Effects of light regime on the hatching performance, body development and serum biochemical indexes in Beijing You Chicken

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ABSTRACT The paper aimed to study the effects of light regime during the incubation on hatching performance, body development and serum biochemical indexes in Beijing You Chicken (**BYC**). A total of 1,408 BYC eggs were randomly allocated into 4 groups: 24 h dark as the control (Inc_{24D}); 3 light regimes were 8 h light and 16 h dark group ($Inc_{8L:16D}$); 12 h light and 12 h dark group ($Inc_{12L:12D}$); 16 h light and 8 h dark group ($Inc_{16L:8D}$), respectively. There were 352 eggs in each group, 4 replicates each group and 1 tray each replicate, 88 eggs each tray. Light-emitting diode (**LED**) strips, white light with temperature of 4,500 to 5,000 K, 150 to 200 lx were set up. The results showed that light regimes had no significant effects on hatching rate of eggs, hatching rate of fertile eggs and healthy rate of chicks ($P > 0.05$), but the hatching rate of eggs was the lowest in the Inc_{24D} group (87.22%), and the highest in the Inc12L:12D group (93.64%); Lighted incubation significantly affected the incidence of leg problems of 1-day-old chicks ($P < 0.05$). The incidence rate of leg problems was the highest in the Inc_{24D} group (4.21%), and was decreased in $Inc_{12L:12D}$ and $Inc_{16L:8D}$ groups ($P < 0.05$). Femur length in the $Inc_{12L:12D}$ group was greater than that in the Inc_{24D} and $Inc_{16L:8D}$ groups ($P = 0.011$), but there were no differences between $Inc_{8L:16D}$ group and other three groups ($P > 0.05$). The relative brain weight of 1-day-old chicks was higher in Inc_{24D} and $Inc_{16L:8D}$ groups than in $Inc_{8L:16D}$ group ($P = 0.052$), but had no difference with $Inc_{12L:12D}$ group. Light regimes during incubation had no effects on serum total protein, albumen, globulin, and urea nitrogen content of 1-day-old chicks ($P > 0.05$), while the globulin content in $Inc_{12L:12D}$ group was numerically greater than in other three groups ($P = 0.063$). Lysozyme content in $Inc_{12L:12D}$ group was higher than that in the Inc_{24D} and $Inc_{8L:16D}$ groups ($P < 0.05$), but had no difference with that in $Inc_{16L:8D}$ group. Light regime had no effect on serum total antioxidant capability (**T-AOC**) ($P > 0.05$), but significantly affected the activities of glutathione peroxidase (**GSH-Px**) and total superoxide dismutase (**T-SOD**) ($P < 0.05$). The GSH-Px and T-SOD activities in the $Inc_{12L:12D}$ group were higher than those in the Inc_{24D} and $Inc_{8L:16D}$ groups. The MDA content of Inc_{24D} was the highest (9.67 nmol/mL) compared to the others, which was very close to the significant level ($P = 0.056$). In conclusion, 12 h light and 12 h dark incubation has the potential to improve the hatching performance of BYC eggs, benefit for the long bone development, improve some serum immune and antioxidant indexes, and reduce the leg problems in 1-day-old chicks.

Key words: incubation, light regime, hatching performance, serum biochemical indexes

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INTRODUCTION

The incubation period is very important in the life cycle of chickens, and the incubation environment has a lasting effect on the health and welfare of chickens throughout their lives (Archer et al., 2009). Recent studies have shown that incubation light can affect hatchability (Huth and Archer, 2015; Archer et al., 2017), improve embryonic growth (Rozenboim et al., 2004; Zhang et al., 2016), shorten hatching time (Shafey and Almohsen, 2002), improve feed conversion (Zhang et al., 2012), reduce fear response of chicks after hatching (Archer and Mench, 2014, 2017), and affect the chick behavior after hatching (Riedstra and Groothuis, 2014), etc. Özkan et al. (2012) found that lighting during incubation was helpful for chicks to adapt to the new environment after hatching. However, the incubation is still completely dark in current commercial hatchery, which may be due to the harmful

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effects caused by previous light sources, the secondary heat caused by incandescent light bulbs, which reduced the hatching rate (Gold and Kalb, 1976; Tong et al., 2017). With the implementation and application of modern LED lamp technology, the temperature in the incubator is not or less affected (Sabuncuoglu et al., 2018; Yameen et al., 2020), and it is cheap and durable, and closer to the effects of daylight (Benson et al., 2013).

Chen et al. (2018) reviewed the lighting during incubation, including different light intensity, light length and color, and indicated that lighting during incubation can affect the embryonic development and hatching performance, such as hatching rate, etc. Previous studies on lighting during incubation were mostly conducted in fast-growing broilers or high-egg-production layers, while there are relatively fewer studies on dual-purpose chicken.

Beijing You Chicken (**BYC**), a dual-purpose native chicken in Beijing district, is known for its characteristic appearance (having crest, beard, shank feathers, and five-toes) and high quality of meat and egg (Liu and Xu, 2001), was listed as one of the most important chicken breeds by Ministry of Agriculture, China (National Livestock and Poultry Genetic Resources Committee, 2011), has developed rapidly in recent years due to the increasing demand for good quality eggs and meat. Nevertheless, there remains a great limitation regarding the lower hatching performance and the chick quality for this bird. The purpose of this study was to study the effects of different light regimes during incubation on the hatching performance, body development and serum biochemical indexes, and to provide an important reference for suitable light treatment during incubation for the bird.

MATERIALS AND METHODS

Experimental Design and Birds

The experiment was conducted at BYC Breeding Farm, Daxing district, Beijing. A total of 1,408 BYC eggs from 38-wk-old breeders (storage <7 d) were prewarmed for 6 h at 25°C and randomly allocated into 4 groups: 24 h dark group (Inc_{24D}) as the control, 3 light regimes were 8 h light and16 h dark group ($Inc_{8L:16D}$), 6:00 to 14:00; 12 h light and 12 h dark group ($Inc_{12L:12D}$), 6:00 to 18:00; 16 h light and 8 h dark group ($Inc_{16L:8D}$), 6:00 to 22:00, respectively. There were 352 eggs in each group, 4 replicates each group with 1 tray each replicate, 88 eggs per tray.

Four automatic two-purpose incubators (Weiqian, Shandong) were calibrated using a standard thermometer and hygrometer before incubation, with a capacity of 352 eggs each, and the technician of a professional company were invited to design and modify the incubators in advance, and an electronic display remote controller was used to flexibly set up the lighting length, intensity, color and temperature, etc. LED light strip, white light with temperature of 4,500 to 5,000 K, and 150 to 200 lx were set up. All the incubators were then calibrated using a standard light meter before incubation. The eggs are incubated after routine disinfection procedures. In addition to light regime, all the incubation conditions such as temperature, relative humidity, ventilation and egg turning were maintained equally, and were managed by a professional incubation technician. The incubation was maintained at a temperature of 38.1°C and a relative humidity (**RH**) 65% from 0 to 5 d, then adjusted the temperature of 38.0°C and RH 60% from 6 to 11 d, 37.9°C and 57% from 12 to 16 d, 37.6°C and 70% from 17 to 18 d. From day 19, eggs were transferred to hatcher baskets, and a temperature of 37.6°C and RH of 70% was set. The incubation stopped completely at 510 h.

Egg weight was recorded according to each replicate (tray) when the eggs were put into incubators. Eggs are not allowed to take out of the incubators for candle checking in order not to affect the embryonic development.

Measurement and Methods

After the complete end of incubation, the total number of hatched chicks, healthy chicks, and weak chicks were recorded according to the replicate groups. The incidence of leg problems, mainly including weak legs and deformed legs, were observed and recorded, which were manifested as unstable standing, single leg valgus, pronus, and splayed legs, etc. The number of dead embryo and sperm free eggs for each replicate group was recorded after the hatching ended.

The hatching rate of eggs, the hatching rate of fertile eggs, the rate of healthy chicks and the incidence rate of leg problems in each group were calculated. The hatching rate of eggs is the ratio of hatching chicks to hatching eggs; the hatching rate of fertile eggs is the ratio of hatching chicks to total fertile eggs; the rate of healthy chicks is the ratio of healthy chicks to total hatching chicks. The incidence rate of leg problem was the ratio of the number of chicks with leg problems to the total number of hatching chicks.

Five 1-day-old chicks were randomly selected from each replicate group for individual weight measurement. Body development measurement included body length, femur length, tibia length, and longest phalangeal length (longest toe length on the right side), keel bone length were measured with a vernier caliper. The chicks were killed by cervical dislocation immediately after blood sample were obtained through cardiac puncture. The blood was transferred into coagulation-promoting tubes and centrifuged at 3,000 for 10 min at 4°C. The serum was frozen at −20°C for later analysis of some endocrine hormone and biochemical indexes. The heart, the liver, and the brain were separated and weighed, the relative heart weight (the ratio of heart weight to bird weight), the relative brain weight, and the relative liver weight were calculated. The brain and liver sample were immediately frozen in liquid nitrogen, and then stored in −80°C for later analysis of some gene expression (serum

Table 1. Effects of light regime on hatching performance.

Group	Average egg weight/(g)	Hatching rate of eggs/(%)	Hatching rate of fertile eggs/(%)	Healthy rate of chicks/(%)	Incidence rate of leg problem /(%)
Inc_{24D}	53.41 ± 0.46	87.22 ± 3.13	89.35 ± 4.25	95.12 ± 2.89	4.21 ± 1.14[a]
$Inc_{8L:16D}$	53.84 ± 0.28	89.77 ± 2.45	92.36 ± 3.16	92.39 ± 2.35	2.56 ± 1.82[ab]
$Inc_{12L:12D}$	54.12 ± 0.54	90.34 ± 3.28	93.64 ± 3.43	93.18 ± 4.07	1.54 ± 1.17[b]
$Inc_{16L:8D}$	54.12 ± 0.28	90.34 ± 2.18	93.15 ± 4.30	95.03 ± 2.76	1.57 ± 1.19[b]
P value	0.094	0.373	0.265	0.529	0.054

Data are the mean of 4 replicates.
[a,b]Values with different letter superscripts in the same column mean significant difference ($P < 0.05$).

hormone measurement and gene expression were reported in another manuscript).

Serum biochemical indexes measurement included serum total protein, albumin, globulin, urea nitrogen, lysozyme content, total antioxidant capacity (**T-AOC**), glutathione peroxidase (**GSH-Px**) and total superoxide dismutase (**T-SOD**) activities, malondialdehyde (**MDA**) content. All the kits were purchased from Nanjing Jiancheng Institute of Biological Engineering and determined by a spectrophotometer (Evolution 60, Thermal Fisher Scientific, Shanghai, China) and an ELIASA (Multiskan FC, Thermal Fisher Scientific, Shanghai, China).

The study was performed in accordance with local ethical guidelines and met the requirement of the Institutional Animal Care and Use Committee.

Statistical Analyses

The data were expressed as mean ± SD, and analyzed statistically using the SPSS 25.0 Software for Windows (SPSS Inc. Chicago, IL). One-way ANOVA was used to analyze the effects of light regime on hatching performance, body development, and serum biochemical indexes. Duncan's Test was used for multiple comparisons. The percentage was arcsine transformed before analysis. $P < 0.05$ was regarded as statistically significant.

RESULTS

Hatching Performance

Table 1 shows that there was no difference in average egg weight before hatching ($P > 0.05$). Lighted incubation had no significant effects on the hatching rate of eggs, the hatching rate of fertile eggs and the rate of healthy chicks ($P > 0.05$), but the hatching rate of eggs in the $Inc_{12L:12D}$ and $Inc_{16L:8D}$ groups were numerically greater than the other 2 groups, the hatching rate of eggs was the lowest in the Inc_{24D} group (87.22%), and the highest in the Inc12L:12D group (93.64%). The incidence rate of leg problem in 1-day-old chicks was affected by the lighted incubation, the incidence rate of leg problem in Inc_{24D} group was the highest (4.21%), and reduced in $Inc_{12L:12D}$ and $Inc_{16L:8D}$ groups ($P < 0.05$), while there had no change in the $Inc_{8L:16D}$ group ($P > 0.05$).

Body Development

Table 2 shows that lighted incubation had no effects on body length, tibia length, the longest phalanges length, and keel bone length in 1-day-old chicks ($P > 0.05$), but the femur length in the $Inc_{12L:12D}$ group was greater than those in the Inc_{24D} and $Inc_{16L:8D}$ groups ($P < 0.05$), and femur length in the $Inc_{8L:16D}$ had no difference with other three groups. The body length in the $Inc_{12L:12D}$ group was numerically greater than the other 3 groups (4.42 cm) ($P > 0.05$).

Table 3 shows that lighted incubation had no effects on body weight, the relative heart weight, relative liver weight in 1-day-old chicks ($P > 0.05$), but affected the relative brain weight ($P = 0.052$), the relative heart weight in the Inc_{24D} and $Inc_{16L:8D}$ groups were greater than that in the $Inc_{8L:16D}$ group, while the $Inc_{12L:12D}$ group had no differences with other 3 groups ($P > 0.05$).

Biochemical Indexes

Table 4 shows that lighted incubation had no effects on the serum total protein, albumin, globulin, and urea nitrogen in 1-day-old chicks ($P > 0.05$), but the globulin content in the $Inc_{12L:12D}$ group was numerically greater than the other 3 groups ($P = 0.063$). The lysozyme

Table 2. Effects of light regime during incubation on body size of 1-day-old chicks.

Group	Body length/cm	Femur length/cm	Tibia length/cm	The longest phalanges length/cm	Keel bone length/cm
Inc_{24D}	4.32 ± 0.17	1.59 ± 0.11[b]	2.23 ± 0.14	1.95 ± 0.14	1.81 ± 0.20
$Inc_{8L:16D}$	4.29 ± 0.15	1.67 ± 0.11[ab]	2.28 ± 0.11	1.86 ± 0.17	1.70 ± 0.15
$Inc_{12L:12D}$	4.42 ± 0.18	1.71 ± 0.13[a]	2.27 ± 0.06	1.93 ± 0.10	1.82 ± 0.09
$Inc_{16L:8D}$	4.35 ± 0.13	1.60 ± 0.12[b]	2.22 ± 0.12	1.85 ± 0.09	1.86 ± 0.21
P value	0.127	0.011	0.392	0.132	0.073

Data are the mean of 4 replicates.
[a,b]Values with different letter superscripts in the same column mean significant difference ($P < 0.05$).

Table 3. Effects of light regime during incubation on relative organ weight in 1-day-old chicks.

Group	Body weight/ (g)	Relative brain weight/ (g/g × 100)	Relative heart weight/ (g/g × 100)	Relative liver weight/ (g/g × 100)
Inc_{24D}	33.57 ± 2.51	2.55 ± 0.29[a]	0.67 ± 0.12	2.71 ± 0.32
$Inc_{8L:16D}$	34.68 ± 2.62	2.29 ± 0.39[b]	0.64 ± 0.09	2.59 ± 0.28
$Inc_{12L:12D}$	33.94 ± 1.56	2.47 ± 0.20[ab]	0.69 ± 0.08	2.31 ± 0.08
$Inc_{16L:8D}$	33.33 ± 2.23	2.55 ± 0.23[a]	0.69 ± 0.09	2.42 ± 0.11
P value	0.394	0.052	0.529	0.752

Data are the mean of 4 replicates.
[a,b]Values with different letter superscripts in the same column mean significant difference ($P < 0.05$).

Table 4. Effects of light regime during incubation on serum biochemical indexes in 1-day-old chicks.

Group	Total protein/(g/L)	Albumin/(g/L)	Globulin/(g/L)	Lysozyme/(μg/mL)	Urea nitrogen/(mmol/L)
Inc_{24D}	42.36 ± 2.37	7.43 ± 1.05	34.93 ± 2.10	4.56 ± 0.38[b]	0.05 ± 0.01
$Inc_{8L:16D}$	48.57 ± 3.44	6.13 ± 0.78	42.44 ± 3.03	6.05 ± 0.95[b]	0.11 ± 0.03
$Inc_{12L:12D}$	51.65 ± 3.76	6.58 ± 1.56	45.07 ± 3.56	9.13 ± 0.76[a]	0.10 ± 0.03
$Inc_{16L:8D}$	44.62 ± 4.04	7.79 ± 1.32	36.83 ± 2.05	7.44 ± 0.55[ab]	0.08 ± 0.02
P value	0.215	0.141	0.063	0.041	0.226

Data are the mean of 4 replicates.
[a,b]Values with different letter superscripts in the same column mean significant difference ($P < 0.05$).

Table 5. Effects of light regime during incubation on serum biochemical indexes in 1-day-old chicks.

Group	T-AOC/(U/mL)	GSH-Px/(μmol/L)	T-SOD/(nmol/mL)	MDA/(nmol/mL)
Inc_{24D}	3.46 ± 1.03	881.16 ± 37.69[b]	34.25 ± 12.16[b]	9.67 ± 1.23
$Inc_{8L:16D}$	6.68 ± 2.45	836.45 ± 42.15[b]	33.16 ± 9.85[b]	5.96 ± 2.34
$Inc_{12L:12D}$	11.27 ± 2.74	1144.61 ± 44.78[a]	42.53 ± 12.74[a]	5.65 ± 1.45
$Inc_{16L:8D}$	7.23 ± 1.78	960.78 ± 39.62[ab]	40.08 ± 10.05[ab]	7.03 ± 0.87
P value	0.075	0.037	0.047	0.056

Data are the mean of 4 replicates.
[a,b]Values with different letter superscripts in the same column mean significant difference ($P < 0.05$).
Abbreviations: GSH-Px, glutathione peroxidase; MDA, malondiadehyde; T-AOC, total anti-oxidant capability; T-SOD, total superoxide dismutase.

content in the $Inc_{12L:12D}$ group was greater than those in the Inc_{24D} and $Inc_{8L:16D}$ groups ($P < 0.05$).

Table 5 shows that lighted incubation had no effects on the serum T-AOC ($P > 0.05$), but T-AOC in the $Inc_{12L:12D}$ group (11.27 U/mL) was numerically greater than the other 3 groups. GSH-Px and T-SOD activities in the $Inc_{12L:12D}$ group were higher than those in the Inc_{24D} and $Inc_{8L:16D}$ groups ($P < 0.05$), while the $Inc_{16L:8D}$ had no difference with other 3 groups. The MDA content in the Inc_{24D} group was the highest (9.67 nmol/mL) compared to the other 3 groups, very close to the significant level ($P = 0.056$).

DISCUSSION

Light plays an important role in regulating the circadian rhythms and providing rest and regeneration for poultry (Malleau et al., 2007; Zawilska et al., 2007). There has been a lot of research on the effects of lighted incubation on the hatching performance of breeder eggs, but the results were variable due to different breeds, light intensity, color, length, etc. Huth and Archer (2015) observed that broiler breeders exposed to white LED light during incubation showed better hatching performance compared with the 24 h dark group, while the hatching performance of white Leghorn layer eggs did not improve. Yu (2016) used three intensities of monochromatic green light to study the hatching performance of Lingnan Yellow broiler breeder eggs, and found that green light could improve the hatching rate, and lower light intensities (22−75 lx) were most effective, while higher light intensities (92−208 lx and 150−392 lx) were associated with reduced effects or side effects.

Yameen et al. (2020) compared the effects of 2 light regimes during incubation (24 h dark as the control, 12 h light and 12 h dark, and 24 h light) on hatching performance of three broiler eggs, and found that the hatching traits were improved only in one of the 3 broiler eggs. Wang et al. (2020) compared the effects of monochromatic green light on 4 strains of layer breeder eggs, and showed that no significant difference in hatchability of fertile eggs, or chick quality among the 4-strain eggs. Güz et al. (2021) compared the effect of $Inc_{16L:8D}$ and Inc_{24D} (green LED light) on Ross 308 broiler eggs, and found that the hatchability was not affected.

In this present experiment, we observed 3 light regimes ($Inc_{8L:16D}$, $Inc_{12L:12D}$, $Inc_{16L:8D}$, white LED light, light intensity of 150−200 lx) and found that the hatching performance of BYC eggs was not significantly affected, but the hatching rate of eggs was the lowest in the Inc_{24D} group (87.22%), and the highest in the $Inc_{12L:12D}$ group (93.64%), which indicates that the lighted incubation had the potential to improve the hatching performance for this bird.

The above differences in hatching performance by lighted incubation might be related to the light absorption capability of different eggshells. Eggshell is a critical factor that influences hatching performance of eggs (King'Ori, 2011). The light absorption capability of eggshell is related to eggshell color, eggshell strength and eggshell thickness. Generally, brown eggs have a larger eggshell thickness, such as 0.37 mm for Hyline Brown, 0.38 mm for Roman Brown, and 0.40 mm for Isa Brown (Tang et al., 2014), while the eggshell color is light pink, and the eggshell thickness is 0.325 mm on average in the middle period of laying for BYC eggs (Zhang et al., 2010), which will affect its light absorption capability. The effects of the eggshell characteristics on light absorption capability need to be further studied and compared in future.

Body size is a direct observation index of body development. Measuring the body size of chicks at 1 d of age can not only predict the future performance, but also partly reflect the hatching performance. The femur length and tibia length can reflect the long bone development. Van der Pol et al. (2017) studied 3 lighting during incubation: Inc_{24L}, $Inc_{16L:8D}$, Inc_{24D}, and 2 lighting after incubation: 24L, 16L:8D on the leg bone development of Ross 308 broilers, and found that there had more abnormal growth plate in the Inc_{24L} group than in the $Inc_{16L:8D}$ and Inc_{24D} groups, there had more bacterial cartilage necrosis in the Inc_{24D} than in the $Inc_{16L:8D}$ group, suggesting that $Inc_{16L:8D}$ can improve the leg health of chicken. Van der Pol et al. (2019a) observed the Inc_{24L}, $Inc_{12L:12D}$ and Inc_{24D}, and found that the tibia ossification rate of the $Inc_{12L:12D}$ group was higher for 12 to 14 embryonic age, and the hatched chicks had longer tibia and femur, suggesting that the $Inc_{12L:12D}$ stimulated the leg bone development. Van der Pol et al. (2019b) further studied the Inc_{24L}, Inc_{24D}, $Inc_{16L:8D}$ (cool white, 500 lx) and found that the hatched chicks in Inc_{24D} group had higher femur length, higher femur weight, tibia weight in the $Inc_{16L:8D}$ group, and it also had higher femur length, width, and tibia depth than in the Inc_{24L} group.

This present study found that the femur length in the $Inc_{12L:12D}$ group was higher than in the Inc_{24D} and $Inc_{16L:8D}$ groups ($P < 0.05$), and the tibia length in the $Inc_{12L:12D}$ was numerically greater than in the Inc_{24D} and $Inc_{16L:8D}$ groups, indicating that $Inc_{12L:12D}$ was relatively more conducive to the development of long bone, which supported the results of Yu (2016) and Van der Pol et al. (2019a), but was contrary to Van der Pol et al. (2019b), which may be due to the different breeds and light intensity adopted.

It was said that light perception of avian embryo mainly occurred at first 2 days of incubation, and the light stimulated the meiosis of neural crest mesoderm, quickens the closure of neural tube and promoted the development of central nervous system (Cooper et al., 2011). The relative brain weight can partly reflect the development of the brain tissue. This present study found that the relative brain weight in the Inc_{24D} and $Inc_{16L:8D}$ groups were significantly higher than that in $Inc_{8L:16D}$ group, and there had no difference between $Inc_{12L:12D}$ group and other 3 groups, indicating the relative brain weight in $Inc_{12L:12D}$ was not or less affected, which may be one of reasons affecting the leg bone development.

Serum biochemical indexes can partly reflect the metabolism and health status of the organism, especially serum immune and antioxidant indexes can indirectly reflect the health status of the body. Serum total protein, albumin and urea nitrogen reflect the metabolic status of protein in the body to a certain extent, while serum globulin and the activity of lysozyme are closely related to the immune level of the body. Yameen et al. (2020) compared 3 light regime during incubation on serum biochemical index of broilers at 35 d of age after hatching, and found that serum total protein and albumin content were the highest in the 24 h light group, followed by the 12 h light group and the 24 h dark group, with significant differences. Serum globulin was the highest in the 24 h light group and the lowest in the 24 h dark group. Uric acid levels maintained the same trend, which suggests that 24 h light exposure during incubation may lead to more protein catabolism, higher blood protein levels, which is actually not good for chick health, suggesting 12 h light group was more beneficial to serum biochemical indexes in later periods. In this present study, the serum total protein, albumin, globulin and urea nitrogen in 1-day-old chicks were not significantly affected by the light regime, but the globulin content in 12 h light and 12 h dark group were higher than those in other groups, partly supporting that 12 h light and 12 h dark group was beneficial to the protein catabolism in hatched chicks.

Yameen et al. (2020) measured the serum immune indexes of broilers at 35 d of age, and found that the antibody titers of 12 h light and 12 h dark group were the highest, and the lowest in the 24 h dark group. Thymic weight and spleen weight were also the highest in the 24 h light group, followed by the 12 h light group and the 24 h dark group. In this experiment, we found that the serum lysozyme content in the $Inc_{12L:12D}$ group was higher than those in the Inc_{24D} and $Inc_{8L:16D}$ groups, and $Inc_{16L:8D}$ had no difference with the other three groups, indicating both the 12 h light and 16 h light were beneficial to the immune functions of chicks.

Oxidative stress is a state of imbalance between the oxidant system and the antioxidant system in the body, which is generally manifested as the oxidants prevailing. Wan (2017) injected different concentrations of 2,2′-azobis[2-methylproionamidine] dihydrochloride (**AAPH**) into embryo at the early stage to induce the oxidative stress environment inside the chicken embryos, found AAPH could cause increasing death, growth retardation, and shorter tibia length, suggesting that the oxidative stress can cause the embryonic bone injury. Özkan et al. (2012) found that, compared with the Inc_{24D}, the lower MDA content in brain tissue of $Inc_{16L:8D}$ birds on d 6 may indicate lower lipid peroxidation and thus lower oxidative stress. This present study found that serum MDA content in 1-day-old chicks was

the highest in Inc_{24D} group ($P = 0.056$), suggesting there might exist some peroxidation, the serum GSH-Px and T-SOD activities in $Inc_{12L:12D}$ groups were significantly higher than other groups, indicating that 12 h light and 12 h dark group could increase the serum antioxidant level in 1-day-old chicks, indirectly reduce oxidative stress and then the possibility of the related bone injury, along with the previously mentioned that the incidence rate of leg problem reduced in 12 h light and 12 h dark incubation group, which may be a good reason to promote the future application of lighting incubation in commercial hatchery.

CONCLUSIONS

The present study indicated that lighted incubation had no significant effects on the hatching rate of eggs, the hatching rate of fertile eggs, and healthy rate of 1-day-old chicks, but the hatching rate of eggs was the highest in the $Inc_{12L:12D}$ group, indicating that 12 h light and 12 h dark incubation has the potential to improve the hatching performance of BYC eggs, benefit for the long bone development, improve some serum immune and antioxidant indexes, and reduce leg problems in 1-day-old chicks.

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DISCLOSURES

The authors declared that we have no conflicts of interest to this work.

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