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Circadian calcium feeding regime in laying hens related to zinc concentration, gene expression of circadian clock, calcium transporters and oxidative status

Xue Lin[a,b], Yilin Liu[a,1], Tiantian Meng[a,b], Chunyan Xie[a], Xin Wu[a,b,*], Yulong Yin[a,b]

[a] *Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, The Chinese Academy of Sciences, National Engineering Laboratory for Pollution Control and Waste Utilization in Livestock and Poultry Production, Hunan Provincial Engineering Research Center for Healthy Livestock and Poultry Production, Scientific Observing and Experimental Station of Animal Nutrition and Feed Science in South-Central, Ministry of Agriculture, Changsha 410125, China*

[b] *Hunan Co-Innovation Center of Safety Animal Production, College of Animal Science and Technology, Hunan Agricultural University, Changsha 410128, China*

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ABSTRACT

The study was conducted to investigate the effects of different circadian calcium feeding regimes on parameters of Zn status and gene expression of circadian clock, calcium transporters and oxidative status in laying hens. In total, 180 of 41-weeks Brown Hy-line laying hens were assigned randomly into three groups, 1-CON group (Control Ca, diets contained 3.4% Ca at both 0730 and 1530 h), 2-HL group (High-low Ca, diets contained 3.6%–3.2% Ca respectively) and 3-LH group (Low-high Ca, diets contained 3.2%–3.6% Ca respectively), which were fed a certain amount of control diet at 0730 h and 1530 h. Blood, tibia, jejunum and kidney samples were collected at 4 h intervals with initial starting at 0800 h after 10 weeks of experiment. Compared with the CON group: 1) the serum zinc in HL group increased at 2000 h, but lower at 1600 h in LH group ($P < 0.05$). 2) in jejunum, circadian clock genes including CLOCK and BMAL1 expression of HL group were down-regulated at 0000 h and 1600 h, as well as CLOCK, BMAL1, Cry2, Per3 and calcium transporter gene NCX1 in LH group at 2000 h ($P < 0.05$). 3) in kidney, CLOCK, Cry1, Cry2 and Per3 of LH group were up-regulated at 0400 h, CLOCK at 0000 h as well, while CLOCK at 2000 h were down-regulated ($P < 0.05$). 4) in kidney, the calcium transporters including PMCA, CaBP and CA of LH group were up-regulated at 0000 h, but PMCA and CaBP of LH group were down-regulated at 0800 h, 1200 h and 1600 h, as well as CA at 1600 h and PMCA at 2000 h of LH group ($P < 0.05$), and the oxidative gene SOD of the LH group was up-regulated at 0400 h, as well as CAT at 0400 h, SOD and GPX1 at 1200 h in HL group, but SOD at 1600 h and 2000 h, and GPX1 at 1600 h were down-regulated in LH group ($P < 0.05$). These results demonstrated that the dynamic circadian calcium feeding regime affected circadian rhythms of serum zinc concentration as well as the expression of certain genes related to the circadian clock, calcium transport and antioxidative capacity, and circadian calcium feeding regimes may therefore be considered with regard to improving the calcium usability.

1. Introduction

Circadian rhythms regulate a wide array of metabolic and physiologic activities in a periodicity of about 24 h [1,2]. The rhythm is regulated by a circadian oscillator in the chicken pineal gland [3], and the circadian pacemaking systems in birds, requires the interaction of the pineal gland which mediates various physiological functions [4]. And the circadian clock mechanism in the brain and peripheral tissues includes CLOCK, BMAL1, core clock proteins period (PER) and cryptochrome (CRY) gene expression [5–9]. Circadian timekeeping system synchronizes slave clocks in peripheral organs to adapt to daily alternating periods during which animals either forage and feed or sleep and fast, and to regulate metabolic homeostasis corresponded to environmental changes, most notably light/dark cycles [10,11]. Urinary calcium excretion decreases at night in normal individuals, associated with the general decrease in excretion of water and minerals at night [12]. In the kidney, there are more clock regulated genes than most other organs and every cell type in the kidney follows its own circadian clock [13], and numerous genes critical for renal functions have been identified to be clock controlled, which comprises of central and peripheral

* Corresponding author at: Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Changsha 410125, China.
E-mail address: wuxin@isa.ac.cnm (X. Wu).

[1] Contribute equal to first author.

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Table 1
Sequence of Primers for Real-Time PCR.

Name of target gene	Accession number	Nucleotide sequence of primers (5′–3′)
CLOCK	AF246959.1	F: TTCTGTCTTCTCATCTGCTGGA R: GGTTGCTCTTTTGGGTCTATTG
BMAL1	AF193070.1	F: CCATCCTACTGTCCCTGGAA R: TGGCGATGATCCTCTTATCC
CRY1	NM_204245.1	F: AGACCACGAATGAACGCCAA R: CAGGACAAACAGCCAAAGCG
CRY2	NM_204244.1	F: AGTCCCTACCTGCGTTTTGG R: CTTCGGGTTGTTTGTGGCTG
PER2	NM_204262.1	F: ACCATCCCAGTTTTCTGCCC R: GTTGTGGGAAAGGGCTAGGG
PER3	NM_001289779.2	F: TTCCCTTTTCCTTTGGCTTT R: GGAAACAACCTCCCAGTGAA
PMCA	NM_204891.1	F: AGCTCAAGATGGTGCAGCTA R: TCCTAGACTGTGGGGGACTG
CaBP-d28k	NM_205513.1	F:GACTTCATGCAGACATGGAGA R:CTTGATCGTACATCTCAAAGGC
NCX1	DQ987923.1	F:TGCCCTGACCATCATTCGTC R:CGGGCTTGAAGACCACTGTC
TRPV6	XM_004938143.2	F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGCCTCGTG
CA	M60853.1	F: GCCGTCGTAGGCATCTTCA R: GCAGCAGTCCAGTAGGGTCA
OPN	NM_204535.4	F: AGGTGGACGGAGGAGACA R: ACGGGTGACCTCGTTGTT
CAT	NM_001031215.2	F: TATCAGAGGGACGGGCCAAT R: GCACTACTGAAACGCTGCAC
SOD	NM_205064.1	F: TGACTGCAAAGGGAGGAGTG R: CCCCCTCTACCCAGGTCATC
GPX1	NM_001277853.2	F: TGACCAACCCGCAGTACATC R: TTGTATGACAGAGGTGCGGG
β-actin	L08165.1	F: TTACTCGCCTCTGTGAAGGC R: TCCTAGACTGTGGGGGACTG

components, i.e. CLOCK, BMAL1, Cry1/2 and Per1/2 [14].

Calcium (Ca) is a critical nutrient which ensures the production of egg with good quality eggshell [15,16]. A definite circadian rhythm for serum calcium exists on randomly selected hens [17]. The serum calcium exhibits a circadian rhythm which varied with the changed light: dark cycle in laying hens [3], and in our previous studies, dynamic feeding low and high methionine or calcium diets regimes altered the circadian variation of serum Ca [18,19]. And the Ca transport proteins including calbindins, plasma membrane calcium ATPases (PMCAs), Na^+/Ca^{2+} exchangers (NCXs) and epithelial calcium channels (TRPVs) were considered to be involved in calcium transport [20]. It is well known that a mineral intake at high levels has interaction effects on other minerals [21]. Zinc, as a cofactor in over 300 zinc metalloenzymes, is one of the most abundant trace minerals in cells, and is essential for growth and development of nearly all organisms [22]. Previous study demonstrated that calcium addition may cause or accentuate poor utilization of zinc from soy products [23], and excessive calcium decreased zinc absorption because of the competition for mineral binding ligands [24,25].

It is speculated that the dynamic feeding time of Ca coupling with circadian rhythms may affect serum trace minerals concentration in laying hens. No attempt was made to determine relationships between circadian rhythms and calcium transport in laying hens. Therefore, the aim of the present study was conducted to study the relationship of serum Zn concentration, gene expression levels of circadian clock, Ca transporters and oxidative status in laying hens under the circadian feeding regimes with low and high Ca supplementation diets in a daily cycle.

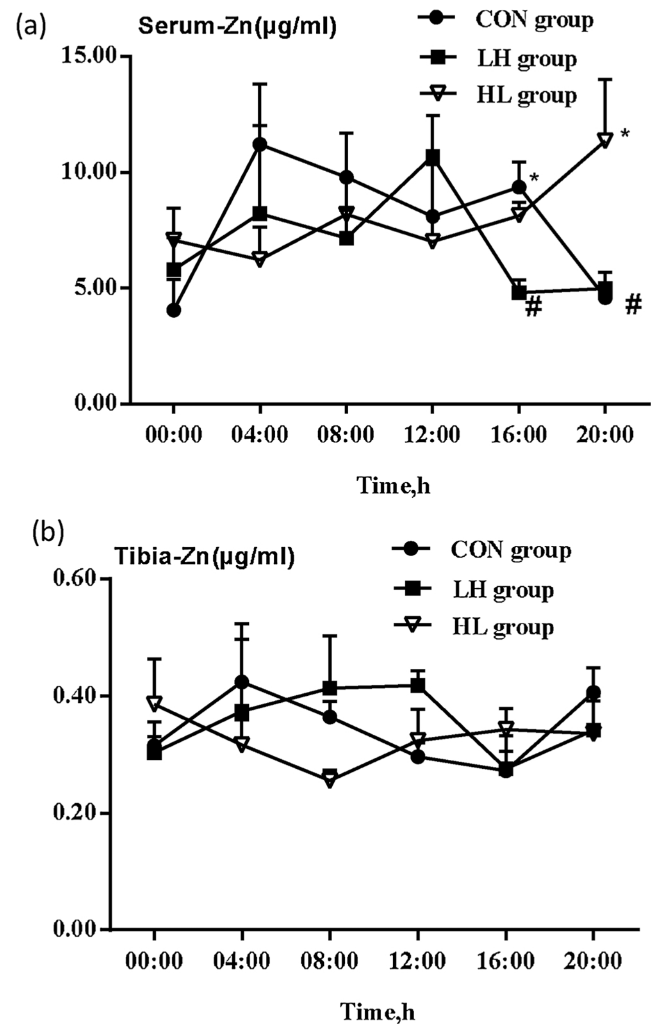

Fig. 1. Effects of dynamic calcium feeding regime on serum and tibia Zn concentration of hens. The serum Zn value of HL group was higher at 2000 h, but lower at 1600 h in LH group, when compared with the CON group ($P < 0.05$). (Data are means of 6 hens per dietary treatment per time point, * and # denote significant ($P < 0.05$) differences for: either: a) the LH group from the CON group; or b) the HL group from the CON group. Abbreviations represented: CON = Control; LH = Calcium: low-high; HL = Calcium: high-low).

2. Materials and methods

2.1. Ethics statement

Animal experiments were approved by the Animal Care Committee of the Institute of Subtropical Agriculture, Chinese Academy of Science. And all experiments were performed in accordance upon the regulations and guidelines established by this committee.

2.2. Birds and management

Birds and management were designed and performed as the same as the study [19]. A total of 180 Brown Hy-line laying hens were selected from a commercial flock at 41 weeks of age(Changsha County, Changsha City, China), then divided into 3 equal dietary treatments with 6 replications of 10 hens each, the average EP ($P = 0.989$) of