Impacts of colored light-emitting diode illumination on the growth performance and fecal microbiota in goose

Xiujin Li,* Zhaobo Zheng,* Jianqiu Pan,* Danli Jiang,* Yunbo Tian,* Lingzhao Fang,¹ and Yunmao Huang*,¹

*Guangdong Provincial Key Laboratory of Waterfowl Healthy Breeding, College of Animal Science & Technology, Zhongkai University of Agriculture and Engineering, Guangzhou Guangdong 510225, China; and ¹Medical Research Council Human Genetics Unit at the Medical Research Council, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, United Kingdom

ABSTRACT Besides on the reproductive performance, the light also has an important effect on the growth in birds. In the present study, we for the first time investigated effects of colored light-emitting diodes (LED) on both growth performance and fecal microbiota in meat geese. We randomly selected a total of 120 geese at birth (0-day), divided them into 3 groups evenly (i.e., 40 geese each group), and then reared them under 3 colored light-emitting diodes (i.e., blue, red, and white) with the same photoperiod for 9 wk, respectively. We collected fecal samples at the experimental day 35 and 63, respectively. We observed that geese in blue light had higher body weight than those in red and white lights at the early stage of the experiment but showed lower body weight at the late stage, particularly at day 63 ($P < 0.05$). Interestingly, we found that the relative abundances of 3 dominant bacteria phyla, Firmicutes, Proteobacteria, and Cyanobacteria, were comparable among 3 groups at day 35, while at day 63, the blue light group had the significantly ($P < 0.05$) lowest and highest abundance for Firmicutes and Proteobacteria, respectively. Functional enrichment analyses revealed that the fecal microbiota in the red light group was mainly involved in metabolism at day 35, whereas at day 63, the fecal microbiota were engaged into membrane transportation and transcription. In contrast, the blue light group had more enriched pathways relevant with membrane transportation at day 63 than day 35 and had several pathways involved in metabolism at day 63 as well. Collectively, our results revealed that the light with different colors affects the growth performance of geese via the gut microbiota, which in turn influences the digestion and absorption of geese.

Key words: meat goose, light color, growth performance, fecal microbiota

INTRODUCTION

Artificial light, as an external environmental factor, is crucial for the release of various hormones, which play key roles in the activity, growth, immunity, and reproduction of birds (Patel et al., 2016). For laying birds, light plays an important role in the development and functioning of their reproductive systems, significantly influencing the age when they start laying and how many eggs they could lay in a given period (Min et al., 2012; Huber-Eicher et al., 2013). For broiler birds, light shows a big impact on their growth performance and other relevant activities (Rozenboim et al., 2004).

The light color is determined by different wavelengths of the visible spectrum. Previous studies have explored effects of the monochromatic light on the behavior, welfare, and performance of birds but mainly focusing on laying and broiler chickens. It has been reported that laying hens had the best egg production when exposed to red light (Min et al., 2012; Hassan et al., 2014). However, effects of light colors on broiler growth are largely unknown yet. By examining 4 different light colors, Firouzi et al. (2014) reported that broiler chickens reared under yellow and blue light had the largest and smallest body weight gain, respectively. Conversely, Morrill et al. (2014) found 6.0 and 8.9% increases in the final body weight of broilers reared under blue and green monochromatic light when compared with those reared under red and white light. Yet, the
impact of light colors on the growth performance in meat geese has not been investigated.

Nowadays, the rapid development of 16S rRNA sequencing technology has enabled the in-depth analysis of gut microbiota in humans and animals at an unprecedented scale (Sweeney and Morton, 2013; Hacquard et al., 2015). Previous studies showed that changes in gut microbiota had a close relationship with many fundamental biological processes in humans and animals, such as development and diseases (Dominguez-Bello et al., 2011; Lee and Hase, 2014). Therefore, it could be of great interest to investigate the effects of light colors on gut microbiota in meat geese using 16S rRNA sequencing technology, enabling us to further explore the underlying microbiota mechanisms of correlation between growth performance and light colors.

Monochromatic light-emitting diode (LED) sources have been widely used in poultry management because of reducing electricity consumption and being environmentally friendly (Rozenboim et al., 1998). In summary, the main objective of this study was to investigate the impact of different colored LED on both growth and fecal microbiota (an indicator of gut microbiota) in meat geese, which provides a foundation for future studies targeting the specific effects of important bacterial populations on the growth performance in geese.

MATERIALS AND METHODS

Ethics Statement

This study was carried out in strict accordance with the Animal Management Rule of the National Health and Family Planning Commission, People’s Republic of China (documentation 55, 2001). The research protocol was reviewed and approved by the Animal Care and Use Committee of Zhongkai University of Agriculture and Engineering.

Animals and Experimental Design

A total of 120 Magang geese after birth at 0-day-old were provided by a commercial company in Qingyuan city of Guangdong. These geese were selected randomly and divided into 3 groups evenly, resulting in 40 geese per group, and each group was reared in 1 of 3 equally matched rooms except for light colors being studied. Three monochromatic LED lights, that is blue, red, and white lights, were used for these 3 groups, labeling as B, R, and C, respectively (Figure 1). The light intensity was fixed at 40 lux and at the height of about 1.5 m off the ground in all 3 groups to ensure that light color is the only variable factor. The entire experimental period was 9 wk. The photoperiod was adjusted to 24 L: 0 D for the first 1 wk. Thereafter, the photoperiod was adjusted to 16 L: 8 D for the following 8 wk. Feeding, water, and other environmental factors were kept as similar as possible across 3 groups to avoid the systematic effects on growth performance.

Individual body weight was recorded at the end of each week. Blood samples were randomly collected from 8 out of 40 geese in each of the 3 groups at the experimental day 63. Growth hormone (GH), insulin-like growth factor 1 (IGF-1), triiodothyronine (T3), and adrenaline (ADR) were then measured from serum. Fecal samples were randomly selected from 6 out of 40 geese in each of 3 groups at the experimental day 35 and 63, respectively. These 36 fecal samples were then immediately snap-frozen in liquid nitrogen and stored at −80°C for microbiota analyses.

DNA Extraction and 16S rRNA Gene Sequencing

Microbial DNA was extracted from the 36 fecal samples using the E.Z.N.A. stool DNA Kit (Omega Bio-tek, Norcross, GA) based on the manufacturer’s protocols. The 515 F (5’-GTGCCAGCMGCC GCGG-3’) and 806R (5’-GGACTACH VGGG TWTCTAAT-3’) conventional primers were used to amplify the V4 variable region of 16S rDNA of total bacterial DNA (Whiteley et al., 2012). PCR was performed in a 20 μL reaction system in which 0.8 μL of each primer, 10 ng template DNA, 4 μL 5 × FastPfu buffer, 2 μL 2.5 mM dNTPs, and 0.4 μL FastPfu polymerase were added, and ddH2O was then added to reach 20 μL. PCR was set as follows: 95°C for 5 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 10 min. The amplicons were extracted from 2% agarose gels, purified using the Axy-Prep DNA Gel Extraction Kit (Axygen Bioscience,
Union City, CA) and quantified using QuantiFluor-ST quantitative system (Promega, Madison City, WI). Purified amplicons were pooled to construct sequencing libraries, and these sequencing libraries were then sequenced (~50,000 paired end reads per sample) by an Illumina Miseq sequencer in the Beijing Genomics Institute (BGI, Guangdong, China), using MiSeq Reagent Kit v2-HS 500 cycles.

Data Analysis

The microbiota sequences were analyzed by the open-source QIIME2 pipeline (Berg-Lyons et al., 2010). Raw sequences data were demultiplexed and quality filtered using the demux plugin in QIIME2, followed by denoising with DADA2 (Callahan et al., 2016) to identify the amplicon sequence variants. A masked alignment using MAFFT plugin were then conducted, followed by a phylogeny analysis with FastTree and Midpoint-root plugins (Katoh, 2002). The core-metrics command of diversity plugin was used to calculate \( \alpha \)-diversity (intragroup diversity) and \( \beta \)-diversity (diversity between groups). The taxonomic assignment was performed by using the feature-classifier plugin, adopting a confidence threshold of 0.80 and using the SILVA database release 132 as a reference. Phylum and genus relative abundance were calculated by using the taxa plugin.

The functional profiles of bacterial metagenomes were predicted by using PICRUSt (http://huttenhower.sph.harvard.edu/galaxy/) (Langille et al., 2013). Briefly, the individual sequences and the vsearch plugin were used to get individual operational taxonomic units. These operational taxonomic units were normalized by 16 rRNA gene copy numbers, and then the predicted Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs were summarized to level-3 functional categories. The difference in abundances of these categories among 3 groups was compared by using the linear discriminant analysis effect size (LEfSe) method (Segata et al., 2011).

The differences in body weights, \( \alpha \)-diversity, and specific bacterial abundance among 3 groups were analyzed by one-way ANOVA using R software, and Turkey multiple comparisons were then employed.

RESULTS

Growth Performance

As shown in Table 1, from the experimental day 14 to 42, the same trend were observed that B group had the largest body weight, whereas C group had the smallest body weight. However, the difference in body weight did not reach the statistical significance among the 3 groups \((P > 0.05)\). Of interest, from the experimental day 49 to 63, R group started to have the largest body weight, followed by C and B groups. At the experimental day 63, body weights between R and C groups were comparable, but both of them were significantly larger than B group \((P < 0.05)\). In other 2 scenarios of day 49 and 56, no significant differences in body weights were observed among 3 groups \((P > 0.05)\).

In addition, levels of 4 hormones (GH, IGF-1, T3, and ADR) relevant with growth were measured and compared among 3 groups (Table 2). Compared with C group, B and R groups had lower levels of these 4 hormones, particularly for ADR. While, B group had higher levels of these 4 hormones than R group. The standard error of estimates were large because of the small sample size.

Microbial Diversity

The rarefaction curve of the Shannon index approached the plateau at the sequencing depth of 3330 (Figure S1). Four indexes (Shannon, Evenness, Faith_pd, and Observed_otus) were calculated as the \( \alpha \)-diversity to evaluate the microbial diversity within group (Table 3). At the experimental day 35, R group had largest values for 3 out of 4 indexes, whereas C group had the smallest ones for all 4 indexes. The significant differences in 3 indexes were observed among 3 groups \((P < 0.05)\). At the experimental day 63, B group had largest values for all 4 indexes, followed by R and C groups. However, differences in 4 indexes were not significant among 3 groups \((P > 0.05)\). Principal coordinate analysis showed that samples in C group tended to be discrepant with those in other 2 groups at the experimental day 35, and B group was different from other 2 groups at the experimental day 63 (Figure 2). The PREMANOVA test also confirmed significant differences between group B and R \((P < 0.05)\) and at day 63 significant differences between group B and C \((P < 0.05)\) and suggestively significant differences between group B and R \((P = 0.06)\). This suggested that the associations of the fecal bacterial compositions among 3 light colors were distinct between day 35 and 63.

Characterization of Microbiota Composition

A total of 17 phyla and 120 genera were detected at the experimental day 35 and 19 phyla and 212 genera at day 63, respectively. At the phylum level, the first 3 dominant bacteria were Firmicutes, Proteobacteria, and Cyanobacteria in all 3 groups at both day 35 and

| Table 1. Body weight (unit: g) ± standard error in the experimental period. |
| Days | Blue light | White light | Red light |
|------|-----------|-------------|----------|
| 14   | 263.68 ± 8.70 | 242.68 ± 6.93 | 258.64 ± 6.96 |
| 21   | 490.30 ± 17.94 | 439.46 ± 15.64 | 484.11 ± 14.41 |
| 28   | 922.53 ± 29.78 | 856.23 ± 27.30 | 911.10 ± 24.13 |
| 35   | 1,413.05 ± 39.39 | 1,352.91 ± 38.61 | 1,398.00 ± 32.75 |
| 42   | 2,025.48 ± 49.51 | 1,942.36 ± 45.40 | 1,997.73 ± 46.02 |
| 49   | 2,025.48 ± 49.51 | 2,339.23 ± 52.43 | 2,366.67 ± 49.49 |
| 56   | 2,482.74 ± 70.92 | 2,041.39 ± 59.33 | 2,693.79 ± 67.70 |
| 63   | 2,636.71 ± 67.73<sup>a</sup> | 2,847.64 ± 64.53<sup>b</sup> | 2,888.39 ± 79.40<sup>b</sup> |

<sup>a,b</sup>Body weights within a row with no common superscript differ significantly \((P < 0.05)\) among 3 groups.
was observed between day 35 and 63. In the R group, the top 3 enriched pathways were involved in amino acid and carbohydrate metabolism at day 35, while the top pathways at day 63 were related to membrane transport and transcription. For the B group, more enriched pathways regarding membrane transport were obtained at day 63 than day 35. These results suggested that different colors of light affect growth performance potentially through influencing the composition of fecal microbiota in meat geese.

**DISCUSSION**

To our knowledge, we for the first time reported effects of light colors on both growth performance and fecal microbiota in meat goose. Lighting is reported to be an essential factor for poultry growth. Blue light tends to keep birds calm down while red light promotes

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**Table 2. The level of hormone (mean ± standard error, unit: ng/mL) associated with growth performance at the experimental day 63.**

| Hormone | Blue light | White light | Red light | P-value |
|---------|------------|-------------|-----------|---------|
| GH      | 2.29 ± 0.42 | 2.85 ± 0.40 | 1.98 ± 0.15 | 0.13    |
| IGF-1   | 9.81 ± 1.47 | 12.82 ± 2.28 | 8.22 ± 0.51 | 0.07    |
| T3      | 242.02 ± 52.44 | 321.68 ± 66.20 | 186.56 ± 16.49 | 0.09    |
| ADR     | 1.38 ± 0.18   | 1.93 ± 0.33* | 1.20 ± 0.10b | 0.04    |

*a-b* Hormone levels within a row with no common superscript differ significantly (*P* < 0.05) among 3 groups.

**Table 3. The alpha diversity indexes of bacteria at the sequencing depth of 3330 in different groups at the experimental day 35 and 63.**

| Days | Group | Shannon | Evenness | Faith_pd | Observed_otus |
|------|-------|---------|----------|----------|---------------|
|      |       |         |          |          |               |
|      | B     | 5.34 ± 0.32a | 0.83 ± 0.02 | 9.29 ± 0.87a | 87.67 ± 10.88a |
| 35   | C     | 4.85 ± 0.13a | 0.83 ± 0.02 | 6.26 ± 0.72b | 59.17 ± 5.88b  |
|      | R     | 5.73 ± 0.13b | 0.88 ± 0.01 | 8.68 ± 0.43a | 94.33 ± 7.69a  |
|      |       | 0.02       | 0.16      | 0.01      | 0.01          |
|      | B     | 5.96 ± 0.22  | 0.91 ± 0.01 | 11.74 ± 1.05 | 95.83 ± 12.21 |
| 63   | C     | 5.11 ± 0.28  | 0.86 ± 0.02 | 8.15 ± 1.31  | 67.50 ± 12.66  |
|      | R     | 5.60 ± 0.27  | 0.89 ± 0.02 | 9.54 ± 1.20  | 81.67 ± 10.53  |
|      |       | 0.06       | 0.08      | 0.09      | 0.21          |

*a-b* Index within a column with no common superscript differ significantly (*P* < 0.05) among 3 groups.

**Abbreviations:** B, blue light; C, white light; OTU, operational taxonomic unit; R, red light.

**P-value:** *P*-value from one-way analysis of variance among 3 groups.
birds more activities. However findings on the effects of lights on the growth performance of birds are not consistent (Firouzi et al., 2014; Hassan et al., 2014; Seo et al., 2015; Patel et al., 2016). In the current study, geese reared under blue light had a higher body weight than those under red and white lights at the early stage but had a lower body weight at the late stage, especially at the experimental day 63 ($P < 0.05$). Fecal samples at both early stage (day 35) and late stage (day 63) were collected to approximate the gut microbiota. Distinct differences in microbiota diversity, microbiota composition, and the functionality prediction of microbiota were found between B and R groups at day 63, whereas similarities in microbiota diversity and microbiota composition were observed at day 35. Functional enrichment analysis showed that the composition and functionality of microbiota in the B group served for the basic metabolism, whereas those in the R group improved the metabolism activity of the host.

Few studies explored effects of light colors on meat geese. We found only 1 study on Roman breeder geese, in which they reported no significant difference in body weight among blue, red, and white groups reared for about 6 mo. However, the red LED light led to a longer laying period and a higher total egg number, and thus, it was a better choice for the management of breeding geese compared with other colors of light (Chang et al., 2016). Red light was also reported to have similar positive effects on laying hens (Min et al., 2012; Borille et al., 2013; Hassan et al., 2014). However, effects of red and blue lights were inconsistent on broilers. Most of the previous studies reported that blue light had the advantage of improving growth performance over red light because blue light kept birds calmer than red one (Prayitno et al., 1997a; Seo et al., 2015; Patel et al., 2016). However, some studies found opposite results that red light improved growth performance in the late rearing period, as red light increased activity and reduced locomotion disorders (Prayitno et al., 1997b; Firouzi et al., 2014; Hassan et al., 2014). By consistently observing the body weight of meat geese in a long experimental period, our study demonstrated that at the early stage, blue and red lights improved growth compared with white light, and blue light was better than the red one. However, at the late stage, red light had a positive effect on growth compared with white light, whereas blue light showed a negative effect. Therefore, it seems better to use red LED light rather than blue and white lights in meat geese industries.

Although significant differences in body weight between B and other 2 groups were observed, the levels of 4 hormones (GH, IGF-1, T3, and ADR) among 3 groups were not consistent with the trend of body weight at day 63 (Tables 1 and 2). Owing to the small sample size (8 samples in each group), we did not explore the link between hormone levels and fecal microbiota in this study.

Because the Shannon index was less sensitive to the experimental conditions of PCR and sequencing platforms, the Shannon index was used as the rarefaction curve for alpha-diversity. These rarefaction curves approached the plateau phase with more than 3,330 sequences per sample. At day 35, geese in white light showed a clear distinction in microbiota diversity (both alpha and beta diversity) compared with those in blue and red ones. Whereas, at day 63, there were no statistically significant differences in alpha diversity among 3 groups, but a clear difference was observed in beta diversity between B and the other 2 groups. This was consistent with the phenomenon that geese have the lowest body weight in white light at day 35 and in blue light at day 63.
Differences in the composition of fecal microbiota between blue light group and other 2 groups were observed at day 35 and 63, providing more evidence that blue light had a positive effect on growth performance at day 35 but a negative one at day 63. The phylum Firmicutes was the top abundant bacteria across all samples, which was in line with previous reports that Firmicutes was the most ubiquitous and common phylum observed within gut environments of many birds (Waite and Taylor, 2014; Wang et al., 2016). We observed similar proportions of Firmicutes among 3 groups at day 35 and a significantly lower proportion of Firmicutes in the B group compared with other 2 groups at day 63. This was in line with previous reports that Firmicutes was associated with the ability of energy harvest and nutrient absorption (such as insoluble fiber degradation) from the feed components (Turnbaugh et al., 2008; Jumpertz et al., 2011). In contrast, we found that Proteobacteria had a similar proportion among 3 groups at day 35, but a significantly higher proportion in B group compared with other 2 groups at day 63. This indicated that geese in blue light had

Figure 3. Relative abundance of the 7 major bacterial phylum compositions and genus compositions in 3 groups. A: Phylum compositions at the experimental day 35, B: genus compositions at the experimental day 35, C: phylum compositions at the experimental day 63, and D: genus compositions at the experimental day 63.
serious dysbiosis in gut microbiota at day 63, because *Proteobacteria* was reported as microbial signature of dysbiosis in gut microbiota (Shin et al., 2015). High levels of *Cyanobacteria*, an artifact as chloroplasts in plant matter, might be the indicative of undigested plant material. The difference in *Cyanobacteria* among 3 groups (15.85, 10.41, and 2.14% for B, C, and R groups, respectively) at day 63 suggested that geese in blue light had more undigested plant material than those in red and white ones.

At the genus level, the top 2 bacteria (*Bacillus* and *Lactococcus*), belonged to *Firmicutes*, had similar proportions among 3 groups at day 35, whereas at day 63, they had much lower proportions in B group than R and C groups. This was consistent with the result that similar body weights were found among 3 groups at day 35 ($P > 0.05$), whereas the B group had significantly lower body weights than R and C groups at day 63 ($P < 0.05$). Additionally, it should be noted that a large number of unclassified genera were detected for all groups in our study, which ranged from 12.18 to 32.83%. Further study should be required to better characterize these unknown bacteria and their special functions in the hosts.

Gut microbiota plays an important role in the digestion and absorption of the host. In the present study, functional prediction for fecal microbiota (the proxy as gut microbiota) in each group was conducted based on KEGG and LEfSe. The results revealed remarkable differences in microbiota functions among 3 groups, indicating that the bacterial compositions were important for growth performance in geese. The significantly and specifically enriched pathways in the R group were related to metabolism at day 35, and those at day 63 were engaged in metabolism. This was consistent with the phenomenon that geese in red light were more activities for compared with other 2 lights. Whereas, significantly and specifically enriched pathways in the B group were involved in membrane transport and cellular processes, which were involved in the fundamental maintenance of the host. All these results provided further evidence that geese reared under blue and red lights had similar body weights at day 35 and significantly different body weights at day 63.

The important biological role of the ceca in chicken productivity, health, and well-being has made the majority of chicken microbiota studies to use the ceca as a sampling site (Torok et al., 2008; Stanley et al., 2012, 2013, 2015). In the current study, fecal sampling were used rather than cecal sampling because fecal sampling does not require sacrifice and thus allows the same goose to be sampled repeatedly at day 35 and 63. Sampling the same bird is a more meaningful way than selecting a subset of birds at each time point because of the known animal variation in microbiota. Furthermore, Stanley et al. (2015) also found that regardless of community structure differences, both cecal and fecal microbiota analyses are likely to accurately report whether a treat or condition induces changes in gut microbiota.

**CONCLUSIONS**

A preliminary investigation on the effects of light colors on growth performance and fecal microbiota in meat geese was carried out in this study. We observed that geese reared in blue light had a higher body weight than those in red and white lights at the early stage of growth but showed a lower body weight at the late stage, especially at day 63. The further analysis of the fecal microbiota among 3 groups at 2 experimental points (day 35 and 63) revealed that the gut microbiota could markedly influence the digestion and absorption of geese reared under red and blue lights, resulting in a better growth performance at day 63 compared with those reared under blue light.

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The authors confirm that all data underlying the findings are fully available without restriction and declare that they have no competing interests.
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