excess dietary P should be avoided during egg-laying periods [40]. Many factors play pivotal roles in the regulation of Ca and P metabolism, including VD_3, PTH, and CT; therefore, we further determined the serum levels of 25-(OH)VD_3, 1,25-$(OH)_2VD_3$, CT, and PTH in hens during different egg-laying periods. Consistent with previous reports [31], 25-(OH)-VD_3 and 1,25-$(OH)_2$-VD_3 levels during the early egg-laying period were markedly lower than those during the peak and late egg-laying periods, while PTH level was comparable across the three periods. VD_3 promotes Ca and P absorption in the small intestine and kidney through the biologically active vitamin D hormones 25-$(OH)_2$-VD_3 and 1,25-$(OH)_2$-VD_3. Our results indicated that high Ca levels during the peak and late egg-laying periods may be attributed to the absorption promoting actions of 25-$(OH)_2$-VD_3 and 1,25-$(OH)_2$-VD_3 in the intestine and nephridium. Dietary VD_3 is converted to 25-$(OH)_2$-VD_3 by the action of 25-hydroxylase in the mammalian liver, and 25-$(OH)_2$-VD_3 is hydroxylated to 1,25-$(OH)_2$-VD_3 in the kidney through 1-hydroxylase encoded by *Cyp27b1*. Meanwhile, 1, 25-$(OH)_2$-VD_3 can be inactivated to a highly hydrophilic metabolite by the nephritic *Cyp24a1* gene product 24-hydroxylase [41]. Consistently, the present study showed that nephritic *Cyp24a1* expression during the peak and late egg-laying periods was significantly lower than that during the early egg-laying period, but we did not detect *Cyp27b1* expression in the kidney. These data suggest that high serum 1,25-$(OH)_2$-VD_3 levels during the peak and late egg-laying periods may partly be attributed to the reduced enzymatic activity of 24-hydroxylase in the kidney. Interestingly, the CT content during the peak and late egg-laying periods was also dramatically higher than that during the early egg-laying period. CT secreted by the ornithic ultimobranchial gland can decrease serum Ca and P levels by inhibiting nephritic reabsorption and bone mineral dissolution. The abnormal elevation of CT during the peak and late egg-laying periods may be attributed to the stimulation of higher Ca levels, although the exact mechanism warrants further investigation.

Ca and P metabolism in laying hens is a very complex process involving the small intestine, kidneys, bones, and eggshell gland. Ca and P absorption from diet in the small intestine plays key roles in maintaining the Ca and P homoeostasis in the body. To determine whether the high levels of Ca during the peak and late egg-laying periods were associated with changes in intestinal Ca and P absorption capacity, we analysed the expression of duodenal Ca and P absorption-related genes. *VDR*, *CaBP-D28k*, and *PMCA1b* expression was significantly higher but *NCX1* expression was significantly lower during the peak egg-laying period than during the early egg-laying period. Meanwhile, *VDR* and *CaBP-D28k* expression was markedly higher but *PMCA1b* and *NCX1* expression was significantly lower during the late egg-laying period than during the early egg-laying period. There are two major Ca absorption pathways in the intestinal tract, namely transcellular and paracellular absorption, regulated by the VD–VDR signalling pathway [42]. In poultry, transcellular Ca absorption mainly occurs in the duodenum and accounts for approximately 90% of total Ca absorption; it involves three processes, including Ca entry into the epithelium, intracellular Ca transport, and Ca discharge, and *TRPV5/6*, *CaBPs*, *PMCA1b*, and *NCX1* are involved in these processes [43]. Paracellular absorption occurs throughout the intestinal tract and depends on the close connections between the intestinal epithelial cells. Our findings suggest that the VD–VDR signalling pathway activation is responsible for the high Ca levels during the peak and late egg-laying periods via the promotion of duodenal transcellular absorption.

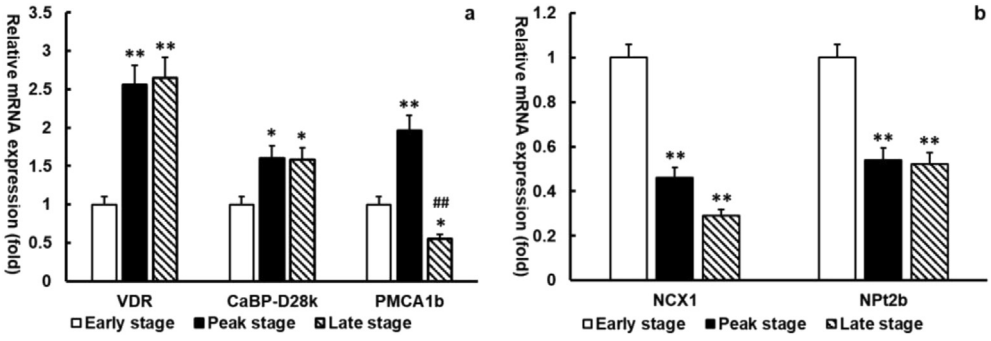

Figure 2. Changes in the expression of Ca and P transporter genes in the duodenum during different egg-laying periods. Results are presented as mean ± SD ($n = 6$). $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with the early egg-laying period. $^{\#\#}P < 0.01$, compared with the peak egg-laying period.

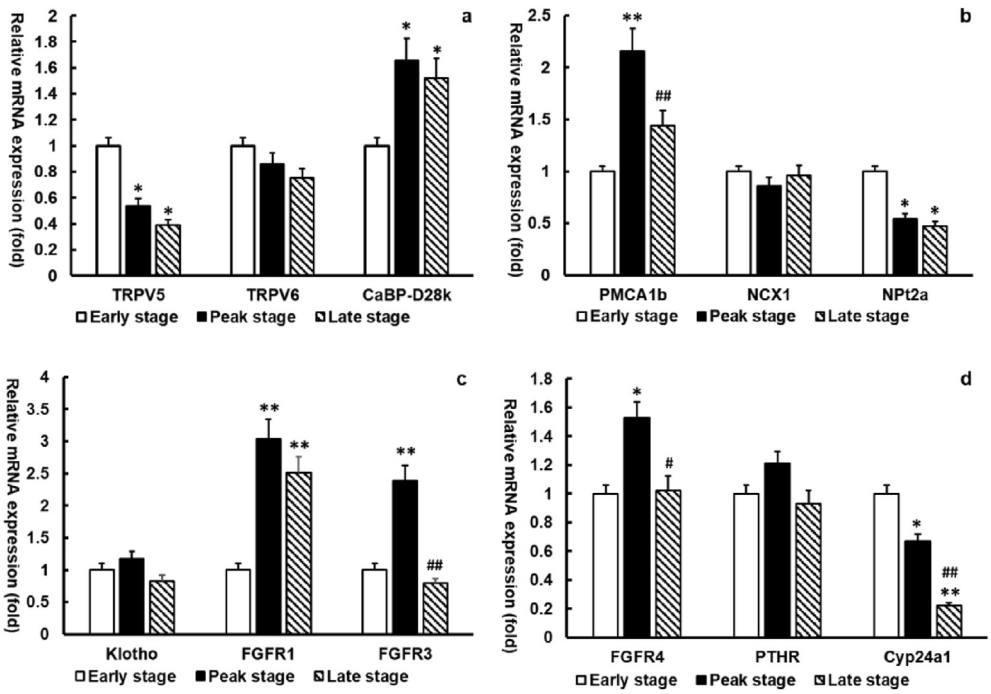

Figure 3. Changes in the expression of Ca and P reabsorption-related genes in the kidney during different egg-laying periods. Results are presented as mean ± SD ($n = 6$). $^{*}P < 0.05$ and $^{**}P < 0.05$, compared with the early egg-laying period. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with the peak egg-laying period.

Table 3. Laying rate at three different laying periods.

Laying period	Early	Peak	Late
Laying rate	91.7%	100%	91.7%

Note: Laying rate (%) = (total number of eggs/total number of laying hens) × 100%.

Furthermore, the duodenal expression of *NPt2b*, a unique factor mediating intestinal P absorption [16], was lower during the peak and late egg-laying periods than that during the early egg-laying period. These results suggest that the intestinal absorption capacity of P was reduced, which is contrary to the stable plasma P levels in the peak and late egg-laying periods, and this may be attributed to the stimulation of high serum P derived from osteolysis. In chickens, during the egg-laying period, specifically the peak and late phases, Ca and P are released into the bloodstream through osteolysis to maintain egg production [44].

The kidney is the key organ responsible for the regulation of Ca and P homoeostasis through reabsorption and excretion in the renal and collecting tubules. To investigate changes in the renal Ca and P reabsorption capacity of hens during different egg-laying periods, we examined the expression levels of Ca and P reabsorption-related genes in the kidney. *CaBP-D28k* and *PMCA1b* expression during the peak and late egg-laying periods was significantly higher than that during the early egg-laying period, whereas *TRPV6*, *NCX1*, and *PTHR* expression was comparable across all periods. These results indicate that renal Ca reabsorption increased during the peak and late egg-laying periods, which may be one of the reasons for increased blood Ca levels during these two periods. Moreover, 1,25-$(OH)_2D_3$ promotes the absorption of Ca in the kidney by activating the VDR pathway and *CaBP-D28K* expression [45]. Together with the above 1,25-$(OH)_2VD_3$ detection results, the present findings suggest that increased renal Ca reabsorption capacity may be attributed to the increased plasma 1,25-$(OH)_2VD_3$ levels. Interestingly, *TRPV5* expression during the late egg-laying period was markedly lower than that during the early egg-laying period. *TRPV5* is a key factor involved in renal Ca reabsorption [46], suggesting that the increased serum Ca levels in hens during the late egg-laying period may also be associated with osteolysis, although the precise mechanism needs to be elucidated.

Similar to that in the duodenum, P reabsorption in the kidney depends on active transport through sodium/phosphate co-transporters [22]. In poultry, a single sodium/phosphate co-transporter is present in the kidney, namely *NPt2a*, mainly expressed in the proximal tubules [46]. The present study showed that nephritic *NPt2a* expression during the peak and late egg-laying periods was obviously lower than that during the early egg-laying period, suggesting that the renal P resorption capacity was reduced. Nephritic *NPt2a* expression is regulated by the *Klotho–FGFR* complex pathway. *FGF23*, a phosphaturic hormone primarily produced by osteocytes, binds to the *Klotho–FGFR* complex to inhibit *NPt2a* transcription [47]. Therefore, we detected *Klotho* and *FGFR1*, *-3*, and *-4* expression in the kidney during the three egg-laying periods. The results revealed that *FGFR1*, *-3*, and *-4* expression during the peak egg-laying period and *FGFR1* expression during the late egg-laying period were significantly higher than those during the early egg-laying period; these results indicate that the *Klotho–FGFR* signalling pathway was upregulated, leading to *NPt2a* inhibition. Our results are consistent with previous reports [48, 49], although the experimental subjects were different. However, these results contradicted the stable plasma P levels during the peak and late egg-laying periods, further implying that the serum P levels of laying hens are, at least partly, derived from bone degradation. The upregulation of the *Klotho–FGFR* pathway during the peak and late egg-laying periods may be attributed to *VDR* upregulation, as activated *VDR* may initiate the transcription of osteocyte-derived *FGF23* and renal *Klotho*, further triggering *FGFR* expression [50].

5. Conclusions

Collectively, our data indicate that the increased serum Ca levels during the peak and late egg-laying periods may be attributed to the increased renal and duodenal Ca absorption through the activation of the VD–VDR signalling pathway. Moreover, decreased duodenal and nephritic P absorption capacity with the progression of the egg-laying period despite stable serum P levels may be attributed to osteolysis,

although the precise mechanism remains to be elucidated. Our data provide a theoretical evidence that high amounts of Ca should be supplemented to peak- and late-laying hens to meet the demands for egg production and reduce bone dissolution while simultaneously reducing the amount of P supplied to avoid wastage in poultry farming.

Declarations

Author contribution statement

Jishuang San, Zaixiang Zhang: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Shuyang Bu, Mingxi Zhang, Jianmin Hu: Analyzed and interpreted the data.

Jiancheng Yang, Gaofeng Wu: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

[1] T. Li, et al., Dietary calcium or phosphorus deficiency impairs the bone development by regulating related calcium or phosphorus metabolic utilization parameters of broilers, Poultry Sci. 99 (6) (2020) 3207–3214.
[2] J.J. Anderson, Calcium, phosphorus and human bone development, J. Nutr. 126 (4 Suppl) (1996) 1153S–1158S.
[3] S. Schmitt, B. Dobenecker, Calcium and phosphorus metabolism in peripartal dogs, J. Anim. Physiol. Anim. Nutr. 104 (2) (2020) 707–714.
[4] M.R. Wilkens, A.S. Muscher-Banse, Review: regulation of gastrointestinal and renal transport of calcium and phosphorus in ruminants, Animal 14 (2020) S29–S43.
[5] M. Schreiweis, et al., The use of densitometry to detect differences in bone mineral density and content of live White Leghorns fed varying levels of dietary calcium, Poultry Sci. 82 (8) (2003) 1292–1301.
[6] F. Guinotte, Y. Nys, Effects of particle size and origin of calcium sources on eggshell quality and bone mineralization in egg laying hens, Poultry Sci. 70 (3) (1991) 583–592.
[7] S.C. Bolukbasi, S. Celebi, N. Utlu, The Effects of Calcium and Vitamin Din Diet on Plasma Calcium and Phosphorus, Eggshell Calcium and Phosphorus Levels of Laying Hens in Late Laying Production Period, 2005.
[8] E. Kebreab, et al., Development and evaluation of a dynamic model of calcium and phosphorus flows in layers, Poultry Sci. 88 (3) (2009) 680–689.
[9] M. Proszkowiec-Weglarz, et al., Effect of early neonatal development and delayed feeding post-hatch on jejunal and ileal calcium and phosphorus transporter genes expression in broiler chickens1, Poultry Sci. 98 (4) (2019) 1861–1871.
[10] Yan, et al., Effects of vitamin D receptor inactivation on the expression of calbindins and calcium metabolism, Am. J. Physiol. Endocrinol. Metabol. 281 (3) (2001) E558.
[11] J.C. Fleet, R.D. Schoch, Molecular mechanisms for regulation of intestinal calcium absorption by vitamin D and other factors, Crit. Rev. Clin. Lab Sci. 47 (4) (2010) 181–195.
[12] Proszkowiec-Weglarz, et al., Calcium and phosphorus metabolism in broilers: effect of homeostatic;mechanism on calcium and phosphorus digestibility, J. Appl. Poultry Res. 22 (3) (2013) 609–627.
[13] I. N, et al., Redistribution of calbindin-D28k in chick intestine in response to calcium transport*, Endocrinology (6) (1991) 6.
[14] X. Qin, H. Klandorf, Effect of estrogen in relation to dietary vitamin D3 and calcium on activity of intestinal alkaline phosphatase and Ca-ATPase in immature chicks, Gen. Comp. Endocrinol. 90 (3) (1993) 318–327.
[15] Y. Sabbagh, et al., Intestinal Npt2b plays a major role in phosphate absorption and homeostasis, J. Am. Soc. Nephrol. Jasn 20 (11) (2009) 2348–2358.
[16] F. Yan, R. Angel, C.M. Ashwell, Characterization of the chicken small intestine type IIb sodium phosphate cotransporter, Poultry Sci. 86 (1) (2007) 67–76.
[17] P.A. Friedman, Calcium transport in the kidney, Curr. Opin. Nephrol. Hypertens. 8 (5) (1999) 589–595.
[18] M.B. Moor, O. Bonny, Ways of calcium reabsorption in the kidney, Am. J. Physiol. Ren. Physiol. 310 (11) (2016) F1337–F1350.
[19] J. Lytton, Na+/Ca2+ exchangers: three mammalian gene families control Ca2+ transport, Biochem. J. 406 (3) (2007) 365–382.
[20] V. Abel, 1,25-Dihydroxyvitamin D3-independent stimulatory effect of estrogen on the expression of ECaC1 in the kidney, J. Am. Soc. Nephrol. 13 (8) (2002) 2102–2109.
[21] P. Manghat, R. Sodi, R. Swaminathan, Phosphate homeostasis and disorders, Annals Clin. Biochem. An Int. J. Biochem. Laboratory Med. 51 (6) (2014) 631–656.
[22] J. Biber, et al., Regulation of phosphate transport in proximal tubules, Pflueg. Arch. Eur. J. Physiol. 458 (1) (2009) 39–52.
[23] H.M. Kronenberg and, NPT2a — the key to phosphate homeostasis, N. Engl. J. Med. 347 (13) (2002) 1022–1024.
[24] L. Underland, M. Markowitz, R. Gensure, Calcium and phosphate hormones: vitamin D, parathyroid hormone, and fibroblast growth factor 23, Pediatr. Rev. 41 (1) (2020) 3–11.
[25] S.K. Rao, D.A. Roland, F.J. Hoerr, Response of early- and late-maturing commercial leghorn pullets to low levels of dietary phosphorus, Poultry Sci. 71 (4) (1992) 691–699.
[26] M. Clunies, D. Parks, S. Leeson, Calcium and phosphorus metabolism and eggshell thickness in laying hens producing thick or thin shells, Poultry Sci. 71 (3) (1992) 490–498.
[27] C.C. Whitehead and, Overview of bone biology in the egg-laying hen, Poultry Sci. 83 (2) (2004) 193–199.
[28] H. Segawa, et al., Internalization of renal type IIc Na-Pi cotransporter in response to a high-phosphate diet, Ann. N. Y. Acad. Sci. 1043 (1) (2005) 933.
[29] L. Peng, et al., Effects of dietary phosphorus level on the expression of calcium and phosphorus transporters in laying hens, Front. Physiol. 9 (2018) 627.
[30] S.H. An, D.W. Kim, B.K. An, Effects of dietary calcium levels on productive performance, eggshell quality and overall calcium status in aged laying hens, Asian Austr. J. Animal ences 29 (10) (2016) 1477–1482.
[31] B. Arie, H. Shmuel, Vitamin D metabolism and calbindin (Calcium-Binding protein) in aged laying hens, J. Nutr. 117 (10) (1987) 1775–1779.
[32] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative CT method, Nat. Protoc. 3 (6) (2008) 1101–1108.
[33] M.J. Berridge, M.D. Bootman, H.L. Roderick, Calcium signalling: dynamics, homeostasis and remodelling, Nat. Rev. Mol. Cell Biol. 4 (7) (2003) 517–529.
[34] E. Takeda, et al., Inorganic phosphate homeostasis and the role of dietary phosphorus, J. Cell Mol. Med. 8 (2) (2004) 191–200.
[35] M. Clunies, D. Parks, S. Leeson, Calcium and phosphorus metabolism and eggshell formation of hens fed different amounts of calcium, Poultry Sci. 71 (3) (1992) 482–489.
[36] J. Davidson, et al., The calcium and phosphorus requirements of laying hens, British Poultry ence 11 (2) (1970) 231–240.
[37] M. Clunies, D. Parks, S.J.P. Leeson, Calcium and phosphorus metabolism and eggshell thickness in laying hens producing thick or thin shells 71 (3) (1992) 490–498.
[38] R.H. Harms, O.M. Junqueira, R.D. Miles, Plasma calcium, phosphorus, 25-dihydroxyvitamin D3, and 1-25-dihydroxyvitamin D3 of hens with fatty liver syndrome, Poultry Sci. 64 (4) (1985) 768–770.
[39] B. Arie, H.J.J.o.N. Shmuel, Vitamin D metabolism and calbindin (Calcium-Binding protein) in aged laying hens 117 (10) (1987) 1775–1779.
[40] E.L. OUSTERHOUT and, Effects of calcium and phosphorus levels on egg weight and egg shell quality in laying hens, Poultry Sci. 59 (7) (1980) 1480–1484.
[41] A.W. Norman, R. Jürgen, O. Lelio, The vitamin D endocrine system: steroid metabolism, hormone receptors, and biological response (calcium binding proteins)*, Endocr. Rev. (4) (1982) 4.
[42] M. Hewison, et al., Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells 170 (11) (2003) 5382–5390.
[43] A. G, et al., Candidate genes of the transcellular and paracellular calcium absorption pathways in the small intestine of laying hens 98 (11) (2019) 6005–6018.
[44] W.J. Mueller, S. Rosemary, S. Harald, Calcium metabolism and skeletal dynamics of laying pullets, J. Nutr. (1) (1964) 1.
[45] M. Gross, R. Kumar, Physiology and biochemistry of vitamin D-dependent calcium binding proteins, Am. J. Physiol. 259 (2) (1987) 195–209.
[46] T. Nijenhuis, et al., (Patho)physiological implications of the novel epithelial Ca2+ channels TRPV5 and TRPV6 446 (4) (2003) 401–409.
[47] F. Perwad, et al., Fibroblast growth factor 23 impairs phosphorus and vitamin D metabolism in vivo and suppresses 25-hydroxyvitamin D-1 -hydroxylase expression in vitro, Am. J. Physiol. Ren. Physiol. 293 (5) (2007) F1577–F1583.
[48] Dërmaku-Sopjani, et al., Downregulation of NaPi-IIa and NaPi-IIb Na-coupled phosphate transporters by coexpression of klotho, Cell. Physiol. Biochem. 28 (2) (2011) 251–258.
[49] M. Sopjani, M. Dërmaku-Sopjani, Klotho-dependent cellular transport regulation, Vitam. Horm. 101 (2016) 59–84.
[50] H. Mark R, The role of vitamin D in the FGF23, klotho, and phosphate bone-kidney endocrine axis, J. Rev. Endocrine Metabol. Disorders 13 (1) (2012) 57–69.