Effects of different light intensities on the transcriptome changes of duck retina and pineal gland

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ABSTRACT The light intensity can affect the production performance of animals. The retina and pineal gland, closely linked, are directly photosensitive organs. This study evaluated the effect of light intensity on duck growth and investigated the effects of varying light intensities on retina and pineal gland transcriptome changes. The increase of light intensity will significantly decrease production performance, such as body weight, eviscerated weight, breast muscle weight, percentage of abdominal fat, etc. The RNA-seq revealed 967 and 201 differentially expressed genes (**DEG**s) in the retina and pineal gland under different light intensities, respectively. The Gene Ontology (**GO**) and Kyoto Encyclopedia of Genes and Genomes (**KEGG**) in the retina showed the DEGs were enriched in ECM-receptor interaction, Focal adhesion, Cell adhesion molecules (**CAM**s), Cytokine-cytokine receptor interaction, Melanogenesis, etc. Meanwhile, the DEGs in the pineal gland were mainly enriched in pathways associated with the mTOR signaling pathway, melanin production pathway, etc. Our results indicated that different light intensities might affect the function of the retina and pineal gland, including the melanin production of the retina and the secretion of melatonin in the pineal gland. Our study can provide a theoretical basis for the molecular mechanism of the effects of different light intensities on the retina and pineal gland.

Keywords: duck, retina, pineal gland, light intensity, transcriptome

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INTRODUCTION

Poultry production is affected by environmental factors. Light intensity is one of the most critical environmental factors. Light can affect poultry behavior, development, feed conversion efficiency, production performance, and animal welfare (Ke et al., 2011; Parvin et al., 2014). Too high or too low are unsuitable for poultry production (Deep et al., 2013). A reasonable light schedule can improve ducks' production performance by reducing stress and enhancing their immunity functions. There are 2 primary tissues and organs related to light for birds, including the retina and pineal gland. The former can synthesize melanin, and the latter can synthesize and secrete melatonin. Birds' circadian rhythms involve 3 nerve pacemakers: the Suprachiasmatic nucleus (**SCN**), the pineal gland, and the retina.

The retina is a major photosensory organ for animals. As an essential receiving organ of environmental information, the retina plays a crucial role in feeling light in mediating circadian rhythm. Compared with mammals, birds are more sensitive to light. Many light receptors are distributed in the Rod cone layer (**RCL**) of the retina in birds, which can cooperate with nonretinal light receptors to receive light information from the surroundings and influence the birds' growth and behaviors (Dawson et al., 2001). For birds, light stimulation can stimulate the light receptor on the retina to produce nerve impulses, which can be transmitted to the visual center. Light stimulation can also directly stimulate the light receptor outside the retina through the skull to produce nerve impulses, stimulating the secretion of hormones related to growth and development, and the hormones act on the corresponding target organs through the blood circulation system, to adjust the growth and reproduction of the body.

The pineal gland, which mainly contains glial cells and pineal cells, is one of the central organs that regulate the physiological system. The pineal gland is dominated by synaptic pathways originating in the SCN of the hypothalamus (Simonneaux and Ribelayga, 2003). The pineal gland is a photosensitive

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organ, which participates in circadian and seasonal rhythms, and plays a vital role in regulating many behavioral and physiological phenomena (Kwiecińska et al., 2017).

For mammals, the retina is closely connected with the pineal gland. The secretion of melatonin in the pineal gland is closely related to retinal photoreceptor cells, and this relationship plays a vital role in endocrine regulation (Wiechmann, 1986). The synthesis and release of melatonin in the pineal gland are affected by retinal light stimulation, mainly retinal ganglion cells (Ostrin, 2019). Melatonin biosynthesis is also located in the retina outside the pineal gland (Iuvone et al., 2005). For birds, the avian pineal organ is directly photosensitive, and it can also be the locus of a circadian pacemaker (Underwood et al., 2001). Studies have shown obvious compensatory changes in melatonin levels do not occur in the pineal gland following blinding in the Japanese quail (Underwood et al., 1984).

More attention has been paid to the retina and pineal gland. For mice, studies have shown that 100 lux low-intensity white light irradiation can cause changes in the retinal function of mice after dark adaptation (Yun-Zhi et al., 2014). Some scholars detected the transcriptome changes of the rat retina, showing that *Ecel1, Tnfrsf12a, Gpnmb, Chac1, CREB1*, and *NUPR1* were the most promising candidates for the development of novel neuroprotection strategies against axonal injury-driven RGC death after the optic nerve was excised for 2 d (Yasuda et al., 2016). Studies on quails have demonstrated that light stimulation can increase the *PER2* level in the pineal gland (Yoshimura et al., 2000). In maintaining the circadian rhythm, melatonin is synthesized by the pineal gland and retina in Turkey, and light plays a vital role in preserving melatonin secretion (Zawilska et al., 2006). The chick retina is a circadian oscillator relatively independent of the pineal gland. Greenlight can increase melatonin and cAanat by enhancing the circadian expression of melanopsin and positive clock genes on the chick retinal tissue (Bian et al., 2020).

Previous studies mainly focused on the retina and pineal gland gene expression under monochromatic light. But our research is focused on the effect of light intensity on the retina and pineal gland transcriptome. In meat duck production, the application of the multilayer cage is gradually widespread. But the light is unevenly distributed. Therefore, we need to pay attention to the uneven illumination in the vertical direction between different cage layers, especially the bottom cage. Since ducks are more sensitive to light information than chickens (Porter et al., 2018), 52 d old ducks were used as experimental animals. The retina and pineal gland were used as experimental observation organs. By setting up different light intensity experimental groups, the effects of light intensity on the retina and pineal gland transcriptome were analyzed by molecular biotechnology.

MATERIALS AND METHODS

Experimental Design and Sampling

Four hundred twenty-eight Cherry Valley ducks (214 males and 214 females) were provided by Sichuan New Mianying Agriculture and Animal Husbandry Co. Ltd (Mianyang, China). All ducks were reared in the same environment in the first two weeks. After the rearing period, 428 ducks were randomly divided into 2 groups. The feed intake, feeding pattern, and temperature were consistent except for light intensity during the experiment. All ducks were reared in cages (2.0 × 1.3 × 0.7 m) in a temperature- and humidity-controlled breeding house, drinking water and feeding were free at 14 to 43 d of age, and feeding was restricted at 44 to 51 d of age, with a daily limit of 120 g, according to the nutritional information of the diet (Table S1). Each group was exposed to white light-emitting diodes (**LED**) for 24 h (24 L:0 D) a d. The Treatment 1 (**T1**) group was exposed to 47,810 watts/m^2 from 14 to 43 d of age, 1,366 watts/m^2 from 44 to 51 days of age; the Treatment 2 (**T2**) group was exposed to 1,366 watts/m^2 light intensity from 14 to 51 d of age respectively (Figure S1). After 44 d of duck age, the light intensity decreased significantly due to the removal of the light.

The ducks were weighed at 1, 13, 37, 43, and 52 d, respectively. One-day-old and 13-day-old ducks were weighed randomly, and the average weight of the whole group was taken as the average weight; After 13 d of age, each individual was weighed at 37 d, 43 d, and 52 d of duck age.

Total 48 individuals were involved in the slaughter performance test, 24 in the T1 and 24 in the T2 groups. At 52 d old, one healthy male duck with similar body weight was selected from each replicate. According to the following order, the slaughter performance includes dressing weight, eviscerated carcass, semieviscerated carcass, thigh, breast, abdominal fat, skin fat, heart, liver, spleen, bursa, thymus, proventriculus, was weighed, respectively. Each duck was killed, and the eyes were immediately removed and hemisected. Retinal tissue was then removed from each eye using fine forceps. The retina was placed into the sterile Petri dishes containing PBS buffer, waggled slightly, to obtain relatively homogeneous and complete retinal tissue. After opening the duck head, immediately cut the pineal tissue with sterile scissors, wash it in a sterile Petri dish containing PBS buffer. Then quickly put the RNA sample of the retina and pineal gland into a 1.5 mL EP tube, store it in liquid nitrogen for a short time, and transfer it to an ultra-low temperature refrigerator at −80 °C for long-term storage.

All animals used have cared following the Institutional Animal Care and Use Committee (**IACUC**) guidelines of Sichuan Agricultural University.

Table 1. Effect of light intensity on body weight between the T1 group and the T2 group.

Age of day	Group	N	Average value	Standard deviation	CV (%)	*P*-value
37	T1	214	2.22	0.23	10.36%	<0.01
	T2	212	2.32	0.29	12.50%	
43	T1	213	2.55	0.34	13.33%	<0.01
	T2	212	2.76	0.36	13.04%	
52	T1	199	2.63	0.55	20.91%	<0.01
	T2	170	2.92	0.40	13.70%	

Total RNA Extraction and RNA-Seq

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Poly (A) + mRNA was purified with mRNA capture beads, and then the mRNA was randomly segmented into small fragments by divalent cations in a fragmentation buffer. These short fragments were used as templates to synthesize the first-strand cDNA using random hexamer primers. Second-strand cDNA was synthesized using RNaseH and DNA polymerase I. Short cDNA fragments were purified with VAHTSTM DNA Clean Beads. According to an Illumina protocol, the cDNA fragments were connected with sequencing adapters. After agarose gel electrophoresis, the 300 to 500 bp target fragments were selected for PCR amplification to create the final cDNA library.

The RNA libraries were prepared using the VAHTS mRNA-seq V3 Library Prep Kit for Illumina, according to the product instructions. First, the quality and size of the cDNA libraries for sequencing were checked using the Agilent 2200 Tape Station system (Agilent). Then, nine cDNA libraries were sequenced on the Illumina sequencing platform (Nova Seq 6000, Illumina, San Diego, CA). FastQC analyzed raw reads for quality, and high-quality reads with Q > 20 were obtained using NGSToolkits (version: 2.3.3) (Fumagalli et al., 2014). Functions of the Unigenes were annotated based on sequence similarities to sequences in the public UniProt database (Grabherr et al., 2011).

Differentially expressed genes (**DEGs**) between the 3 groups were identified using the EdgeR package. *P* value <0.05 and $|\text{Log}_2$ (fold change)| > 1 was set as the threshold for significantly differential expression.

Statistical Analysis

Data were analyzed using the SPSS statistical software (version 20.0, Windows, SPSS Inc., Chicago, IL) and Simca software. T-test and one-way ANOVA were used to analyze the differences between groups. The graph is drawn with GraphPad Prism (version 8.3) and R studio (version 4.0.2).

RESULTS AND ANALYSIS

Body Weight and Slaughtering Performance

In this experiment, the individual bodyweight of 428 ducks was measured at 37, 43, and 52 d old of duck age. SPSS software was used to analyze the differences in body weight between the T1 and T2 group, where the results showed that the bodyweight of the T2 group in the 3 periods was significantly larger than those in the T1 group ($P < 0.01$) (Table 1).

In terms of production performance, there were more significant differences between the T1 and T2 group in all 10 determined items, including bodyweight before slaughter, dressing weight, breast muscle weight, abdominal fat rate, etc. ($P < 0.01$). The weight of the bursa of Fabricius in the T1 group was significantly larger than the T2 group, and the weight of the glandular stomach bursa was on the contrary ($P < 0.05$). However, there was no significant difference in both groups in five items, including dressed percentage, leg muscle weight, etc. (Table 2).

In both periods, the feed conversion ratio of the T1 group was higher than that of the T2 group (Table 3). Thus, the changing trend of feed conversion ratio was consistent with body weight change.

Quality Control of RNA Sequencing Data

Paired-end sequencing was performed with HiSeTM 2000 (Illumina, USA) and, after quality control, an average of 22,664,670 and 23,182,582 clean reads was obtained from the retina and pineal gland, respectively. The Q30 value was higher than 92% for each sample, indicating that the sequencing quality was good and could be used for subsequent analysis (Table S2).

Partial least squares discriminant analysis (**PLS-DA**) was conducted by Simca software to analyze the retina and pineal gland of the 6 ducks (Figure 1). For a significant biological model, the expected R^2 and Q^2, highly dependent on their application model, should be more than 0.5 and 0.4, respectively (Boulesteix and Strimmer, 2007). In our established model, the value of R^2 and Q^2 meet the requirements (Table S3). It suggested that the samples were clustered well within the group, which indicated that the repeatability is well and the quality of the sample is reliable.

Screening DEGs

According to *P* value <0.05 and $|\text{Log}_2$ (fold change)| >1, 962 DEGs were screened out in the retina. Among them, there were 927 up-regulated genes and 35 down-regulated genes (Figure 2A). And 201 DEGs were

Table 2. Effect of light intensity on slaughter performance of ducks.

Phenotype	Group	N	Average value	Standard deviation	CV	*P* value
Body weight before slaughter (g)	T1	24	2631.25	486.96	19.00%	<0.01
	T2	24	2940.08	262.94	9.00%	
Dressing weight(g)	T1	24	2082.42	347.31	17.00%	<0.01
	T2	24	2339.96	230.55	10.00%	
Dressed percentage (%)	T1	24	79.41	2.59	3.26%	0.848
	T2	24	79.57	3.31	4.16%	
Eviscerated weight (g)	T1	24	1825.04	299.86	16.43%	<0.01
	T2	24	2054.58	180.63	8.79%	
Half eviscerated yield(g)	T1	24	1966.17	325.55	16.56%	<0.01
	T2	24	2219.92	197.22	8.88%	
Breast muscle weight(g)	T1	24	252.80	78.45	31.03%	<0.01
	T2	24	310.05	48.42	15.62%	
Leg muscle weight (g)	T1	24	247.87	51.19	20.65%	0.247
	T2	24	262.19	30.94	11.80%	
Heart (g)	T1	24	12.91	2.41	18.67%	<0.01
	T2	24	14.38	2.45	17.04%	
Liver (g)	T1	24	50.85	15.22	29.93%	0.051
	T2	24	60.10	16.68	27.75%	
Bursa of Fabricius (g)	T1	24	1.16	0.57	49.14%	0.045
	T2	24	1.48	0.48	32.43%	
Muscular stomach (g)	T1	24	49.76	10.61	21.32%	0.302
	T2	24	52.65	8.39	15.94%	
Glandular stomach (g)	T1	24	6.35	1.89	29.76%	0.026
	T2	24	7.89	2.67	33.84%	
Spleen(g)	T1	24	1.31	0.74	56.49%	0.300
	T2	24	1.53	0.73	47.71%	
Abdominal fat (g)	T1	24	17.04	7.91	46.42%	<0.01
	T2	24	27.83	10.26	36.87%	
Percentage of abdominal fat (%)	T1	24	0.91	0.37	40.66%	<0.01
	T2	24	1.34	0.44	32.84%	
Skin fat (g)	T1	24	325.96	85.90	26.35%	<0.01
	T2	24	410.13	65.93	16.08%	
Percentage of skin fat rate (%)	T1	24	18.55	2.33	12.56%	<0.01
	T2	24	21.23	2.67	12.58%	

Note: CV, the coefficient of variation; significance levels.

Table 3. Analysis of feed conversion ratio in different stages.

	One to 43 d of age		One to 52 d of age	
Group	T1	T2	T1	T2
Consumption (kg)	1175.90	1192.10	1312.25	1327.85
Total weight gain (kg)	531.38	571.68	510.81	554.69
Feed to meat ratio	2.21	2.09	2.57	2.39

detected in the pineal gland, including 92 up-regulated and 109 down-regulated genes (Figure 2B).

Functional Enrichment of DEGs

We performed a Gene Ontology (**GO**) analysis of DEGs identified in the retina and pineal gland to compare the functions of DEGs under different light intensities. The David website analyzed our experiment data, and DEGs were divided into three main GO categories: biological process, molecular function, and cell composition. The results showed 20 top GO terms in the retina (Figure 3A), according to *P* value <0.05. Within the biological process category, "substrate adhesion-dependent cell spreading" "positive regulation of angiogenesis" "wound healing" were counted as the top 3 categories; In addition, cell composition category, genes belong to extracellular space and focal adhesion, and "proteinaceous extracellular matrix," was highly enriched. In the molecular function category, "extracellular matrix structural constituent," "calcium ion binding," "extracellular matrix binding" accounted for a significant proportion.

There are 13 GO terms in the pineal gland according to *P* value <0.05 (Figure 3B). The results of GO showed that most of the DEGs were related to biological process, namely, in the biological process category, "T cell receptor signaling pathway," "cardiac muscle tissue morphogenesis," "skeletal muscle contraction," were significantly higher; In addition, "FK506 binding" and "heme binding" were enriched in the molecular functional category; In cell composition, "myofibril," "alpha-beta T cell receptor complex" "troponin complex" were enriched. In GO terms, "cAMP-mediated signaling" is involved in melatonin synthesis in the pineal gland.

KEGG Pathway Analysis

The KEGG enrichment showed that TOP 20 significantly enriched signaling pathways were enriched (Figure 3C) based on the DEGs in the retina ($P < 0.05$). These pathways included ECM-receptor interaction, Focal adhesion, Cell adhesion molecules (CAMs), Cytokine-cytokine receptor interaction, Melanogenesis, etc.

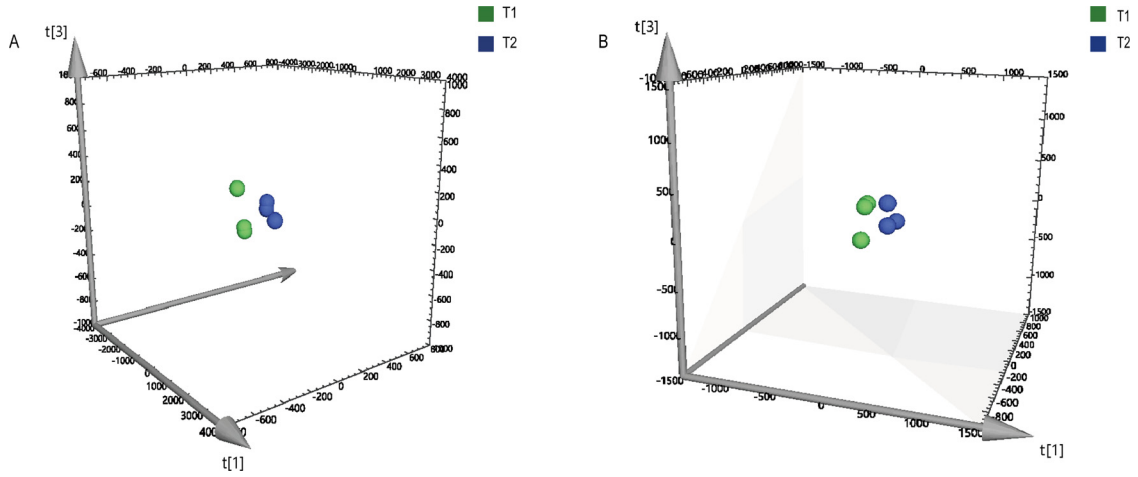

Figure 1. the PLS-DA model of the retina and the pineal gland. A represents the retina, and B represents the pineal gland. Each point represents one sample, and the same color represents the same group.

There are 48 pathways in the pineal gland. Tryptophan metabolism plays a vital role in the pineal gland. The substrate of melatonin synthesis is tryptophan, closely related to the synthesis and secretion of melatonin in the pineal gland. The KEGG enriched 5 significantly signaling pathways ($P < 0.05$) (Figure 3D): mTOR signaling pathway, melanin production pathway, Wnt signaling pathway, vitamin B6 metabolism, and cell adhesion molecule. According to the KOBAS website, the mTOR signaling pathway, melanin production pathway, and Wnt signaling pathway have an obvious correlation.

The Potential Relationships Between the Retina and Pineal Gland

There are 35 DEGs in the retina and pineal gland, including 25 annotated genes and ten unannotated genes (Figure 4A). Since the variables of this experiment are different light intensities, the retina and pineal gland are organs that directly sense light stimulation. Therefore, it was speculated that there is a relationship between the DEGs and light stress, and the relationship between them is the regulation of the clock. Thus, they are involved in the regulation of circadian rhythm. By using the gene cards database, there are 19 genes related to light stress, and the chart shows the most relevant 10 genes, including *HMOX1, MYL1, FKBP5*, etc. (Table S4); Eight genes are related to clock genes, including *DIO1, DIO3, FKBP5, HMOX1, PHOSPHO2*, etc. (Table S5).

Their CPMs, in tissues, were then performed for the gene expression cluster analysis. It was found that light stress genes in the retina, such as *DIO1, MSH4, RPL34*, can be clustered with clock gene *PHOSPHO2* (Figure 4C). In the pineal gland, the gene expression cluster analysis of light stress genes, including *DIO1* and *RPL34*, was the same as the clock gene *PHOSPHO2* (Figure 4D). We performed a PPI analysis based on the light stress and clock genes in all DEGs to further explore light stress and clock genes between the retina and pineal gland under different light intensities. According to the obtained data on the *STRING,*

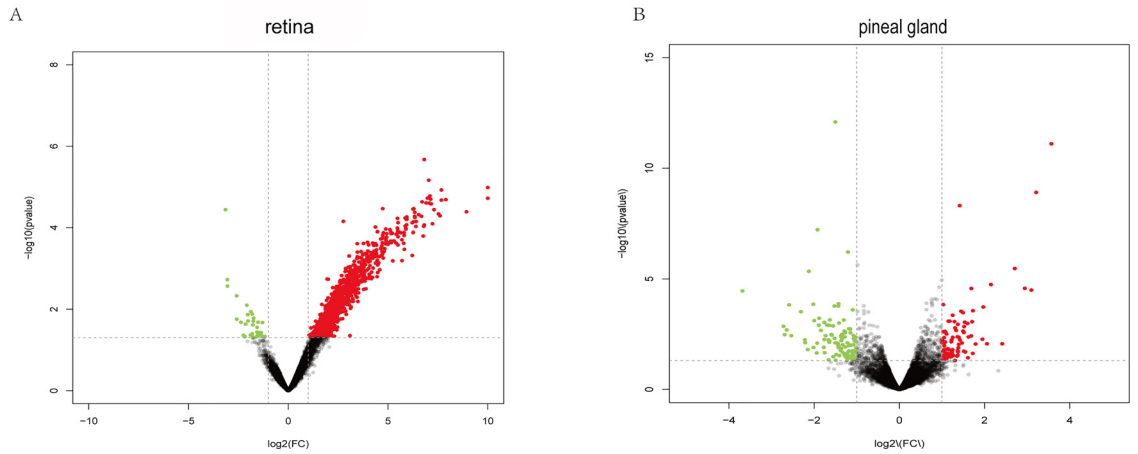

Figure 2. Volcano maps of differentially expressed genes. Each point in the differential expression map represents a gene. The green dots represent down-regulated genes, the red dots represent up-regulated genes, and the black dots represent non-differentially expressed genes. (A) Volcano map of differentially expressed genes for the retina. (B) Volcano map of differentially expressed genes for the pineal gland.

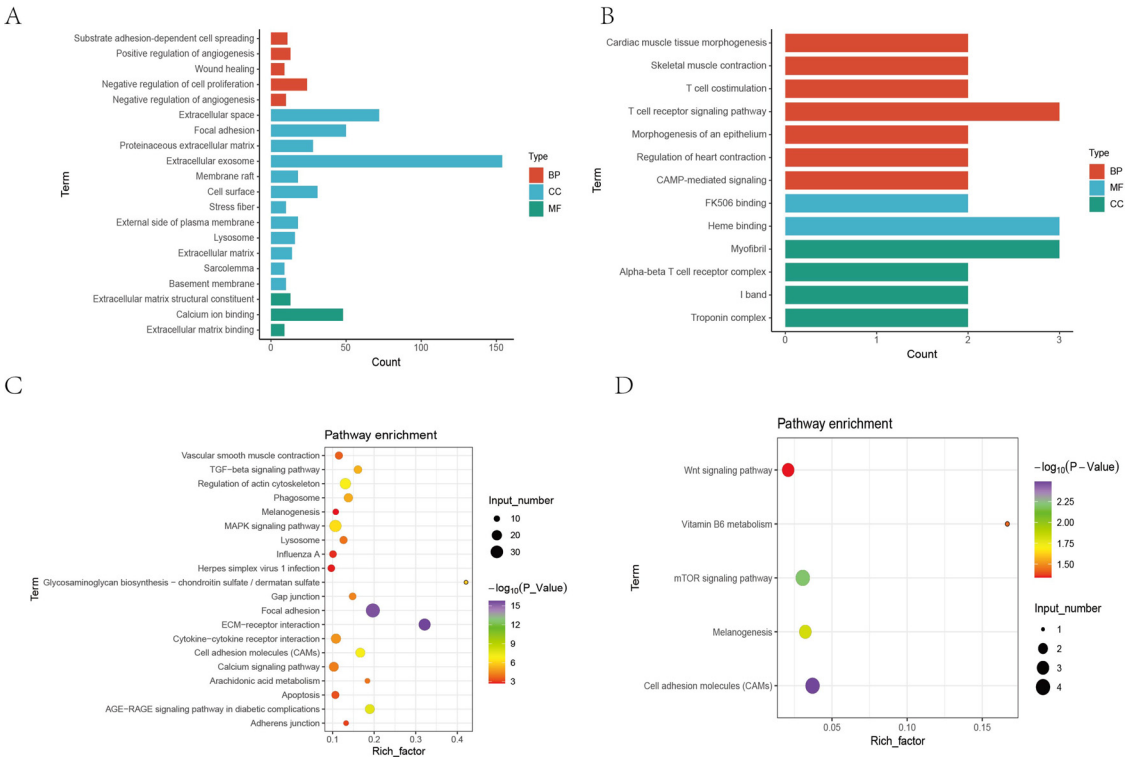

Figure 3. Enrichment analysis of DEGs between the different light intensity groups in the retina and pineal gland. (A) Gene ontology (GO) annotation of differentially expressed genes (DEGs) in the retina. (B) Gene ontology (GO) annotation of DEGs in the pineal gland. (C) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment scatter plot of DEGs in the retina. (C): KEGG enrichment scatter plot of DEGs in the pineal gland. All differentially expressed genes in the retina and pineal gland were compared under different light intensities

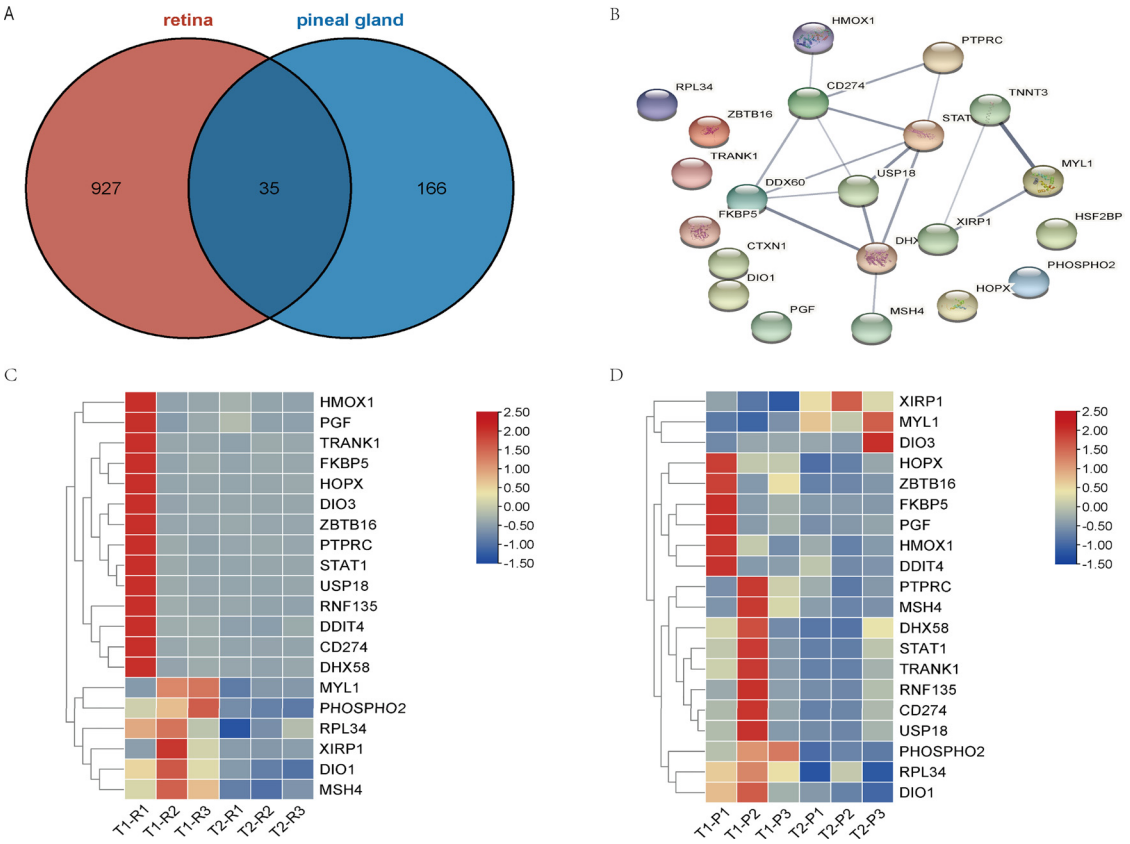

Figure 4. Venn map of DEGs in the retina and pineal gland Clustered heatmap of light stress genes and clock genes and Map of protein-protein interaction (PPI) networks. (A) Venn map of DEGs in the retina and pineal gland. The red region represents the DEGs in the retina. The blue area represents the DEGs in the pineal gland. The cross-section represents the DEGs shared by the two tissues, and the number represents the number of DEGs. (B) PPI network of the same DEGs between the retina and the pineal gland. (C) Gene expression cluster of light stress genes and clock genes in the retina. (D) Gene expression cluster of light stress genes and clock genes in the pineal gland.

STAT2, CD274, and *USP18* were the top 3 critical genes (Figure 4B). We also found that the gene expression of the top three genes was clustered together.

DISCUSSION

In meat duck production, the application of the multilayer cage is gradually widespread. The lighting belt is usually installed directly above the top cage layer. The illumination intensity of the top cage layer is significantly higher than that of the other cages. Therefore, in the production, we need to pay attention to the uneven illumination in the vertical direction between different cage layers, especially the bottom cage. Still, the different light intensity significantly impacts the upper layer and lower layer meat duck production. As the retina and pineal gland is directly affected by light intensity, we used the retina and pineal gland as experimental tissues. There are several critical processes in birds' retina and the pineal gland: both are directly photosensitive, contain circadian oscillators, and rhythmically synthesize and release the indoleamine neurohormone melatonin. In addition, it is reported that different types of retinal ganglion cells transmit external light signals to the tectum, thalamus, and hypothalamus of chickens and then enter the pineal gland (Lien et al., 2008).

In terms of production performance, the results showed that the body weight, breast muscle weight, abdominal fat rate, and other slaughter performance items of the T1 group were significantly lower than those of the T2 group. We speculated that the increase of light intensity could lead to duck activity, so the slaughter performance items such as body weight decreased significantly. Some scholars (Schallreuter et al., 2008) showed that the weight of the chickens in the dim group is higher, and some slaughter items such as total blast, fillets, tendons, and legs were higher than other groups. Our result was consistent with this. Some studies showed that broilers in a 200 lx environment were more active than a 1 lx environment (Blatchford et al., 2012), consistent with our view. Therefore, we thought that the decrease in production performance in the T1 group was due to duck activity.

Based on this, we took the retina and its pineal gland for RNA-seq. The KEGG enrichment based on the DEGs in the retina between different light intensity groups includes melanogenesis, an important retina function. This is consistent with the view that different intensity white light irradiation can cause changes in the retinal function of mice after dark adaptation (Yun-Zhi et al., 2014). Melanocytes are related to endocrine and immunity, and it has a critical role in photoprotection (Videira et al., 2013). Even though the classical and well-known protective role of melanin is well established, emerging evidence is showing that melanin may participate in DNA damage in response to UVA radiation and white light(Orlando et al., 2014; Premi et al., 2015). Therefore, this suggests that light intensity may affect melanin synthesis in the retina.

Besides, in the pineal gland, the KEGG enrichment based on the DEGs demonstrated that the tryptophan metabolism plays a vital role in melatonin synthesis in the pineal gland because tryptophan is the substrate melatonin synthesis. It is well established that the pineal gland is photoresponsive and that the retina of the eye produces melatonin. Consistent with our results, the melatonin release from the pineal gland is also susceptible to dark light (Faluhelyi and Csernus, 2007). Melatonin levels in Turkey's pineal gland, retina, and plasma were significantly decreased after acute exposure to light at night. Some studies showed dim light T1 at night can reduce the melatonin level of rodents (Rumanova et al., 2020). These are consistent with our results. Therefore, we think different light intensities can lead to changes in the Melatonin synthesis of the pineal gland.

There are 35 same DEGs in the retina and pineal gland. These 2 organs are sensitive to light, and we found 19 light stress genes. A study in mice exposed the eyes to low-energy UVB radiation and found 537 DEGs, and the 177 filtered overlapping DEGs were classified into stress and signaling (An et al., 2018). These all show that the light stress genes have different effects under different light conditions. On the other hand, it has been demonstrated that continuous light conditions in the early postnatal period have a long-term impact on the development of circadian oscillations of the primary circadian clocks, brain structures, and activity rhythms (Kubištová et al., 2020). However, there was little clock-related gene among the DEGs in the 2 issues. We speculated that the reason might be that the 2 groups had a different intensity of light for a long time without dark control.

CONCLUSION

In conclusion, our results indicated that the increase of light intensity would reduce the body weight, breast muscle weight, abdominal fat rate, and other product performance. Meanwhile, different light intensities might affect the retina and pineal gland function, including the melanin production of the retina and the secretion of melatonin in the pineal gland.

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DISCLOSURES

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in

part. All the authors listed have approved the manuscript that is enclosed.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.101819.

REFERENCES

An, M. J., C. H. Kim, G. Y. Nam, D. H. Kim, S. Rhee, S. J. Cho, and J. W. Kim. 2018. Transcriptome analysis for UVB-induced phototoxicity in mouse retina. Environ. Toxicol. 33:52–62.

Bian, J., Z. Wang, Y. Dong, J. Cao, and Y. Chen. 2020. Effect of monochromatic light on the circadian clock of cultured chick retinal tissue. Exp. Eye Res. 194:108008.

Blatchford, R. A., G. S. Archer, and J. A. Mench. 2012. Contrast in light intensity, rather than day length, influences the behavior and health of broiler chickens. Poult. Sci. 91:1768–1774.

Boulesteix, A. L., and K. Strimmer. 2007. Partial least squares: a versatile tool for the analysis of high-dimensional genomic data. Brief. Bioinform. 8:32–44.

Dawson, A., V. M. King, G. E. Bentley, and G. F. Ball. 2001. Photoperiodic control of seasonality in birds. J. Biol. Rhythms 16:365–380.

Deep, A., C. Raginski, K. Schwean-Lardner, B. I. Fancher, and H. L. Classen. 2013. Minimum light intensity threshold to prevent negative effects on broiler production and welfare. Br. Poult. Sci. 54:686–694.

Faluhelyi, N., and V. Csernus. 2007. The effects of environmental illumination on the in vitro melatonin secretion from the embryonic and adult chicken pineal gland. Gen. Comp. Endocrinol. 152:154–158.

Fumagalli, M., F. G. Vieira, T. Linderoth, and R. Nielsen. 2014. ngsTools: methods for population genetics analyses from next-generation sequencing data. Bioinformatics (Oxford, England) 30:1486–1487.

Grabherr, M. G., B. J. Haas, M. Yassour, J. Z. Levin, D. A. Thompson, I. Amit, X. Adiconis, L. Fan, R. Raychowdhury, Q. Zeng, Z. Chen, E. Mauceli, N. Hacohen, A. Gnirke, N. Rhind, F. di Palma, B. W. Birren, C. Nusbaum, K. Lindblad-Toh, N. Friedman, and A. Regev. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat. Biotechnol. 29:644–652.

Iuvone, P. M., G. Tosini, N. Pozdeyev, R. Haque, D. C. Klein, and S. S. Chaurasia. 2005. Circadian clocks, clock networks, arylalkylamine N-acetyltransferase, and melatonin in the retina. Prog. Retin Eye Res. 24:433–456.

Ke, Y. Y., W. J. Liu, Z. X. Wang, and Y. X. Chen. 2011. Effects of monochromatic light on quality properties and antioxidation of meat in broilers. Poult. Sci. 90:2632–2637.

Kubištová, A., V. Spišská, L. Petrželková, L. Hrubcová, S. Moravcová, L. Maierová, and Z. Bendová. 2020. Constant light in critical postnatal days affects circadian rhythms in locomotion and gene expression in the suprachiasmatic nucleus, retina, and pineal gland later in life. Biomedicines 8:579.

Kwiecińska, K., M. Hanuszewska, and M. Petrusewicz-Kosińska. 2017. Pineal organ of the Muscovy duck. Med. Weter 73:549–555.

Lien, R. J., J. B. Hess, S. R. McKee, and S. F. Bilgili. 2008. Effect of light intensity on live performance and processing characteristics of broilers. Poult. Sci. 87:853–857.

Orlando, C.-N., F. A. Silva, M. W. Kerllen, P. Christiane, S. Divinomar, F.-F. Fernanda, M.-E. S. Stuchi, A. Eduardo, G. R M, D. M. Paolo, M. H. G M, and M. S B. 2014. Melanin photosensitization and the effect of visible light on epithelial cells. PloS one 9:e113266.

Ostrin, L. A. 2019. Ocular and systemic melatonin and the influence of light exposure. Clin Exp. Optom. 102:99–108.

Parvin, R., M. Mushtaq, M. Kim, and H. Choi. 2014. Light emitting diode (LED) as a source of monochromatic light: a novel lighting approach for behaviour, physiology and welfare of poultry. World's Poult. Sci. J. 70:543–556.

Porter, L., A. Porter, H. Potter, E. Alenciks, S. M. Fraley, and G. S. Fraley. 2018. Low light intensity in Pekin duck breeder barns has a greater impact on the fertility of drakes than hens. Poult. Sci. 97:4262–4271.

Premi, S., S. Wallisch, C. M. Mano, A. B. Weiner, A. Bacchiocchi, K. Wakamatsu, E. Bechara, R. Halaban, T. D Ouki, and D. E. J. S. Brash. 2015. Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. Photochemistry 347:842–847.

Rumanova, V. S., M. Okuliarova, and M. Zeman. 2020. Differential effects of constant light and dim light at night on the circadian control of metabolism and behavior. Int. J. Mol. Sci. 21:5478.

Schallreuter, K. U., S. Kothari, B. Chavan, and J. D. J. E. D. Spencer. 2008. Regulation of melanogenesis - controversies and new concepts. Exp. Dermatol. 17:395–404.

Simonneaux, V., and C. Ribelayga. 2003. Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. Pharmacol. Rev. 55:325–395.

Underwood, H., S. Binkley, T. Siopes, and K. J. G. Mosher. 1984. Melatonin rhythms in the eyes, pineal bodies, and blood of Japanese quail (Coturnix coturnix japonica). Gen. Comp. Endocrinol. 56:70–81.

Underwood, H., C. T. Steele, and B. J. M. r. Zivkovic. 2001. Circadian organization and the role of the pineal in birds. Microsc. Res. Tech. 53:48–62.

Videira, I. F., D. F. Moura, and S. Magina. 2013. Mechanisms regulating melanogenesis. Anais Bras Dermatol. 88:76–83.

Wiechmann, A. F. 1986. Melatonin: parallels in pineal gland and retina. Exp. Eye Res. 42:507–527.

Yasuda, M., Y. Tanaka, K. Omodaka, K. M. Nishiguchi, O. Nakamura, S. Tsuda, and T. Nakazawa. 2016. Transcriptome profiling of the rat retina after optic nerve transection. Sci. Rep. 6:28736.

Yoshimura, T., Y. Suzuki, E. Makino, T. Suzuki, A. Kuroiwa, Y. Matsuda, T. Namikawa, and S. Ebihara. 2000. Molecular analysis of avian circadian clock genes. Brain Res. Mol. Brain Res. 78:207–215.

Yun-Zhi, L., and X. Ping. 2014. Effect of low intensity white light irradiation on the retinas of mice. Int. Eye Sci. 12:1394–1398.

Zawilska, J. B., A. Lorenc, M. Berezińska, B. Vivien-Roels, P. Pévet, and D. J. Skene. 2006. Diurnal and circadian rhythms in melatonin synthesis in the turkey pineal gland and retina. Gen. Comp. Endocrinol. 145:162–168.