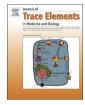
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Circadian calcium feeding regime in laying hens related to zinc concentration, gene expression of circadian clock, calcium transporters and oxidative status

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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 upregulated 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

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X. Lin et al

Table 1

Sequence of Primers	for Real-Time PCR.
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CLOCKAF246959.1F: TTCTGTCTTCTCATCTGCTGGA R: GGTTGCTCTTTTGGGTCTATTGBMAL1AF193070.1F: CCATCCTACTGTCCCTGGAA R: TGGCGATGATCCTCTTATCCCRY1NM_204245.1F: AGACCACGAATGACGCCAA R: CAGGACAAACAGCCAAAGCGCRY2NM_204244.1F: AGTCCTACCTGGGTTTTGG R: CTTCGGGTTGTTTGTGGCTGPER2NM_204262.1F: ACCATCCCAGTTTTCGGCC R: GTTGTGGGAAAGGGCTAGGGPER3NM_001289779.2F: TTCCCTTTCTTTGGTCTT R: GGAAACACCTCCCAGTGAAPMCANM_204891.1F: AGCTCAAGATGGTGCAGCTA R: TCCTAGCAGCATGGGGGAACTGCaBP-d28kNM_205513.1F:GACTTCATGCAGACATGGAGA R:CTTGATCGTACATCTCAAAGGCNCX1DQ987923.1F:GGCGTGAAGCACATGGTC R:GGGGTGAAGCACTGTCTRPV6XM_004938143.2F: GGCGTCGTAGGCATCTCA R: GCAGCATCTTCA R: GCAGCAGTCAGGAGACA R: AGCATCTGGAAGACCATGTTCOPNNM_204535.4F: AGGTGGACGAGGAGACA R: AGCATCTGGAAGACCACTGTT CATOPNNM_205064.1F: TGACTGCAAAGGGGGCCAAT R: GCACTACTGAAAGGGGGGCGAAT R: GCACTACTGAAAGGGGGGGGGGGGGGGGGGGGGGGGGGG	Name of target gene	Accession number	Nucleotide sequence of primers (5'-3')
BMAL1AF193070.1F: CCATCCTACTGTCCCTGGAA R: TGGCGATGATCCTCTTATCCCRY1NM_204245.1F: AGACCACGAATGAACCGCAA R: CAGGACAAACAGCCAAAGCGCRY2NM_204244.1F: AGTCCTACCTGCGTTTTGG R: CTTCGGGTTGTTTGTGGCTGPER2NM_204262.1F: ACCATCCCAGTTTTCGGCCT R: GTTGTGGGAAAGGGCTAGGGPER3NM_001289779.2F: TTCCCTTTTCCTTTGGCTGTT R: GGAAACACCTCCCAGTGAAPMCANM_204891.1F: AGCTCAAGATGGTGCAGCTA R: TCCTAGACTGTGGGGGACTGCaBP-d28kNM_205513.1F:GACTTCATGCAGACATGGAGA R:CTTGATCGTACATCTCAAAGGCNCX1DQ987923.1F:TGCCCTGAACATCGTCGTG R:GGGGTGAAGCCACTGTCTRPV6XM_004938143.2F: GGTGATGCGTCTTACCAGCA R: GCGGCGTGAAGGCACATGGTCA R: GCGGGGAGCGGCCAAT R: GCGGGGGACGGCCAAT R: GCGGGGGACGGCCAAT R: GCGGGGGACGGCCAAT R: GCAGCAGTCGCTTGTTCATNM_001031215.2F: TATCAGAGGGAGAGACA R: ACCCCCTGTAACGCGCGCCAAT R: GCACTACTGAAAGGCGGGCCAAT R: GCACTACTGAAAGGCGGCCAAT R: GCACTACTGAAAGGCGGGCCAAT R: GCACTACTGAAAGGGAGAGACA R: ACCCCCCTTACCCAGGGGGGGGGGGGGGGGGGGGGGGGG	CLOCK	AF246959.1	F: TTCTGTCTTCTCATCTGCTGGA
R: TGGCGATGATCCTCTTATCCCRY1NM_204245.1F: AGACCACGAATGAACGCCAA R: CAGGACAAACAGCCAAAGCGCRY2NM_204244.1F: AGTCCCTACCTGCGTTTTGG R: CTTCGGGTGTTTTGGGCTGPER2NM_204262.1F: ACCATCCCAGTTTTCTGGCCC R: GTTGTGGGAAAGGGCTAGGGPER3NM_001289779.2F: TTCCCTTTTCCTTTGGCTTT R: GGAAACAACCTCCCAGTGAAPMCANM_204891.1F: AGCTCCAAGATGGTGCAGCTA R: TTCATAGCAACATGGAGACTG R: GTTGTGGGAACAGGCTA R: TCCTAGACTGTGGGGAGATGCaBP-d28kNM_205513.1F:GACTTCATGCAACATGAGAGA R: CTTGATCGTACATCTCAAAGGCNCX1DQ987923.1F:TGCCCTGACCATCATTCGTC R:CGGGCTTGAAGACCACTGTCTRPV6XM_004938143.2F: GGTGATGCGTCTTACCAGCA R: ACCATCTGGAAGCCTCCTGCAM60853.1F: GCCGTCGTAGGCATCTCA R: GCAGTCCATGATGGGCAACA R: ACGGGTCAATGCAACAGCACA R: ACGGGTCAATGCAAAGGCAGACA R: ACGGGTGACCTCGTTGTTCATNM_001031215.2F: TATCAGAGGAGGAGACA R: CCCCTCTACCCAGGTCATC R: CCACACCCAGGTCAATC R: CCCCCTCTACCCAGGTCATC R: TTGTATGACAGAGGTGCGGG GF-actinL08165.1F: TTACTGCCCTGTGGAAGGCF: TTACTGCCAAGGGG			R: GGTTGCTCTTTTGGGTCTATTG
CRY1NM_204245.1F: AGACCACGAATGAACGCCAA R: CAGGACAAACAGCCGAAAGCGCRY2NM_204244.1F: AGTCCTACCTGCGTTTTGG R: CTTCGGGTGTTTTGGGCTGPER2NM_204262.1F: ACCATCCCAGTTTTCTGGCCG R: GTTGTGGGAAAGGGCTAGGGPER3NM_001289779.2F: TTCCCTTTTCCTTTGGCTTT R: GGAAACAACCTCCCAGTGAAPMCANM_204891.1F: AGCTCAAGATGGTGCAGCTA R: TTCATGCGACATGGGGAGAGGCGCaBP-d28kNM_205513.1F:GACTTCATGCGACATGGAGANCX1DQ987923.1F:TGCCTGACCATCATTCGTC R:CGGGCTTGAAGACCACTGTCTRPV6XM_004938143.2F: GGTGATGCGTCTTACCAGCA R: ACCATCTGGAGGAGACGOPNNM_204535.4F: AGCTGGACGGAGGAGCA R: ACGGTGCACCTGTTTCATNM_001031215.2F: TATCAGAGGGAGGGGCCAAT R: GCCATCATGGAAAGCGCTGCACSODNM_205064.1F: TGACTGCAAAGGGAGGAGCA R: CCCCCTCTACCAGGGAGACA R: CCCCCTCTACCAGGGAGACAC R: CCCCCTCTACCCAGGTCATC R: CCCCCTCTACCCAGGTCATC R: TTGTATGAAAGGAGGGGGGGGGGGGGGGGGGGGGGGGG	BMAL1	AF193070.1	F: CCATCCTACTGTCCCTGGAA
R: CAGGACAAACAGCCAAAGCGCRY2NM_204244.1F: AGTCCCTACCTGCGTTTTGG R: CTTCGGGTTGTTGTGGCTGPER2NM_204262.1F: ACCATCCCAGTTTCTGGCCC R: GTTGTGGGAAGGGCTAGGGPER3NM_001289779.2F: TTCCCTTTGCCTTTGGCTTT R: GGAAACAACCTCCCAGTGAAPMCANM_204891.1F: AGCTCAAGATGGTGCAGCTA R: TCCTAGACTGTGGGGGACTGCaBP-d28kNM_205513.1F:GACTTCATGCAGACATGGGGANCX1DQ987923.1F:TGCCTGACCATCTCAAAGGCNCX1DQ987923.1F:GGCGTGAAGACCACTGTC R:CGGGCTTGAAGACCACTGTCTRPV6XM_004938143.2F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGCCTCGTGCAM60853.1F: GCCGTGAGCGATGAGGGCAA R: AGCGTCCAGTAGGGTCAOPNNM_204535.4F: AGCGGGGAGGAGACA R: ACGGGTGACCTGGTTTCATNM_001031215.2F: TATCAGAGGGACGGGCCAAT R: GCACTACTGAAAGGCGGCGCAAT R: GCACTACTGAAAGGCGGGCCAAT R: GCACTACTGAAAGGAGGAGGAGG R: CCCCCTCTACCCAGGTGATC GPX1NM_001277853.2β-actinL08165.1F: TATCAGGCTCTGTGAAGGC			R: TGGCGATGATCCTCTTATCC
CRY2NM_204244.1F: AGTCCCTACCTGCGTTTTGG R: CTTCGGGTTGTTGTGGCTGPER2NM_204262.1F: ACCATCCCAGTTTTCGTGCCC R: GTTGTGGGAAAGGCTAGGGPER3NM_001289779.2F: TCCCTTTTCCTTGGCTTT R: GGAACCACCTCCCAGTGAAPMCANM_204891.1F: AGCTCAAGATGGTGCAGCTA R: TCCTAGACTGTGGGGGACTGCaBP-d28kNM_205513.1F:GACTTCATGCAGACATGGAGANCX1DQ987923.1F:TGCCTGACATCTCAAAGGCNCX1DQ987923.1F:GGTGATGCGTCTTACCAGCA R: GGGGCTTGAAGACCACTGTCTRPV6XM_004938143.2F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGCCTCGTGCAM60853.1F: GCCGTCGTAGGCATCTTCA R: GCAGCAGTCAGTAGGGTCAOPNNM_204535.4F: AGCGGGTGAAGGAGACA R: AGCGTGCACCTGCTTTTCATNM_001031215.2F: TATCAGAGGGAGGGGCCAAT R: GCACTACTGAAAGCCTGCACSODNM_205064.1F: TGACTGCAAAGGGAGGACG R: CCCCCTCTACCCAGGTGATC R: TGCACAACGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	CRY1	NM_204245.1	F: AGACCACGAATGAACGCCAA
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PER2NM_204262.1F: ACCATCCCAGTTTTCTGCCC R: GTTGTGGGAAAGGGCTAGGGPER3NM_001289779.2F: TTCCCTTTTCCTTTGGCTTT R: GGAACAACCTCCCAGTGAAPMCANM_204891.1F: AGCTCAAGATGGTGGAGACTGCaBP-d28kNM_205513.1F:GACTTCATGCAGCATGAGGGNCX1DQ987923.1F:TGCCCTGACACATCGTGGGGACA R:CTTGATCGTACATCTCAAAGGCTRPV6XM_004938143.2F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGACCACTGTGCAM60853.1F: GGCTGCAAGGAGAGACA R: AGCATCTGGAAGACCACTGTCOPNNM_204535.4F: AGGTGGACGGAGGAGACA R: ACGGGTGACCTCGTTGTTCATNM_001031215.2F: TATCAGAGGAGGAGGAGCA R: GCACTACTGAAAGGCTGCACCSODNM_205064.1F: TGACCAAAGGAGGAGGAGTG R: CCCCCTCTACCCAGGTCATC GPX1L08165.1F: TTATCAGAGGAGGAGGGGGGGGGGGGGGGGGGGGGGGGG	CRY2	NM_204244.1	F: AGTCCCTACCTGCGTTTTGG
R: GTTGTGGGAAAGGGCTAGGGPER3NM_001289779.2F: TTCCCTTTTCCTTTGGCTTT R: GGAAACAACCTCCCAGTGAAPMCANM_204891.1F: AGCTCAAGATGGTGCAGCTA R: TCCTAGACTGTGGGGGACTGCaBP-d28kNM_205513.1F:GACTTCATGCAGACATGGAGA R:CTTGATCGTACATCTCAAAGGCNCX1DQ987923.1F:TGCCCTGACCATCATTCGTC R:CGGGCTTGAAGACCACTGTCTRPV6XM_004938143.2F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGACCACTGTCCAM60853.1F: GCGGTCGTAGGCATCATCCA R: GGCAGCCAGTAGGAGACA R: AGCATCTGGAAGACCACTGTGOPNNM_204535.4F: AGGTGACGCAGAGAGACA R: ACGGGTGACCTCGTTGTTCATNM_001031215.2F: TATCAGAGGAGGAGCACA R: GCACTACTGAAAGGCTGCACSODNM_205064.1F: TGACCAAAGGAGGAGGAGAGG R: CCCCCTCTACCCAGGTCATC R: TCGACAACGGAGGAGAGTG R: CCCCCTCTACCCAGGTCATC R: TTGTATGACAGAGGTGCGGGGPX1NM_001277853.2F: TTACTCGCCCTGTGGAAGGCβ-actinL08165.1F: TTACTCGCCTCTGTGAAGGC			R: CTTCGGGTTGTTTGTGGCTG
PER3NM_001289779.2F: TTCCCTTTCCTTTGGCTTT R: GGAAACAACCTCCCAGTGAAPMCANM_204891.1F: AGCTCAAGATGGTGCAGCTA R: TCCTAGACTGTGGGGACTGCaBP-d28kNM_205513.1F:GACTTCATGCAGACATGGAGA R:CTTGATCGTACATCTCAAAGGCNCX1DQ987923.1F:TGCCCTGACCATCATTCGTC R:CGGGCTTGAAGACCACTGTCTRPV6XM_004938143.2F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGCCTCGTGCAM60853.1F: GCGGTCGTAGGCATCTTCA R: GCAGTCCAGTAGGGTCAOPNNM_204535.4F: AGGTGGACGGGGGGGACA R: ACGGTGGACGGGCCAAT R: GCATCTGTAAAAGGCTGCACSODNM_205064.1F: TGACCAAAGGGAGGAGATG R: CCCCCTCTACCCAGGTCATC R: TGACTACAAGGAGGGGGGGGGGGGGGGGGGGGGGGGGGG	PER2	NM_204262.1	F: ACCATCCCAGTTTTCTGCCC
R: GGAAACAACCTCCCAGTGAAPMCANM_204891.1F: AGCTCAAGATGGTGCAGCTA R: TCCTAGACTGTGGGGGACTGCaBP-d28kNM_205513.1F:GACTTCATGCAGACATGGGAGA R:CTTGATCGTACATCTCAAAGGCNCX1DQ987923.1F:TGCCCTGACCATCATTCGTC R:CGGGCTTGAAGACCACTGTCTRPV6XM_004938143.2F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGCCTCGTGCAM60853.1F: GCCGTGGTAGGCATCTTCA R: GCAGCAGCAGAGAGACA R: AGCGTGCAGCAGCAGGAGACA R: ACGGTGACGGGCAAT R: GCAGGGAGGAGACA R: ACGGGTGACCTGTTGTTCATNM_001031215.2F: TATCAGAGGGAGGGGCCAAT R: GCCACTCGTGAAAGCCTGCACSODNM_205064.1F: TGACTGCAAAGGGAGGAGATG R: CCCCCTCTACCCAGGTCATC R: TGACCAACCGCAGTACATC R: TGACCAACCGCAGTACATC R: TGACCAACGCGGGGGGACATC R: TGACCAACGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGG			R: GTTGTGGGAAAGGGCTAGGG
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R: TCCTAGACTGTGGGGGACTG CaBP-d28k NM_205513.1 F:GACTTCATGCAGACATGGAGA R:CTTGATCGTACATGCAAAGGC NCX1 DQ987923.1 F:TGCCCTGAACATCATCGTAAGGC RCGGGCTTGAAGACCACTGTC TRPV6 XM_004938143.2 F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGACCACTGTC CA M60853.1 F: GCGGTGGAGGCACTCTTCA R: CGCAGCAGTCCAGTAGGTCA OPN NM_204535.4 F: AGGTGGACGGAGGAGACA R: AGCATCTGGAAACCTCGTTGTT CAT NM_001031215.2 F: TATCAGAGGGACGGGCCAAT R: GCACTACTGAAACGCTGCAC SOD NM_205064.1 F: TGACTGCAAAGGGAGGAGTG R: CCCCCTCTACCCAGGTCATC GPX1 NM_001277853.2 F: TGACCAACCGCAGTACATC R: TTGTATGACAAGAGGTGGGGG β-actin L08165.1			R: GGAAACAACCTCCCAGTGAA
CaBP-d28k NM_205513.1 F:GACTTCATGCAGACATGGAGA R:CTTGATCGTACATCTCAAAGGC NCX1 DQ987923.1 F:TGCCCTGAACATCTCCAAAGGC TRPV6 XM_004938143.2 F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGCCTCGTG CA M60853.1 F: GCCGTCGTAGGCATCATCGTC R: CGGGCTGGAGGCATCATCAC R: AGCACTCTGCAAGCCTCGTG OPN NM_204535.4 F: AGGTGGACGGAGGAGACA R: ACGGGTGACCTCGTTGTT CAT NM_001031215.2 F: TATCAGAGGGAGGGGCCAAT R: GCACTACTGGAAAGGCTGCAC SOD NM_205064.1 F: TGACCTACAAGGGAGGAGTG R: CCCCCTCTACCCAGGTCATC GPX1 NM_001277853.2 F: TGACCAACAGGGGGGGGGGGGGGGG β-actin L08165.1 F: TTACTGCCCTCTGTGAAGGC	PMCA	NM_204891.1	F: AGCTCAAGATGGTGCAGCTA
R:CTTGATCGTACATCTCAAAGGC NCX1 DQ987923.1 F:TGCCCTGACCATCATTCGTC R:CGGGCTTGAAGACCACTGTC TRPV6 XM_004938143.2 F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGACCACTGTC CA M60853.1 F: GCCGTCGTAGGCATCTTCA R: GCAGCAGTCCATAGGGTCA OPN NM_204535.4 F: AGGTGGACGGAGGAGACA R: ACGGGTGACCTCGTTGTT CAT NM_001031215.2 F: TATCAGAGGGAGGGGCCAAT R: GCACTACTGAAAGGCTGCAC SOD NM_205064.1 F: TGACCAAAGGAGGAGAGTG R: CCCCCTCTACCCAGGTCATC GPX1 NM_001277853.2 F: TGACCAACAGAGGTGCGGG β-actin L08165.1			R: TCCTAGACTGTGGGGGGACTG
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R:CGGGCTTGAAGACCACTGTC TRPV6 XM_004938143.2 F: GGTGATGCGTCTTACCAGCA R: AGCATCTGGAAGCCTCGTG R: AGCATCTGGAAGCCTCTGTG CA M60853.1 F: GCCGTCGTAGGCATCTCA OPN NM_204535.4 F: AGGTGACGGAGGAGACA R: ACGGGTGACCTCGTTGTT R: ACGGGTGACCTCGTTGTT CAT NM_001031215.2 F: TATCAGAGGGACGGGCCAAT R: GCACTACTGAAAGCCTGCAC SOD NM_205064.1 F: TGACTGCAAAGGGAGGGGCCAAT R: CCCCCTCTACCCAGGTCATC R: CCCCCCTCTACCCAGGTCATC R: TGCCAACCGCAGTACATC GPX1 NM_001277853.2 F: TGACCAACCGCCAGTACATC β-actin L08165.1 F: TTACTGCCCTCTGTGAAGGC			R:CTTGATCGTACATCTCAAAGGC
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CA M60853.1 F: GCCGTCGTAGGCATCTTCA OPN NM_204535.4 F: AGGTGGACGGAGGAGACA CAT NM_001031215.2 F: TATCAGAGGGACGCACTGTTT CAT NM_205064.1 F: TGACTGCAAAGGGAGGAGGAG SOD NM_001277853.2 F: TGACCACCCGCAGTCACTCC GPX1 L08165.1 F: TATCAGAAGGGTGCGGGG			R:CGGGCTTGAAGACCACTGTC
CA M60853.1 F: GCCGTCGTAGGCATCTTCA R: GCAGCAGTCCAGTAGGGTCA R: GCAGCAGTCCAGTAGGGTCA OPN NM_204535.4 F: AGGTGGACGGAGGAGACA R: ACGGGTGACCTCGTTGTT R: ACGGGTGACCTCGTTGTT CAT NM_001031215.2 F: TATCAGAGGACGGGGCCAAT R: GCACTACTGAAAGGCTGCAC R: GCACTACTGAAAGGCTGCAC SOD NM_205064.1 F: TGACCAAAGGGAGGAGGAGGTG R: CCCCTCTACCCAGGTCATC R: CCCCCTCTACCCAGGTCATC GPX1 NM_001277853.2 F: TGACCAACGGCGGGG β-actin L08165.1 F: TTACTCGCCTCTGTGAAGGC	TRPV6	XM_004938143.2	F: GGTGATGCGTCTTACCAGCA
R: GCAGCAGTCCAGTAGGGTCA OPN NM_204535.4 F: AGGTGGACGGAGGAGACA R: ACGGGTGACCTCGTTGTT CAT NM_001031215.2 F: TATCAGAGGGACGGGCCAAT R: GCACTACTGAAAGGCTGCAC SOD NM_205064.1 F: TGACCACAAGGGAGGAGTG R: CCCCCTCTACCCAGGTCATC R: CCCCCCTCTACCCAGGTCATC GPX1 NM_001277853.2 F: TGACCAACAGGGGGGGG β-actin L08165.1 F: TTACTCGCCTCTGTGAAAGGC			R: AGCATCTGGAAGCCTCGTG
OPN NM_204535.4 F: AGGTGGACGGAGGAGACA R: ACGGGTGACCTCGTTGTT R: ACGGGTGACCTCGTTGTT CAT NM_001031215.2 F: TATCAGAGGGACGGGCCAAT R: GCACTACTGAAACGCTGCACC R: GCACTACTGAAACGCTGCACC SOD NM_205064.1 F: TGACCAAAGGGAGGAGTG R: CCCCTCTACCCAGGTCATC R: CCCCCTCTACCCAGGTCATC GPX1 NM_001277853.2 F: TGACCAACGAGGGGGGG β-actin L08165.1 F: TTACTCGCCTCTGTGAAAGGC	CA	M60853.1	F: GCCGTCGTAGGCATCTTCA
R: ACGGGTGACCTCGTTGTT CAT NM_001031215.2 F: TATCAGAGGGACGGGCCAAT R: GCACTACTGAAACGCTGCAC SOD NM_205064.1 F: TGACTGCAAAGGGAGGGGCGAGTG R: CCCCCTCTACCCAGGTCATC GPX1 NM_001277853.2 β-actin L08165.1 F: TTACTGCCCTCTGTGAAGGC			R: GCAGCAGTCCAGTAGGGTCA
CAT NM_001031215.2 F: TATCAGAGGGACGGGCCAAT R: GCACTACTGAAACGCTGCAC SOD NM_205064.1 F: TGACTGCAAAGGGAGGAGTG R: CCCCCTCTACCCAGGTCATC GPX1 NM_001277853.2 F: TGACCAACCCGCAGTACATC R: TTGTATGACAGAGGTGCGGGG β-actin L08165.1 F: TTACTCGCCTCTGTGAAGGC	OPN	NM_204535.4	F: AGGTGGACGGAGGAGACA
R: GCACTACTGAAACGCTGCAC SOD NM_205064.1 F: TGACTGCAAAGGGAGGAGTG R: CCCCCTCTACCCAGGTCATC R: CCCCCTCTACCCAGGTCATC GPX1 NM_001277853.2 F: TGACCAACCCGCAGTACATC β-actin L08165.1 F: TTACTCGCCTCTGTGAAAGGC			R: ACGGGTGACCTCGTTGTT
SOD NM_205064.1 F: TGACTGCAAAGGGAGGAGTG GPX1 NM_001277853.2 F: TGACCAACCCGCAGTACATC β-actin L08165.1 F: TTACTCGCCTCTGTGAAGGC	CAT	NM_001031215.2	F: TATCAGAGGGACGGGCCAAT
GPX1 NM_001277853.2 F: TGACCAACCCGCAGTACATC β-actin L08165.1 F: TTACTCGCCTCTGTGAAGGC			R: GCACTACTGAAACGCTGCAC
GPX1 NM_001277853.2 F: TGACCAACCCGCAGTACATC R: TTGTATGACAGAGGTGCGGG R: TTGTATGACAGAGGTGCGGGG β-actin L08165.1 F: TTACTCGCCTCTGTGAAGGC	SOD	NM_205064.1	F: TGACTGCAAAGGGAGGAGTG
R: TTGTATGACAGAGGTGCGGG β-actin L08165.1 F: TTACTCGCCTCTGTGAAGGC			R: CCCCCTCTACCCAGGTCATC
β-actin L08165.1 F: TTACTCGCCTCTGTGAAGGC	GPX1 NM_001277853.2	F: TGACCAACCCGCAGTACATC	
1			R: TTGTATGACAGAGGTGCGGG
R: TCCTAGACTGTGGGGGACTG	β-actin	L08165.1	F: TTACTCGCCTCTGTGAAGGC
			R: TCCTAGACTGTGGGGGGACTG

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⁺/Ca²⁺ 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. Journal of Trace Elements in Medicine and Biology xxx (xxxx) xxx-xxx

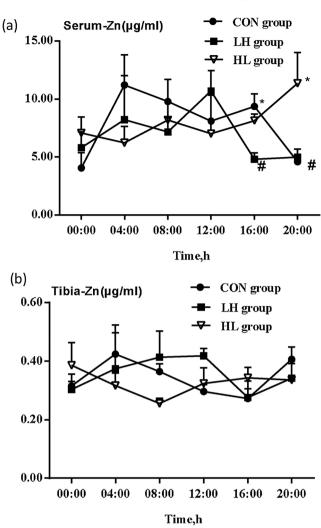


Fig. 1. Effects of dynamic calcium feeding regime on serum and tibia Zn concentration of hens. The serum Zn value of HL group was higher at 2000 h, but lower at 1600 h in LH group, when compared with the CON group (P < 0.05). (Data are means of 6 hens per dietary treatment per time point, * and # denote 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 Download English Version:

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