Effect of photoperiod on eggshell quality and quality characteristics of tibia, femur, and ulna in laying ducks

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ABSTRACT We investigated the effect of photoperiod on eggshell quality, bone quality characteristics and bone metabolism related enzymes and factors in laying ducks. After adaption, 300 Jinding laying ducks (252-day-old) were randomly divided into 5 treatments, receiving 12**L** (hours of light):12**D** (hours of darkness), 14L:10D, 16L:8D, 18L:6D or 20L:4D, respectively. Each group had 6 replicates of 10 birds each. The feeding experiment lasted 8 wk. Compared with 12L:12D, the higher values of eggshell breaking strength occurred in ≥18 h photoperiods at the end of 6 wk, and in ≥16 h photoperiods at the end of 4 wk, with the common highest values in 18 h photoperiod ($P < 0.05$). Besides, 18L:6D had higher values of ultimate load Fu and cortical cross-sectional area A in tibia, femur, and ulna ($P < 0.05$), compared with 12L:12D. The higher values of proximal bone mineral content (**BMC**; tibia), distal BMC (ulna), total Ca (tibia), and cortical volumetric bone mineral density (**vBMC**; tibia and ulna) were observed in 16L:8D and 18L:6D treatments ($P < 0.05$). Meanwhile, 18 h photoperiod group had the higher proximal BMC (femur) and total Ca in ulna ($P < 0.05$). In serum, compared with 12L:12D group, the higher ALP activity occurred in ≥16 h photoperiods (0:00 and 18:00), with the highest values in 18L:6D treatment ($P < 0.05$); the higher values of TGF-β (6:00) and OC (6:00 and 18:00) were simultaneously observed in 18 h photoperiod ($P < 0.05$). Moreover, values of trACP activity, TNF-α and IL-6 contents decreased in ≥18 h photoperiods at 0:00 ($P < 0.05$), compared with 12L:12D group. To sum up, an appropriate photoperiod could improve eggshell quality, bone strength and mineral content through increasing osteogenesis during the light time and decreasing resorption activity during the dark, and 18 h is an adequate photoperiod for the eggshell and bone quality of laying ducks.

Key words: bone quality, eggshell, laying duck, photoperiod

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INTRODUCTION

Laying duck production has been an important industry in China, producing 3,070 thousand t eggs in 2018 which mean 42.3 billion yuan (Liu and Xu, 2019). The avian eggshell is an indispensable part of the egg, as it supplies physical protection and nutrients to the developing embryo and protects the egg contents from microbial contamination (Zhang et al., 2018). Besides, improvement in eggshell quality can decrease economic loss caused by the breakage of eggshells. Raw materials of eggshell formation, for example Ca, mainly derive directly from digestive tract in the day and the remaining portion come from bone resorption during the dark (Miller, 1992). Therefore, different photoperiods mean different manners and percentages of eggshell formation raw materials supply, which may probably have an enormous effect on eggshell quality. Furthermore, in the previous report (Farghly et al., 2019), authors found that appropriate light time and regime could significantly increase eggshell thickness of Rhode Island Red laying hens (during 20−36 wk of age). However, to the best of our knowledge, there are very few reports concerning the effect of photoperiod on eggshell quality of laying ducks. Moreover, there has been no consistent photoperiod protocol for laying ducks in practical production. Hence, more work is needed to evaluate the effect of photoperiod on eggshell quality, and further explore the optimal photoperiod for laying ducks from the standpoint of eggshell quality.

Variation in the degree of bone resorption response to different photoperiods may also affect the bone quality

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of laying birds. In fact, skeletal quality has been an important welfare, health and economic issue in poultry production. Structural bone loss related to osteoporosis may be the major skeletal problem in laying poultry (Kim et al., 2007), which could cause a high incidence of fractures at various sites of the skeleton, resulting in an average of 34% of processed birds exhibiting freshly broken bones (Whitehead and Fleming, 2000). In fact, superior bone quality of laying poultry can be ascribed to 2 factors: more structural bone formation during the growth phase and less bone loss during the laying period (Whitehead, 2004). Therefore, reducing bone loss is crucial to bone health of laying birds during their whole life. However, there were very few studies exploring the effect of photoperiod on bone quality of laying birds, and the only published researches even has been seen in laying ducks. Hence, the effect of extended photoperiods on bone quality will be investigated in this research, and a suitable photoperiod is expected to obtain for bone quality of laying ducks.

Bone resorption aimed to supply for the formation of eggshell seems to be the main reason for bone quality decline of birds during the laying period. In fact, bone tissue undergoes dynamic generation and decomposition every day, and the comprehensive outcome of them is the fundamental reason for the bone quality variation (Whitehead, 2004). Therefore, the effects of photoperiod on bone metabolism related enzymes and factors in serum need to be investigated to reflect the activities of osteoblast and osteoclast, which determines the situation of resorption and remodeling happened in bone tissue (Zarrinkalam et al., 2012). The purpose of this research is to evaluate the effect of photoperiod on eggshell quality, quality characteristics of tibia, femur, and ulna, as well as bone metabolism related enzymes and factors in serum. A suitable photoperiod for eggshell and bone quality of Jinding laying ducks is expected to obtain.

MATERIALS AND METHODS

Birds, Treatments, and Husbandry

All experimental protocols were approved by the Animal Care and Use Committee of the Feed Research Institute of the Chinese Academy of Agricultural Sciences. Laying ducks were fed under natural light before the experiment. Then, they were transferred into light-controlled cages. At the beginning, laying ducks didn't adapt the new environment, evidenced by decreased in average daily feed intake (**ADFI**) and egg production, as well as some molt. One month later, this situation got better. Two months later, the productive performance indicators and mental state had been recovered, evidenced by the normal egg production and ADFI. Then, we started our formal experiment. A total of 300 Jinding laying ducks (252-day-old) were randomly divided into 5 treatments with a corn-soybean meal diet (Cui et al., 2021) for 8 wks. Each group contained 6 replicates with 10 ducks per replicate. An individual room (200 × 90 × 60 cm; length × width × height), containing automatically controlled light timers as well as adjustable light intensity, temperature and ventilation (Cui et al., 2019a), was prepared for each replicate. Ducks received 5 lighting programs: 12**L** (hours of light):12**D** (hours of darkness), 14L:10D, 16L:8D, 18L:6D and 20L:4D, respectively. The beginning times of these 5 photoperiods were 6:00, 5:00, 4:00, 3:00, and 2:00 am, respectively, whereas the ending times were 6:00, 7:00, 8:00, 9:00, and 10:00 pm, respectively. All the birds received light-emitting diode light (white color) with an average intensity of 20 (±1.0) lux at eye level, during the light hours. A programmed ventilation of the whole aviary and cleaning of litters twice a day were adopted to guarantee air quality. Water and diet (in pellet form) were provided ad libitum.

Egg Quality

At the end of 4, 6, and 8 wks of this trial, 5 eggs per replicate were collected for the measurement of egg quality. This determining work was finished within 24 h following eggs being laid. Eggshell breaking strength and thickness were evaluated with the Egg Force Reader (Oraka Food Technology Ltd., Ramat Hasharon, Israel) and Egg Shell Thickness Gauge (ESTG-1, Orka Food Technology Ltd., Ramat Hasharon, Israel), respectively. In detail, 3 points (sharp end, equator, and blunt end) per egg were determined to obtain the value of eggshell thickness. Albumen height, Haugh units, and yolk color were tested by the Egg Multi Tester EMT-500 (Robotmation Co. Ltd., Tokyo, Japan).

Bone Basic Characteristics and Mechanical Traits

Basic characteristics of left femur, tibia, and ulna from 2 ducks each replicate were collected and measured. These samples were weighted and noted (W). Whereafter, they were put into a graduated cylinder with some water. The initial (V1) and final volumes (V2) were noted, and the bone volume was calculated as (V) = V2 − V1. The density of bone was obtained as follows: W/V. Besides, bone index was expressed as a ratio of the bone weight in comparison to body weight.

Following basic characteristics analysis, these bones were adopted to execute bone strength analysis, while the right counterparts (from the sample duck) for geometry and ash content assessment. After thawing overnight at 4℃, 3-point bending test of bone mid-diaphysis was carried out using a machine (TMS-Pro, Food Technology Ltd., SV) equipped with an interchangeable load cell (model ILC-S, range of forces from 0 to 1,000 N), for bone mechanical properties evaluation. The distances (**L**) between 2 rounded support bars were 3, 7, and 7 cm for femur, tibia, and ulna, respectively. The force was loaded in the anterior-posterior (**A-P**) plane of bone at a displacement rate of 2 mm·min^{-1} until fracture (Brzóska et al., 2005).

On the basis of load-displacement curve, the following bone mechanical traits were measured, including yield load (**Fy**), displacement at yield (**d-Fy**), ultimate load (**Fu**), displacement at fracture (**d-Fu**), stiffness (**S**), elastic energy (**Wy**), work to fracture (**Wu**), according to the description in Cui et al. (2019a).

Bone Geometric Properties

Bone samples were cut carefully at midpoint using a precision saw. A digital caliper was adopted to measure the horizontal (medial-lateral, **M-L** plane) external and internal cortical bone diameter (**H** and **h)** as well as the vertical (anterior-posterior, A-P plane) external and internal cortical bone diameter (**B** and **b)**. Based on these results, the mean relative wall thickness (**MRWT**), the mean cortical index (**MCI),** and the cortical cross-sectional area (**A**) were calculated. Besides, when the force loaded in the A-P plane, the radius of gyration (**Rg**) about medial-lateral (M-L) axis and second (cross-sectional) moment of inertia (**Ix**) could be calculated. The second moment of inertia Ix is a critical property in terms of bone bending rigidity evaluation, although it is not a direct bone geometric trait (Regmi et al., 2016). They were calculated from these following equations (Brzóska et al., 2005; Muszyński et al., 2017):

$$MRWT = [(B-b)/b + (H-h)/h]/2; MCI = [(B-b)/B + (H-h)/H]/2;$$

$$A = \pi \cdot (H \cdot B - h \cdot b)/4; Ix = \pi \cdot \left(H \cdot B^3 - h \cdot b^3\right)/64; Rg = \sqrt{\frac{Ix}{A}}$$

After bone geometric properties measurement, these samples were collected to determine the contents of ash, Ca, and P in bone.

Bone Material Properties

Based on the mechanical (Fy, Fu, d-Fy, d-Fu, S) and geometric traits (Ix, B, L), the material properties of the mid-diaphyseal fragment (tibia, femur, and ulna) were obtained. These material properties are used to describe the intrinsic mechanical properties of midshaft cortical tissue, and are independent of bone size as well as the conditions under which mechanical properties are measured. The young modulus of elasticity (**E**), elastic stress (**σy**), and ultimate stress (**σu**) were calculated according to the following formulas descripted in Cui et al (2019a):

$$E = \left(S \cdot L^3\right)/(48 \cdot Ix)$$

$$\sigma y = (Fy \cdot B \cdot L)/(8 \cdot Ix)$$

$$\sigma u = (Fu \cdot B \cdot L)/(8 \cdot Ix)$$

Among them, the young modulus of elasticity (E) is used to reflect the bending resistance of bone. Meanwhile, the elastic stress σy describes the elastic strength, and the ultimate stress σu means the stress of midshaft cortical bone at fracture (Brzóska et al., 2005; Muszyński et al., 2017).

Bone Ash, Ca, and P Contents

The ash content was expressed relative to the fat-free dry weight of bone. Flame atomic absorption spectrophotometry (Zeenit700P, Analytik Jena, Germany) was adopted to determine the content of Ca, and a spectrophotometer (UV-2700, Shimadzu, Japan) was used to analyze P content.

Bone Mineral Measurement

Bone mineral characteristics of femur, tibia and ulna from right side (1 bird per replicate) were measured using dual energy x-ray absorptiometry (**DEXA**) system (DTX-200, Osteometer MediTech, Hawthorne, CA). Bone mineral density (**BMD**) and bone mineral content (**BMC**) were detected at proximal, distal heads, and the mid-diaphyseal region, respectively. All the measurements were finished by the same operator.

Quantitative Computed Tomography

The architectural characteristics of femur, tibia and ulna from right side (1 bird per replicate) were measured by quantitative computed tomography (**QCT**), using a Sky-Scan micro CT scanner (SkyScan 1172 X-ray microtomograph, Antwerp, Belgium). Scan sites at tibia, femur and ulna included the mid-diaphysis for volumetric BMD (**vBMD**) of cortical bone, and metaphyseal for trabecular bone vBMD. The data were obtained at 80 kVp and 112 μA with a resolution of 15 μm. Volumetric analysis was performed with the aid of Skyscan software. For cortical bone, 100 slices were used at mid-diaphysis while 200 metaphyseal slices were used for trabecular bone analysis.

Bone Metabolism Related Enzymes and Factors

Commercial kits were adopted to measure the activities of alkaline phosphatase (**ALP,** Nanjing Jiancheng, Bioengineering Institute, Jiangsu, China) and tartrate-resistant acid phosphatase (**trACP,** Shanghai Meilian Biological Technology Co., LTD., Shanghai, China) in serum. The serum samples were obtained at 0:00, 6:00, 12:00, and 18:00 respectively, at 309 d of age. The levels of bone metabolism related factors were determined with ELISA kits for ducks (Shanghai Meilian Biological Technology Co., LTD., Shanghai, China), including transforming growth factor-β (**TGF-β**), insulin-like growth factor-I (**IGF-I**), osteocalcin (**OC**), tumor necrosis factor-α (**TNF-α**), interleukin-1 (**IL-1**), and interleukin-6 (**IL-6**).

Statistical Analysis

All data were analyzed with SAS, version 9.2. The replicate (5 eggs per replicate) was the experimental unit for the analysis of egg quality. For bone and serum characteristics, the mean of bones (from 2 birds per replicate) served as an experimental unit, except for the QCT and DEXA analyses, which used samples from 1 leg of ducks. The homogeneity of variances and normality of the data were checked first. The Shapiro-Wilk test was used to test the normality. Then, a one-way ANOVA and Duncan's Multiple Range Test were adopted for data analysis. The linear and quadratic effects of photoperiod were evaluated by regression. Differences were supposed to be statistically significant at $P < 0.05$. Data were showed as the mean and pooled SEM.

The PROC REG used in regression analysis and statistical models were as follows (Cui et al., 2018),

$$\mathrm{Yij} = \alpha + \beta_1 \mathrm{Xi} + \mathrm{eij} \text{ (linear)},$$

$$\mathrm{Yij} = \alpha + \beta_1 \mathrm{Xi} + \beta_2 \mathrm{Xi}^2 + \mathrm{eij} \text{ (quadratic)}.$$

Yij was the response variable; α was the intercept (indicators with the 12 h of light); β_1 and β_2 were regression coefficients; Xi was the studied factor effect as hours of light (i = 12, 14, 16, 18, 20), and eij was the observational error for (ij)th observation.

RESULTS

Egg Quality

The effect of photoperiod on egg quality of laying ducks is detailed in Table 1. No significant differences in yolk color and eggshell thickness were observed among all the treatments at the end of 4, 6, and 8 wk ($P > 0.05$). Increment in photoperiod linearly and quadratically decreased albumen height and Haugh units ($P < 0.05$). Remarkably, eggshell breaking strength increased linearly and quadratically in response to the increasing photoperiods, at the end of 4, 6, and 8 wk of the experiment. Compared with 12L:12D, the higher values of eggshell breaking strength occurred in ≥18 h photoperiods at the end of 6 wk, and in ≥16 h photoperiods at the end of 4 wk, with the common highest values occurring in 18 h photoperiod ($P < 0.05$).

Bone Quality Characteristics

Tibia, femur, and ulna quality traits, including basic, mechanical, geometric, and material characteristics are listed in Tables 2–5, respectively. Overall, 12 to 20 h photoperiods had no significant effects on bone basic and material characteristics ($P > 0.05$). Increment in photoperiod linearly and quadratically increased ultimate load Fu (femur and ulna), yield load Fy (ulna), distance at fracture (d-Fu), and cortical cross-sectional area A (femur; $P < 0.05$), while quadratically increased cortical cross-sectional area A of ulna ($P < 0.05$). Compared with 12L:12D treatment, 18L:6D had higher values of ultimate load Fu (tibia and ulna) and cortical cross-sectional area A (tibia, femur and ulna; $P < 0.05$). Besides, the higher ultimate load Fu of femur occurred in ≥ 16 h photoperiods, while ≥ 18 h photoperiods had higher ultimate load Fu of ulna ($P < 0.05$). No significant changes were observed in other bone quality traits ($P > 0.05$), in response to different photoperiods.

Table 1. Effect of photoperiod on egg quality of laying ducks from 37 to 44 wk of age.[1]

	Photoperiod[2]						*P*-value		
Items	12L:12D	14L:10D	16L:8D	18L:6D	20L:4D	SEM	ANOVA	Linear[3]	Quadratic[3]
Albumen height (mm)									
4 wk	8.42[a]	8.34[a]	8.28[ab]	8.05[bc]	7.87[c]	0.05	0.001	<0.001	<0.001
6 wk	8.36[a]	8.33[a]	8.17[ab]	8.01[b]	8.03[b]	0.04	0.027	<0.001	0.001
8 wk	8.42[a]	8.38[a]	8.32[ab]	8.22[ab]	8.13[b]	0.04	0.042	0.002	0.006
Haugh units									
4 wk	87.19	86.99	87.09	86.32	84.54	0.35	0.077	0.013	0.015
6 wk	86.62[a]	86.15[a]	85.31[ab]	83.96[b]	84.13[b]	0.30	0.006	0.001	0.002
8 wk	87.36[a]	86.77[ab]	86.71[ab]	85.53[b]	85.17[b]	0.26	0.032	0.001	0.006
Yolk color									
4 wk	7.41	7.30	7.27	7.13	7.17	0.08	0.86	0.27	0.53
6 wk	7.37	7.30	7.30	7.20	7.23	0.04	0.78	0.23	0.47
8 wk	7.57	7.37	7.34	7.17	7.13	0.06	0.18	0.013	0.045
Eggshell thickness (10^{-2} mm)									
4 wk	42.83	43.07	43.56	43.44	42.77	0.29	0.90	0.91	0.62
6 wk	42.28	41.94	42.60	43.09	42.87	0.22	0.50	0.13	0.33
8 wk	41.99	42.11	42.71	43.42	43.16	0.27	0.36	0.051	0.15
Eggshell breaking strength (N)									
4 wk	45.71[c]	46.57[bc]	48.75[ab]	49.89[a]	49.16[ab]	0.48	0.001	0.001	0.003
6 wk	44.95[b]	45.09[b]	47.31[ab]	49.64[a]	48.80[a]	0.57	0.013	0.001	0.005
8 wk	46.06	46.72	48.48	49.64	49.33	0.49	0.065	0.015	0.047

[1]Data are the mean of 6 replicates (5 eggs of each replicate).
[2]L: hours of light; D: hours of darkness.
[3]Linear and quadratic effects of photoperiod were evaluated using regression analysis.
[a-c]Values within a row with no common superscripts differ significantly ($P < 0.05$).

Table 2. Effect of photoperiod on tibia, femur and ulna basic characteristics of laying ducks (310 d of age).[1]

	Photoperiod[2]						*P*-value		
Items	12L:12D	14L:10D	16L:8D	18L:6D	20L:4D	SEM	ANOVA	Linear[3]	Quadratic[3]
Tibia									
Length (cm)	9.84	9.76	9.78	9.80	9.79	0.06	0.99	0.90	0.96
Weight (g)	5.66	5.74	5.91	6.03	6.02	0.09	0.59	0.099	0.25
Index (%)	0.356	0.344	0.359	0.357	0.363	0.003	0.51	0.27	0.44
Volume (mL)	3.71	3.72	3.77	3.79	3.78	0.05	0.98	0.54	0.82
Density (g/cm^3)	1.53	1.54	1.57	1.59	1.59	0.02	0.56	0.083	0.22
Midpoint perimeter (cm)	1.85	1.87	1.87	1.86	1.82	0.01	0.49	0.33	0.17
Femur									
Length (cm)	6.08	6.07	6.06	6.09	6.06	0.03	0.99	0.90	0.99
Weight (g)	4.70	4.84	4.86	4.87	4.90	0.08	0.95	0.45	0.72
Index (%)	0.296[a]	0.211[b]	0.295[a]	0.289[a]	0.296[a]	0.010	0.008	0.26	0.27
Volume (mL)	3.00	3.04	2.98	3.01	3.00	0.04	0.99	0.92	0.99
Density (g/cm^3)	1.57	1.59	1.63	1.62	1.64	0.02	0.56	0.11	0.25
Midpoint perimeter (cm)	1.94	1.93	1.94	1.92	1.90	0.02	0.95	0.47	0.73
Ulna									
Length (cm)	8.39	8.42	8.38	8.44	8.43	0.03	0.98	0.66	0.91
Weight (g)	3.42	3.51	3.58	3.60	3.63	0.05	0.08	0.19	0.40
Index (%)	0.215	0.211	0.217	0.214	0.219	0.003	0.93	0.65	0.84
Volume (mL)	2.40	2.39	2.33	2.38	2.43	0.03	0.91	0.84	0.69
Density (g/cm^3)	1.43	1.47	1.54	1.51	1.49	0.02	0.36	0.20	0.14
Midpoint perimeter (cm)	1.76	1.76	1.81	1.79	1.84	0.01	0.12	0.018	0.057

[1]Data are the mean of 6 replicates (one-sided leg bone from 2 ducks each replicate).
[2]L: hours of light; D: hours of darkness.
[3]Linear and quadratic effects of photoperiod were evaluated using regression analysis.
[a-b]Values within a row with no common superscripts differ significantly ($P < 0.05$).

BMC

Changes in the total, cortical and trabecular mineral contents of tibia, femur, and ulna are shown in Tables 6–8. The proximal BMC (tibia and femur), proximal BMD (femur), distal BMD (ulna), distal BMC (ulna), total Ca (tibia and ulna), P (tibia) content in ash, cortical vBMC (tibia and ulna), and trabecular vBMC (femur) increased quadratically ($P < 0.05$), in response to the increasing photoperiods. Compared with 12L:12D treatment, the higher values of proximal BMC (tibia), distal BMC (ulna), total Ca (tibia), and cortical vBMC (tibia and ulna) were observed in 16L:8D and 18L:6D treatments; meanwhile, 18 h photoperiod group had the

Table 3. Effect of photoperiod on tibia, femur, and ulna mechanical characteristics of laying ducks (310 d of age).[1]

	Photoperiod[2]						*P*-value		
Items	12L:12D	14L:10D	16L:8D	18L:6D	20L:4D	SEM	ANOVA	Linear[3]	Quadratic[3]
Tibia									
Yield load Fy (N)	148	144	141	142	134	2.46	0.52	0.084	0.23
Distance at yield d-Fy (mm)	2.08	2.05	2.01	2.00	1.97	0.03	0.90	0.30	0.59
Elastic energy Wy (mJ)	154	148	142	143	132	4.34	0.63	0.11	0.29
Ultimate load Fu (N)	136[b]	137[b]	135[b]	151[a]	134[b]	1.90	0.018	0.52	0.41
Distance at fracture d-Fu (mm)	2.31	2.26	2.23	2.22	2.29	0.04	0.92	0.82	0.65
Work to fracture Wu (mJ)	164	169	166	173	161	3.75	0.89	0.96	0.75
Stiffness S (N/mm)	71.27	70.49	70.04	70.99	68.33	1.11	0.94	0.50	0.78
Femur									
Yield load Fy (N)	262	249	267	265	246	5.03	0.63	0.67	0.70
Distance at yield d-Fy (mm)	1.10	1.03	1.05	1.03	1.02	6.02	0.85	0.38	0.63
Elastic energy Wy (mJ)	146	129	141	137	126	5.27	0.77	0.40	0.70
Ultimate load Fu (N)	263[c]	270[bc]	279[ab]	285[a]	277[ab]	1.96	0.003	<0.001	<0.001
Distance at fracture d-Fu (mm)	1.28	1.27	1.26	1.27	1.25	0.04	0.99	0.83	0.98
Work to fracture Wu (mJ)	178	188	192	197	186	3.72	0.63	0.38	0.29
Stiffness S (N/mm)	239	243	257	259	244	4.58	0.58	0.46	0.33
Ulna									
Yield load Fy (N)	103	106	108	111	108	1.05	0.10	0.030	0.031
Distance at yield d-Fy (mm)	1.55	1.53	1.47	1.60	1.47	0.03	0.57	0.66	0.91
Elastic energy Wy (mJ)	79.86	80.74	79.49	89.42	79.33	1.79	0.34	0.56	0.66
Ultimate load Fu (N)	109[b]	113[b]	115[ab]	123[a]	116[ab]	1.44	0.022	0.010	0.012
Distance at fracture d-Fu (mm)	1.71[b]	1.83[ab]	1.82[ab]	1.99[a]	1.96[a]	0.03	0.040	0.004	0.014
Work to fracture Wu (mJ)	96.47	104	107	113	101	2.39	0.24	0.31	0.11
Stiffness S (N/mm)	66.76	70.39	74.07	70.17	73.51	1.43	0.52	0.19	0.35

[1]Data are the mean of 6 replicates (one-sided leg bone from 2 ducks each replicate).
[2]L: hours of light; D: hours of darkness.
[3]Linear and quadratic effects of photoperiod were evaluated using regression analysis.
[a-c]Values within a row with no common superscripts differ significantly ($P < 0.05$).

Table 4. Effect of photoperiod on tibia, femur, and ulna geometric characteristics of laying ducks (310 d of age).[1]

Items	Photoperiod[2]					SEM	*P*-value		
	12L:12D	14L:10D	16L:8D	18L:6D	20L:4D		ANOVA	Linear[3]	Quadratic[3]
Tibia									
M-L plate external diameter H (mm)	5.20	5.16	5.24	5.15	5.17	0.06	0.99	0.87	0.98
M-L plate internal diameter h (mm)	3.44	3.42	3.26	3.28	3.40	0.07	0.90	0.67	0.69
A-P plate external diameter B (mm)	5.73	5.75	5.75	5.77	5.74	0.03	0.99	0.81	0.94
A-P plate internal diameter b (mm)	3.88	3.86	3.85	3.67	3.90	0.06	0.80	0.76	0.79
Cortical cross-sectional area A (mm^2)	12.89[b]	12.92[ab]	13.75[ab]	13.82[a]	12.84[b]	0.15	0.045	0.45	0.20
Mean relative wall thickness MRWT	0.50	0.50	0.56	0.58	0.50	0.02	0.41	0.49	0.34
Cross-sectional moment of inertia Ix (mm^4)	38.01	38.47	39.38	40.35	37.99	0.61	0.73	0.68	0.52
Radius of gyration R_g (mm)	1.72	1.72	1.69	1.71	1.72	0.01	0.95	0.91	0.89
Mean cortical index MCI	33.27	33.32	35.50	36.42	33.24	0.69	0.45	0.54	0.35
Femur									
M-L plate external diameter H (mm)	6.25	6.27	6.30	6.31	6.30	0.07	0.99	0.74	0.94
M-L plate internal diameter h (mm)	4.42	4.37	4.50	4.26	4.29	0.09	0.92	0.56	0.81
A-P plate external diameter B (mm)	5.69	5.77	5.74	5.75	5.74	0.04	0.98	0.79	0.89
A-P plate internal diameter b (mm)	3.96	4.10	3.96	3.99	4.00	0.07	0.97	0.96	0.98
Cortical cross-sectional area A (mm^2)	14.11[b]	14.27[b]	14.39[ab]	15.09[a]	14.82[ab]	0.12	0.033	0.005	0.019
Mean relative wall thickness, MRWT	0.44	0.43	0.42	0.47	0.47	0.01	0.76	0.31	0.55
Cross-sectional moment of inertia Ix (mm^4)	42.84	44.18	44.62	45.45	44.63	0.75	0.88	0.37	0.56
Radius of gyration R_g (mm)	1.74	1.76	1.76	1.73	1.74	0.02	0.98	0.75	0.88
Mean cortical index MCI	30.04	29.73	29.73	31.74	31.39	0.83	0.68	0.34	0.60
Ulna									
M-L plate external diameter H (mm)	5.15	5.20	5.22	5.17	5.18	0.05	0.99	0.90	0.92
M-L plate internal diameter h (mm)	3.87	3.90	3.94	3.80	4.00	0.06	0.88	0.71	0.89
A-P plate external diameter B (mm)	5.51	5.53	5.58	5.55	5.55	0.04	0.99	0.71	0.88
A-P plate internal diameter b (mm)	4.03	4.06	4.00	3.95	3.98	0.06	0.98	0.60	0.87
Cortical cross-sectional area A (mm^2)	9.98[b]	10.13[b]	10.46[ab]	10.73[a]	10.09[b]	0.09	0.022	0.19	0.025
Mean relative wall thickness, MRWT	0.35	0.35	0.36	0.39	0.35	0.01	0.62	0.64	0.61
Cross-sectional moment of inertia Ix (mm^4)	29.72	30.26	32.10	31.92	31.14	0.64	0.75	0.33	0.43
Radius of gyration R_g (mm)	1.72	1.72	1.75	1.72	1.76	0.02	0.92	0.55	0.84
Mean cortical index, MCI	25.92	25.91	26.42	27.81	25.60	0.46	0.60	0.71	0.62

[1]Data are the mean of 6 replicates (one-sided leg bone from 2 ducks each replicate).
[2]L: hours of light; D: hours of darkness.
[3]Linear and quadratic effects of photoperiod were evaluated using regression analysis.
[a-b]Values within a row with no common superscripts differ significantly ($P < 0.05$).

higher proximal BMC in femur, and total Ca in ulna ($P < 0.05$). No differences occurred in the other bone mineral traits ($P > 0.05$) among all the treatments.

Bone Metabolism Related Enzymes and Factors in Serum

Serum samples were collected from laying ducks at 0:00, 6:00, 12:00, and 18:00 of 309 d of age. As shown in Figures 1A and 1B, compared with 12L:12D group, the higher ALP activity occurred in ≥16 h photoperiods (0:00 and 18:00), with the highest values in 18L:6D treatment. Meanwhile, values of trACP activity increased in ≥14 h photoperiods at 6:00, and decreased in ≥18 h photoperiods at 0:00 ($P < 0.05$), compared with 12L:12D group.

Change of bone metabolism related factors response to different photoperiods are illustrated in Figures 2A

Table 5. Effect of photoperiod on tibia, femur, and ulna material characteristics of laying ducks (310 d of age).[1]

Items[2]	Photoperiod[2]					SEM	*P*-value		
	12L:12D	14L:10D	16L:8D	18L:6D	20L:4D		ANOVA	Linear[3]	Quadratic[3]
Tibia									
Young modulus of elasticity E (GPa)	13.46	13.14	12.79	12.56	13.05	0.27	0.88	0.47	0.59
Yield stress σ_y (Mpa)	196	189	180	177	178	3.92	0.51	0.086	0.19
Ultimate stress σ_u (Mpa)	180	181	173	189	177	2.55	0.40	0.93	0.99
Femur									
Young modulus of elasticity E (GPa)	3.16	3.12	3.24	3.22	3.09	0.06	0.94	0.92	0.83
Yield stress σ_y (Mpa)	131	123	129	127	119	2.93	0.75	0.35	0.63
Ultimate stress σ_u (Mpa)	131	133	135	137	134	1.49	0.85	0.35	0.54
Ulna	5.51	5.53	5.58	5.55	5.55	0.04	0.99	0.71	0.88
Young modulus of elasticity E (GPa)	144	147	142	147	144	2.41	0.98	0.97	0.99
Yield stress σ_y (Mpa)	152	157	151	161	156	2.68	0.79	0.54	0.82
Ultimate stress σ_u (Mpa)	5.51	5.53	5.58	5.55	5.55	0.04	0.99	0.71	0.88

[1]Data are the mean of 6 replicates (one-sided leg bone from 2 ducks each replicate).
[2]L: hours of light; D: hours of darkness.
[3]Linear and quadratic effects of photoperiod were evaluated using regression analysis.

Table 6. Effect of photoperiod on ash, Ca and P content in tibia, femur, and ulna of laying ducks (310 d of age).[1]

	Photoperiod[2]						*P*-value		
Items	12L:12D	14L:10D	16L:8D	18L:6D	20L:4D	SEM	ANOVA	Linear[3]	Quadratic[3]
Tibia									
Fat-free dry weight (g)	4.46	4.47	4.49	4.57	4.47	0.08	0.99	0.85	0.96
Ash (g)	1.89	1.89	1.90	1.90	1.87	0.03	0.99	0.93	0.94
Ash content (%)	42.48	42.38	42.55	41.85	42.02	0.66	0.99	0.76	0.95
Total Ca (mg)	711[b]	728[ab]	749[a]	761[a]	728[ab]	5.81	0.046	0.11	0.017
Ca content in ash (mg/g)	379	387	395	402	393	3.95	0.47	0.14	0.19
Total P (mg)	289	296	301	304	293	4.92	0.88	0.64	0.57
P content in ash (mg/g)	153	157	158	160	157	0.89	0.11	0.077	0.028
Femur									
Fat-free dry weight (g)	3.24	3.49	3.48	3.52	3.23	0.10	0.82	0.99	0.48
Ash (g)	1.05	1.14	1.18	1.20	1.05	0.04	0.56	0.84	0.25
Ash content (%)	32.53	32.72	33.91	34.18	32.65	0.56	0.83	0.68	0.60
Total Ca (mg)	399	442	457	485	416	15.38	0.44	0.49	0.20
Ca content in ash (mg/g)	382	387	387	402	396	3.89	0.51	0.12	0.30
Total P (mg)	166	180	187	192	164	5.92	0.51	0.85	0.23
P content in ash (mg/g)	159	158	159	159	157	0.96	0.97	0.89	0.93
Ulna									
Fat-free dry weight (g)	2.25	2.40	2.38	2.43	2.40	0.05	0.81	0.34	0.48
Ash (g)	0.95	1.01	1.01	1.03	1.01	0.02	0.66	0.25	0.32
Ash content (%)	42.00	42.40	42.60	42.66	42.42	0.58	0.99	0.80	0.93
Total Ca (mg)	368[b]	382[ab]	396[ab]	409[a]	384[ab]	4.63	0.046	0.069	0.018
Ca content in ash (mg/g)	392	379	394	400	382	3.98	0.41	0.98	0.87
Total P (mg)	146	160	161	166	160	3.14	0.38	0.44	0.13
P content in ash (mg/g)	155	158	159	161	158	0.88	0.28	0.16	0.10

[1]Data are the mean of 6 replicates (one-sided leg bone from 2 ducks each replicate).
[2]L: hours of light; D: hours of darkness.
[3]Linear and quadratic effects of photoperiod were evaluated using regression analysis.
[a-b]Values within a row with no common superscripts differ significantly ($P < 0.05$).

−2F. Compared with 12L:12D, ≥18 h photoperiods had higher contents of TGF-β at 6:00, accompanied with 16 and 18 h photoperiods having the higher value at 0:00 ($P < 0.05$); ≥16 h photoperiods had higher contents of OC at 6:00 ($P < 0.05$), as well as 18 h photoperiod had the higher value at 18:00 ($P < 0.05$). Besides, 18L:6D and 16L:8D treatments had the lower contents of TNF-α and IL-6 ($P < 0.05$) at 0:00, compared with 12L:12D. No significant changes were observed in contents of IGF-I and IL-1 ($P > 0.05$), in response to the effect of different photoperiods.

DISCUSSION

Photoperiod serves as a vital environmental factor in poultry production, which has biological and physiological significance through regulating circadian rhythms, and changing the time for rest or regeneration (Malleau et al., 2007). The potential benefits of photoperiod on laying ducks have been extensively reported, such as promoting bone and reproductive system development during the pullet phase (Cui et al., 2019a, b), and improving productive performance and reproductive function during the laying phase (Cui et al., 2021). Besides, different photoperiods can affect the manner and percentage of raw materials supply for eggshell formation (Miller, 1992). Therefore, we wondered what would happen in eggshell quality under different photoperiods. In this study, we found that the eggshell quality was improved linearly and quadratically with the increasing photoperiods, at all the observation time points (4, 6, and 8 wk of the experiment). Furthermore, the significant higher values of eggshell breaking strength were synchronously observed in 18 h photoperiod at the end of 4 and 6 wk of the experiment, as well as eggshell breaking strength tended to increase and the numerical highest value occurred in 18 h photoperiod at the end of 8 wk ($P = 0.065$). Similar findings were observed in previous study that 16-h photoperiod treatment brought the higher eggshell breaking strength in egg-type Beijing You Chickens at the end of 28 wk of age, compared with 12L:12D and 14L:10D treatments (Shen et al., 2012). Longer light hours mean more time for feed intake, and thus lead to the longer time of chyme retention in the digestive tract, which can increase the duration of providing raw materials for eggshell formation (e.g., Ca) from intestine and thus obtain superior eggshell quality. This point was evidenced by the findings that limestone with a large particle size provided the stronger eggshell breaking strength than that of the small one (Wang et al., 2014). However, redundant light time had adverse effects on meat turkey from 10 to 126 d of age: decrease in body weight, feed intake and active behavior; increase in mortality, gait score (thought to be associated with pain), the incidence of breast blisters and resting behavior; change in eye size (Vermette et al., 2016a,b). Therefore, there should be an optimal photoperiod for eggshell quality of laying birds. Based on the findings in our research that the highest eggshell breaking strength was observed in 18L:6D photoperiod treatment, 18 h/d light time was supposed to be appropriate for eggshell quality of laying ducks.

Table 7. Effect of photoperiod on tibia, femur and ulna densitometric characteristics of laying ducks (310 d of age).[1]

Items	Photoperiod[2] 12L:12D	14L:10D	16L:8D	18L:6D	20L:4D	SEM	*P*-value ANOVA	Linear[3]	Quadratic[3]
Tibia									
Bone mineral density (BMD, g/cm^2)									
Distal BMD	2.125	2.116	2.119	2.118	2.110	0.008	0.99	0.64	0.90
Midshaft BMD	2.271	2.269	2.266	2.265	2.276	0.008	0.99	0.91	0.90
Proximal BMD	2.159	2.175	2.206	2.229	2.178	0.009	0.12	0.17	0.071
Bone mineral content (BMC, g)									
Distal BMC	1.987	1.979	2.007	2.006	1.995	0.009	0.85	0.51	0.72
Midshaft BMC	1.397	1.395	1.436	1.414	1.393	0.012	0.80	0.91	0.62
Proximal BMC	2.020[b]	2.047[ab]	2.092[a]	2.099[a]	2.057[ab]	0.010	0.034	0.059	0.009
Femur									
Bone mineral density (BMD, g/cm^2)									
Distal BMD	2.148	2.145	2.147	2.141	2.139	0.006	0.99	0.61	0.87
Midshaft BMD	2.115	2.085	2.132	2.109	2.093	0.013	0.82	0.82	0.90
Proximal BMD	2.136	2.161	2.189	2.202	2.164	0.008	0.11	0.11	0.032
Bone mineral content (BMC, g)									
Distal BMC	2.255	2.234	2.295	2.273	2.249	0.013	0.64	0.76	0.69
Midshaft BMC	1.780	1.755	1.812	1.794	1.755	0.011	0.45	0.90	0.57
Proximal BMC	2.010	2.042	2.071	2.114	2.051	0.011	0.049	0.056	0.025
Ulna									
Bone mineral density (BMD, g/cm^2)									
Distal BMD	2.083	2.105	2.132	2.143	2.099	0.008	0.12	0.24	0.044
Midshaft BMD	2.176	2.176	2.177	2.170	2.184	0.007	0.99	0.86	0.93
Proximal BMD	2.054	2.041	2.050	2.046	2.044	0.011	0.99	0.85	0.98
Bone mineral content (BMC, g)									
Distal BMC	1.461[b]	1.483[ab]	1.530[a]	1.543[a]	1.498[ab]	0.010	0.043	0.054	0.016
Midshaft BMC	1.154	1.167	1.182	1.171	1.164	0.005	0.61	0.54	0.30
Proximal BMC	1.398	1.396	1.412	1.405	1.405	0.011	0.99	0.78	0.94

[1]Data are the mean of 6 replicates (one-sided leg bone from 1 duck each replicate).
[2]L: hours of light; D: hours of darkness.
[3]Linear and quadratic effects of photoperiod were evaluated using regression analysis.
[a-b]Values within a row with no common superscripts differ significantly ($P < 0.05$).

It is well-known to all that the raw materials of eggshell formation derive from intestinal tract during the light time, and from bone in the dark (Guinotte et al., 1995; Nys, 2018). Hence, different photoperiods treatments mean different degrees of bone resorption, which may probably affect the bone quality of laying poultry. In this research, the bone quality (including the basic, mechanical, geometric, material, and mineral densitometric property) was further investigated, with ulna standing for wing bone as well as tibia and femur for leg bone. In fact, the superior bone quality in the whole life of laying poultry can be ascribed to 2 aspects, namely a greater amount of structural bone formation during the pullet phase and less bone loss during the laying phase (Whitehead, 2004). Hence, the less amount of bone loss can be supposed to be beneficial for bone quality maintenance, which usually accompanies with a stronger bone strength. Consistently, in our research, higher values of

Table 8. Effect of photoperiod on tibia, femur, and ulna architectural characteristics of laying ducks (310 d of age).[1]

Items[2]	Photoperiod[3] 12L:12D	14L:10D	16L:8D	18L:6D	20L:4D	SEM	*P*-value ANOVA	Linear[4]	Quadratic[4]
Tibia									
Cortical vBMD (g/cm^3)	1.143	1.108	1.142	1.151	1.133	0.01	0.87	0.81	0.97
Cortical vBMC (g)	28.73[c]	30.43[bc]	35.15[ab]	37.03[a]	31.68[abc]	0.98	0.032	0.070	0.017
Trabecular vBMD (g/cm^3)	0.629	0.617	0.622	0.633	0.612	0.01	0.96	0.79	0.94
Trabecular vBMC (g)	26.11	25.01	23.72	24.97	23.37	0.48	0.39	0.10	0.25
Femur									
Cortical vBMD (g/cm^3)	1.503	1.519	1.517	1.508	1.509	0.01	0.44	0.31	0.18
Cortical vBMC (g)	27.30	27.91	27.60	27.55	27.42	0.28	0.62	0.67	0.31
Trabecular vBMD (g/cm^3)	0.512	0.49	0.485	0.473	0.485	0.01	0.24	0.069	0.070
Trabecular vBMC (g)	10.02	9.38	9.05	8.74	9.10	0.15	0.084	0.20	0.016
Ulna									
Cortical vBMD (g/cm^3)	1.586	1.588	1.623	1.634	1.602	0.01	0.15	0.13	0.10
Cortical vBMC (g)	27.24[c]	27.65[bc]	28.77[ab]	29.20[a]	27.52[bc]	0.24	0.020	0.21	0.016
Trabecular vBMD (g/cm^3)	0.228	0.211	0.195	0.206	0.198	0.01	0.26	0.075	0.13
Trabecular vBMC (g)	5.14	4.84	4.40	4.68	4.51	0.12	0.34	0.098	0.16

[1]Data are the mean of 6 replicates (one-sided leg bone from 1 duck each replicate).
[2]Abbreviations: vBMC, volumetric bone mineral content; vBMD, volumetric bone mineral density.
[3]L: hours of light; D: hours of darkness.
[4]Linear and quadratic effects of photoperiod were evaluated using regression analysis.
[a-d]Values within a row with no common superscripts differ significantly ($P < 0.05$).

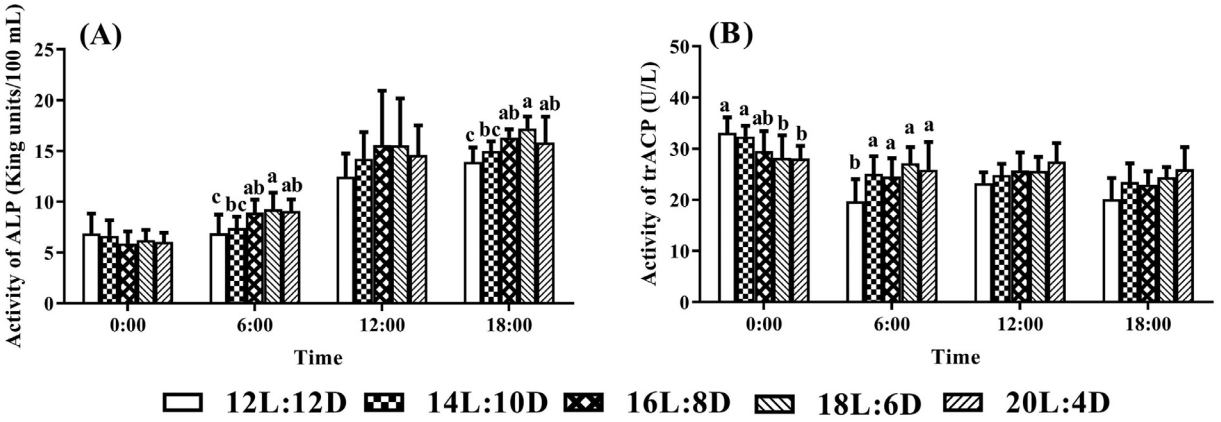

Figure 1. (A, B) Effects of photoperiod on the activities of alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (trACP) in serum of laying ducks at the end of 309 d of age. Means were calculated from 6 replicates (2 ducks/replicate) per treatment. Data were expressed as mean ± SD. [a-c] Values at the same time point with no common superscripts differ significantly ($P < 0.05$).

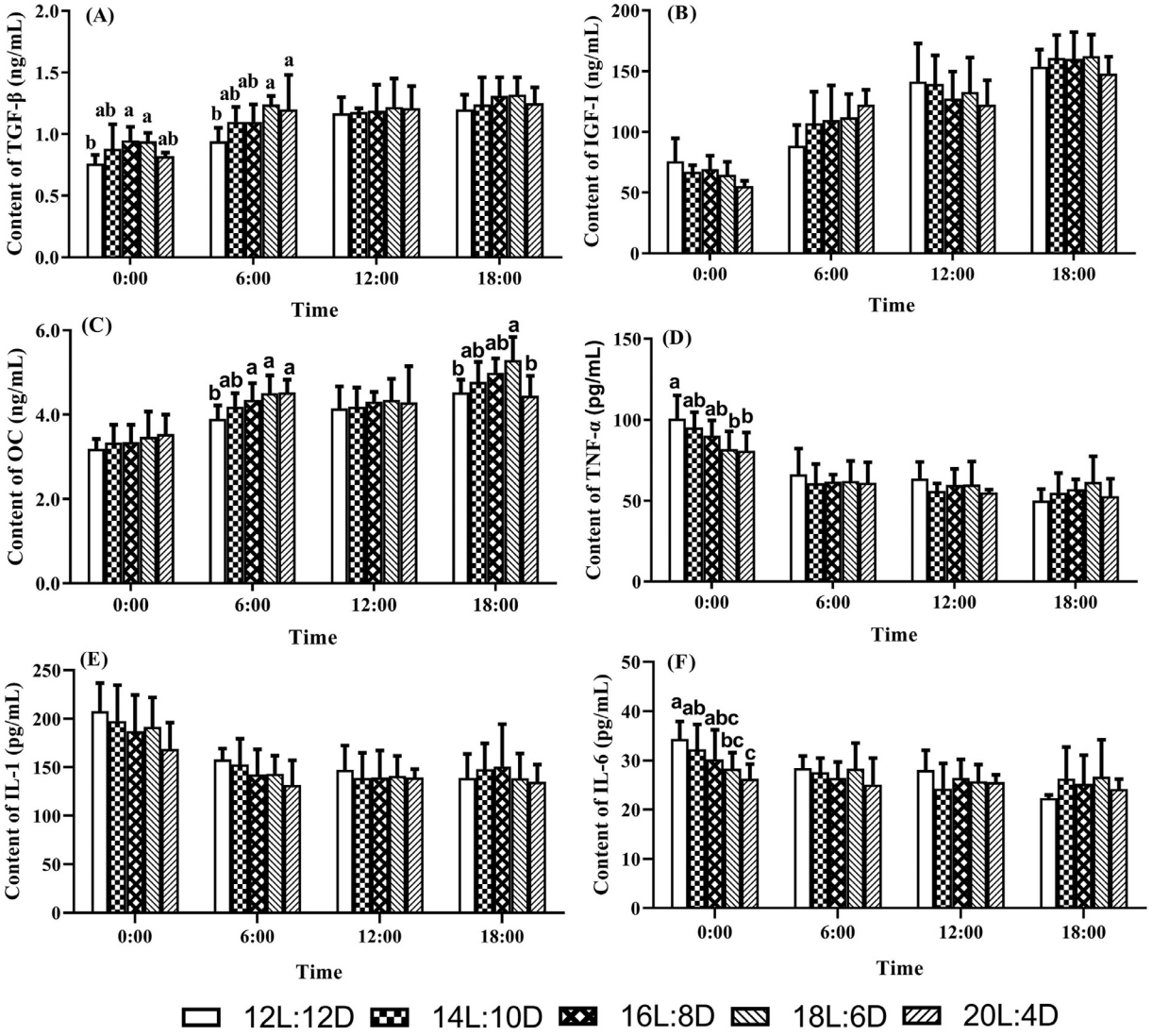

Figure 2. (A−F) Effects of photoperiod on the contents of bone metabolism related factors, including insulin-like growth factor-I (IGF-I), transforming growth factor-β (TGF-β), osteocalcin (OC), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), in serum of laying ducks at 309 d of age. Means were calculated from 6 replicates (2 ducks/replicate) per treatment. Data were expressed as mean ± SD. [a-c] Values at the same time point with no common superscripts differ significantly ($P < 0.05$).

ultimate load Fu associated with cortical cross-sectional area A were observed in tibia, femur, and ulna. These findings indicated that the increase of bone strength (tibia, femur, and ulna) might be attributed to the less bone loss and more structural retention (Silversides et al., 2006). Some doubt may be that medullary bone is the major raw material repository for eggshell formation (Wang et al., 2020), whereas significant differences were observed in cortical bone. In fact, osteoclasts resorb both medullary and structural bone (Whitehead, 2004), and thus a progressive loss may happen in cortical bone. Of note, significantly quadratic responses were observed in ultimate load Fu and cortical cross-sectional area A (femur and ulna) accompanied with the highest values in 18-h photoperiod treatment, indicating mobility capacity firstly increased and then decreased with increasing photoperiods (Regmi et al., 2015; Muszyński at al., 2017), which may support the above explanation that superfluous light brought adverse effect on birds.

Higher content of cortical bone may accompany with a bigger amount of minerals. In fact, the bone strength is closely related to with its chemical constitution. The chemical components of bone can be divided into organic matrix and inorganic minerals. Generally, organic matrix (mainly collagen) provides bone with tensile strength, toughness, elasticity, and structural scaffolds for minerals deposition (Liu et al., 2004), while mineral-dominated inorganic matrix supplies compressional strength (Ferretti et al., 2001). The bone strength can be assessed at two levels: the organ level (mechanical properties) and the tissue level (material properties), and the material properties can be calculated based on the mechanical and geometric properties. Therefore, the minerals contents of bone need to be systematically analyzed. In this study, 3 methods were simultaneously used to measure mineral content, containing ash, dual energy x-ray absorptiometry and quantitative computed tomography. Higher values of total Ca in ash of (tibia and ulna), proximal BMC (tibia and femur), distal BMC (ulna), and cortical vBMC (tibia and ulna) were simultaneously observed in 18L:6D treatment. These results meant higher level of bone mineralization in 18 h photoperiod, which could be responsible for the improvement in bone strength of this treatment (Casey-Trott et al., 2017a). Of note, Ca concentrations in ash of tibia and ulna were not significantly affected, but the total Ca increased in 18-h photoperiod treatment. Consistently, BMC (tibia, femur, and ulna) and cortical vBMC (tibia and ulna) increased significantly in this group while their BMD and vBMD had no differences. These findings may be ascribed to the phenomenon that more mineral content spread out over a greater volume and resulted in no significant change in mineral density (Casey-Trott et al., 2017b). Meanwhile, these results meant less bone loss and more bone mass retention in this group, which was consistent with the former results, indicating the benefits of 18-h photoperiod on bone quality maintenance during the laying phase. Besides, these results also indicated that both wing and leg bone of laying ducks were susceptible to photoperiod. Above all, 18L:6D was supposed to an appropriate photoperiod for ducks during the laying phase, because of greater structure bone retention and less bone minerals loss, and thus a better bone quality maintenance.

Bone quality maintenance is closely related with bone metabolism activity, mainly containing bone resorption and remodeling, which were driven by osteoclast and osteoblast activities (Zhan et al., 2020). Therefore, osteoclast and osteoblast activities related enzymes and factors in serum were further investigated in this current research. The activity of ALP significantly increased in ≥16 h photoperiods with the highest value in 18-h photoperiod, compared with 12L:12D, at 6:00 and 18:00. In fact, ALP is mainly secreted by osteoblast, and supposed to play an important role in osteoblastic activity and osteogenesis (Hsu et al., 1999). Therefore, increase in the activity of ALP indicated ≥16 h photoperiods were beneficial to enhance the osteogenesis (Deng et al., 2010), and 18 h photoperiod performed the best. Besides, higher values of TGF-β and OC, which can be either secreted by osteoblasts or beneficial for the formation of them (Komm et al., 1988; Regmi et al., 2017), were observed simultaneously in 18 h photoperiods at 6:00 and 18:00, compared with 12-h photoperiod. All of these might indicate that 18-h photoperiod could cause more osteogenesis in bone tissue during the light time. However, bone resorption conducts in parallel with osteogenesis (Whitehead, 2004). In this situation, the activity of osteoclast needed to be investigated to explore the reason for the improvement of bone quality above. Tartrate-resistant acid phosphatase is an active component secreted by osteoclast which is conducive to break down bone matrix (Deng et al., 2010). Tumor necrosis factor-α can not only accelerate bone resorption by activation osteoclasts, but also depress osteogenesis through suppression osteoblasts activity (Azuma et al., 2000). Interleukin-6 can be secreted by osteoblast and show inhibiting effect on osteoblast and facilitating effect on osteoclast (Kudo et al., 2003). In this present research, compared with 12L:12D, the lower values of trACP activity, TNF-α and IL-6 contents occurred in 18 and 20 h photoperiods at 0:00, which indicated less bone resorption happened in these 2 groups during the dark. These results may be due to the extension of feeding in these 2 groups (light end at 9:00 and 10:00 pm) and thus more raw materials supply for eggshell formation from intestinal chyme. This point was consistent with the above finding that 18-h photoperiod had more structural bone retention. Hence, the superior bone strength, structural mass and mineral content may be attributed to the more osteogenesis during the light time and less bone resorption during the dark.

In conclusion, an increment in photoperiod could improve eggshell breaking strength of laying ducks in a quadratic manner and 18-h photoperiod performed best. Moreover, photoperiod quadratically increased bone strength, structural bone mass and mineral content, with 18 h as the optimal photoperiod. Furthermore, the higher values of serum ALP activity, TGF-β, and OC

contents (6:00 and 18:00), as well as the lower values of serum trACP activity, TNF-α, and IL-6 contents (6:00) were simultaneously observed in 18-h photoperiod. Therefore, 18 h was the appropriate photoperiod for eggshell and bone quality of laying ducks, which may be attributed to the more intense osteogenesis activity during the light time and the weaker resorption activity during the dark.

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DISCLOSURES

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with this work submitted.

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