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Effects of Light Intensity on Growth, Anti-Stress Ability and Immune Function in Yellow Feathered Broilers

ABSTRACT

An experiment was conducted to investigate the effects of light intensity on growth, anti-stress ability, and immune function of yellow feathered broilers. A total of 480 one-day-old male Lingnan yellow feathered broilers were randomly allocated to 4 treatments based on light intensity (1, 5, 20 and 80 lx) with 8 replicates of 15 chicks each. The experiment lasted for 63 days. Compared with those under high light intensity, broilers exposed to low light intensity had higher ($p < 0.05$) total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), a-Naphthylacetate esterase (ANAE⁺), antibody titer, but lower ($p < 0.05$) malonaldehyde (MDA) levels and heterophil/lymphocyte ratio (H/L). There was a linear effect for T-AOC ($p = 0.002$), GSH-Px ($p \leq 0.047$), MDA ($p = 0.003$), H/L ($p \leq 0.014$), ANAE⁺ ($p \leq 0.044$), and antibody titer ($p \leq 0.021$) with T-AOC, GSH-Px, ANAE⁺, and antibody titer increased significantly as light intensity decreased, whereas MDA and H/L were decreased with the decrease in light intensity. These results suggested that broilers under low light intensity could have similar performance, better anti-stress ability, stronger immune function, and more efficient in energy usage as compared with those exposed to high light intensity environment.

INTRODUCTION

Light intensity plays an important role in broiler's health (Blatchford *et al.*, 2012). Studies showed that low light intensity has negative effects on broiler's carcass traits, early uniformity and meat tenderness, and is in relation to incidence of leg disease, eye defects, dystrophy, skeletal disorders, as well as foot pad health (Blatchford *et al.*, 2009; Deep *et al.*, 2012; Senaratna *et al.*, 2016; Fidan *et al.*, 2017; Rault *et al.*, 2017). High light intensity can improve activity (Blatchford *et al.*, 2012; Rault *et al.*, 2017) and benefit bone health (Blatchford *et al.*, 2009), increase growth and breast muscle percentage (Deep *et al.*, 2013), and give comfort behaviors for broilers (Alvino *et al.*, 2009). However, too strong light may enhance attack behavior of broilers and therefore compromise the health and welfare of broilers (Kjaer & Vestergaard, 1999).

Yellow feathered broiler is a special species in Asia. There were 3.73 billion yellow feathered broilers, accounting for 45.9% of total broilers slaughtered in China in 2015 (Zhang *et al.*, 2016). Yellow feathered broilers usually have longer raising periods and slower growth rate than white feathered broilers, implying that different optimum light regimes could exist between them. Unfortunately, most of the researches involving light intensity were about white feathered broilers and few reports on yellow feathered broilers were published. The purpose of the present study was therefore to investigate the effect of light intensity on growth, anti-stress, and immune function in yellow feathered broilers.



MATERIALS AND METHODS

Bird husbandry and experimental treatments

Four hundred and eighty one-day-old male Lingnan yellow feathered broilers were purchased from a commercial hatchery in Lanzhou, China, and randomly distributed to 4 environmentally controlled rooms (120 chicks per room). Each room had a floor area of 8 m² (2.5 m width × 3.2m length) with a volume of 20m³ (2.5 m height) and was randomly assigned to 1 of 4 light intensities. In each room, 8 cages (1.3 m×0.78 m×0.58 m) were installed in a two tier battery system. Light was provided by light emitting diode.

Chickens were subjected to 1 of the 4 levels of light intensity (1, 5, 20, 80 lx). Light intensity settings were verified at feeder trough (1.3 m×0.15 m, feeder barrels were used before 3 wk of age) level. Light intensity was measured with the photoreceptor sensor of a light meter (Model: ZDS-100W; Shanghai Jiading Xuelian Meter Factory, China). Automatic timers were used for lighting cycle. All birds were provided with 23L:1D from 1 to 3 d of age. Lighting regimes were initiated at 4 d of age and natural photoperiod simulated in Lanzhou (5:47-6:00 sunrise and 20:03-20:21 sunset manual operation). The experiment lasted for 63 d. To keep consistent with the natural day-night rhythm, the lighting period was set as 5:47-20:01 for the first day, and gradually delayed to 6:00-20.21 for the 63rd d). Temperature was set initially at 33°C and gradually reduced by 1°C every 2d until 22°C was reached. The chicks remained in their respective rooms from 1 d of age throughout the experimental period (1 to 63 d of age). Birds were provided a 3-phase feeding program (1 to 21 d, 12.10MJ AME/kg, 19.81 crude protein for starter; 22 to 42 d, 12.40MJ AME/kg, 19.01 crude protein for grower; 43 to 63 d, 12.90MJ AME/kg, 16.09 crude protein for finisher). Feeds were formulated to meet or exceed agricultural industry standard nutrient recommendations (NY/T33-2004, China). Crumbles feed was provided to the starter, and whole pellets were provided as subsequent diets. Broilers had free access to feed and water (each cage contained two nipple drinkers, bell-type drinkers were used before 3 wk of age) during the experiment. Electric exhaust fans were used to provide housing ventilation. Identical conditions except lighting schedule were provided for the 4 rooms. All chicks were vaccinated for Marek's disease at the hatchery, Newcastle disease at 8 and 22 d, and infectious bursal disease at 15 and 28 d of age.

Measurements

Performance

Feed consumption was recorded daily on a per-cage basis. The residual feed was collected once a day before the morning feeding. Body weight of birds was measured in replicate every week. Average daily body weight gain, average daily feed intake and feed/gain from 1 to 21 d, 22 to 42 d and 43 to 63 d were calculated. The incidence of mortality was recorded daily.

Anti-stress ability

At 21, 42 and 63 d, blood samples from 8 randomly-selected chickens in each group (one chick from each cage) were taken via a wing vein, and about 5μL of blood was collected immediately to be smeared on to each of three glass slides. The smears were stained using May-Grunwald, Giemsa stains (Evrin *et al.*, 2017). One hundred leucocytes, including heterophils, lymphocytes, monocytes, basophils and eosinophils, were counted on each slide and the H/L ratio was calculated by dividing the number of heterophils by that of lymphocytes. Three slides were counted and the mean H/L ratio was calculated for each bird. Other blood samples were collected into an anticoagulant-free tube, centrifuged at 3000×g for 10 min to obtain plasma and, stored at -20°C until analysis for other anti-stress and immune parameters. Serum total superoxide dismutase (T-SOD), glutathione peroxides (GSH-PX), total antioxidant capacity (T-AOC), and malonaldehyde (MDA) were measured by using colorimetric assay kits according to manufacturer's instruction (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Immune function assay

Peripheral blood T-lymphocytes were separated by density-gradient centrifugation as described (2000), and T-lymphocyte proliferation was determined using the method by Lin (1999). Peripheral blood T lymphocytes counts were determined by the histochemical demonstration of ANAE on blood films. The lymphocytes with reddish-brown reaction products were considered ANAE positive (Lin, 1999). Antibody titers were determined as described by Olanrewaju *et al.* (2016).

All animal experiments and procedures were performed in compliance with NIH guidelines (NIH Pub. No. 85-23, revised 1996) and were approved by the Animal Care and Use Committee of Gansu Agricultural University, Lanzhou, P.R. China.



Statistical analysis

The data was analyzed by one-way analysis of SPSS 22.0. The main effect is light intensity and the cage was the experimental unit. Significance was designated as $p < 0.05$ with values between $p \geq 0.05$ and $p < 0.10$ being described as a trend. Means were compared by Duncan's multiple-range test when significant difference was detected.

RESULTS

No difference was observed in ADG, ADFI and Feed/Gain ($p > 0.05$, Table 1). Light intensity significantly affected anti-stress ability of chicks ($p < 0.05$, Table 2). Broilers exposed to low light intensity had higher T-AOC and GSH-Px, but lower H/L and MDA as compared with those in broilers under high light intensity. Compared with 20 lx and 80 lx, the treatment of 1 lx increased

Table 1 – Effects of light intensity on growth performance of broilers

Parameters	Light intensity				SEM	<i>p</i> value
	1 lx	5 lx	20 lx	80 lx		
1d Body weight(g)	34.71	34.89	34.75	34.75	0.031	0.103
1-21d						
ADG*(g)	21.19	20.41	20.56	20.79	0.199	0.560
ADFI*(g)	34.11	33.61	33.43	33.58	0.241	0.791
Feed/Gain	1.54	1.57	1.55	1.56	0.007	0.479
22-42d						
ADG *(g)	44.16	43.34	43.93	44.17	0.669	0.972
ADFI*(g)	108.46	106.18	108.65	108.03	0.798	0.701
Feed/Gain	2.34	2.24	2.40	2.34	0.035	0.495
43-63d						
ADG*(g)	52.1	50.19	50.27	56.86	1.506	0.369
ADFI*(g)	142.23	147.23	145.5	151.5	1.956	0.420
Feed/Gain	2.93	3.01	3.12	2.78	0.097	0.678

*ADG: Average daily gain; ADFI: Average daily feed intake.

Table 2 – Effects of light intensity on anti-stress ability of broilers

Parameters	Light intensity				SEM	<i>P</i> value
	1 lx	5 lx	20 lx	80 lx		
21d						
T-AOC*(U/ml)	13.96 ^a	12.76 ^{ab}	11.94 ^{bc}	10.62 ^c	0.355	0.004
T-SOD*(U/ml)	183.95	181.03	162.61	169.97	3.267	0.067
GSH-Px*(U/ml)	1159.18 ^a	1119.39 ^{ab}	1056.33 ^{bc}	1029.08 ^c	15.923	0.009
MDA*(nmol/ml)	2.54	2.58	2.62	2.68	0.052	0.809
H/L*	0.47 ^c	0.51 ^b	0.51 ^b	0.54 ^a	0.005	<0.001
42d						
T-AOC*(U/ml)	17.92 ^a	15.02 ^b	14.76 ^b	14.85 ^b	0.291	<0.001
T-SOD*(U/ml)	178.21	170.76	178.55	169.56	1.696	0.108
GSH-Px*(U/ml)	1191.67 ^a	1198.51 ^a	1119.05 ^{ab}	1070.83 ^b	16.009	0.006
MDA*(nmol/ml)	1.76	1.87	1.77	1.91	0.043	0.532
H/L*	0.31	0.32	0.32	0.34	0.004	0.056
63d						
T-AOC*(U/ml)	16.6	14.99	14.62	16.5	0.396	0.172
T-SOD*(U/ml)	191.09	196.08	189.16	181.39	2.973	0.382
GSH-Px*(U/ml)	1466.28 ^a	1372.31 ^{ab}	1298.28 ^b	1330.50 ^b	20.869	0.019
MDA*(nmol/ml)	1.77 ^b	1.91 ^{ab}	1.99 ^a	2.04 ^a	0.037	0.042
H/L*	0.28 ^b	0.28 ^b	0.30 ^{ab}	0.32 ^a	0.005	0.006

*T-AOC: Total antioxidant capacity; T-SOD: Total superoxide dismutase; GSH-Px: Glutathioneperoxidase; MDA: Malonaldehyde; H/L: Heterophil/lymphocyte ratio.

^{a,b,c} Means with different superscripts within the same line differ significantly ($p < 0.05$).

¹ Linear effect in light intensity (T-AOC at 21 d and 42 d, $p = 0.002$; MDA at 63 d, $p = 0.003$; GSH-Px at 21 d, $p = 0.001$; 42 d, $p = 0.019$ and 63 d, $p = 0.047$; H/L at 21 d, $p < 0.001$; 42 d, $p = 0.014$; and 63 d, $p = 0.002$).



T-AOC at 21d ($p=0.004$), GSH-Px at 21d ($p=0.009$) and 63d ($p=0.019$), and decreased MDA at 63d ($p=0.042$). At 42d, T-AOC in 1lx group was higher than that of other groups ($p<0.001$), GSH-Px in 1lx and 5 lx groups was higher than that in 80lx group ($p<0.001$). There was a linear effect of light intensity on T-AOC ($p=0.002$), GSH-Px ($p\leq 0.047$) and MDA ($p=0.003$). T-AOC and GSH-Px increased significantly as light intensity decreased, whereas MDA decreased as light intensity decreased. The H/L in the 1 lx group was the

lowest ($p\leq 0.056$) and positive linear effect ($p\leq 0.014$) of the magnitude of light intensity on the H/L existed at all the three stages.

Immune functions of broilers varied significantly with light intensity ($p<0.05$, Table 3). ANAE⁺ ($p\leq 0.044$) and antibody titer ($p\leq 0.065$) in 1 lx were the highest. Other than antibody titer at 21 d, there were linear effects for ANAE⁺ ($p\leq 0.044$) and antibody titer at three stages ($p\leq 0.021$), which decreased as light intensity increased.

Table 3 – Effects of light intensity on immune function of broilers

Parameters	Light intensity				SEM	p value
	1 lx	5 lx	20 lx	80 lx		
21d						
ANAE ⁺ (%)	35.09 ^a	33.98 ^a	34.05 ^a	32.51 ^b	0.246	0.001
Antibody titer(log ₂)	6.22 ^a	5.75 ^a	5.81 ^a	4.88 ^b	0.148	0.006
42d						
ANAE ⁺ (%)	43.76 ^a	42.14 ^c	42.97 ^b	41.38 ^d	0.180	<0.001
Antibody titer(log ₂)	5.63	4.88	4.69	4.63	0.151	0.065
63d						
ANAE ⁺ (%)	49.24 ^a	48.17 ^{ab}	48.43 ^{ab}	46.90 ^b	0.303	0.044
Antibody titer(log ₂)	4.59 ^a	4.41 ^{ab}	3.91 ^b	3.91 ^b	0.099	0.015

*ANAE⁺: a-Naphthylacetate esterase.

^{a,b,c,d}Means with different superscripts within the same line differ significantly ($p<0.05$).

[†]Linear effect in light intensity (ANAE⁺ at 21 d, $P=0.004$; 42 d, $p=0.001$ and 63 d, $P=0.021$; Antibody titer at 21 d, $p=0.011$; 63 d, $p=0.007$).

DISCUSSION

In this experiment, no significant difference in ADG might be due to the similar ADFI in four groups and resulted in the similar Feed/Gain. This result indicated that there was no effect of the four light intensities on broiler's performance, with all broilers adapting equally. This result is consistent with several studies in which the light intensity ranged from 1 to 150 lx (Blatchford *et al.*, 2009; Ahmad *et al.*, 2011; Olanrewaju *et al.*, 2014a,b, 2016; Pan *et al.*, 2014; Evrim *et al.*, 2017). In contrast, improved BW and Feed/Gain of birds were found when the light intensities was low (Lien *et al.*, 2008; Blatchford *et al.*, 2012). The causes of the higher body weight were thought to be lower activity of birds when they were kept under lower light intensities.

Light intensity had no significant effect on mortality in this experiment, which is in agreement with other reports (Deep *et al.*, 2010, Olanrewaju *et al.*, 2014 a, b, 2016; Evrim *et al.*, 2017).

The H/L is a sensitive indicator of stress. Increased H/L ratios existed in chickens when exposed to environmental stressors (Evrin *et al.*, 2017). The low,

optimum and high level of stress can be characterized by a H/L ratio of 0.2, 0.5 and 0.8, respectively (Siegel & Gross, 2000). Strong light is considered a stress factor in poultry production. In this study, the highest H/L ratio (0.54) was obtained for broilers in 80lx group at 21d, whereas the lowest H/L ratio (0.28) occurred in 1 and 5lx groups. These results suggested that broilers under low light intensity had a low level of stress and younger chickens were more sensitive to light intensity. Whereas, Fidan *et al.* (2015) and Evrim *et al.* (2017) reported that light intensity did not affect H/L ratios.

SOD, GSH-Px and T-AOC are indicators for assessing oxidative stress status (Wang *et al.*, 2008). Malondialdehyde (MDA) is an end-product of the radical-initiated oxidative decomposition of polyunsaturated fatty acids, and is therefore frequently used as a biomarker of oxidative stress (Grune & Berger, 2007). In the present study, lower MDA and higher SOD, T-AOC and GSH-Px levels under low light intensity suggested that low light intensity may help to maintain the balance of oxidant-antioxidant status and may enhance the ROS scavenging by elevating the concentration of SOD, T-AOC and GSH-



Px. To protect cells from the damage caused by free radicals and related reactants, organisms have evolved several defense mechanisms to rapidly and efficiently remove ROS from the intracellular environment. When the equilibrium between free radicals (oxidants) and antioxidant defense systems is disrupted in favor of oxidants, oxidative stress then occurs (Rodriguez *et al.*, 2004). Yadav *et al.* (2014), reported that low light intensity didn't enhance SOD activity but decreased MDA. Whereas, Ali *et al.* (2005), reported that high light intensity increased SOD activity and MDA.

Enhanced ANAE⁺ percentage and antibody titer in this experiment showed that low light intensity improved the cellular and humoral immune responses of yellow feathered broilers. This could be due either to increased melatonin (Abbas *et al.*, 2007), or activation of peripheral T and B lymphocyte proliferation and energized antibody production (Abbas *et al.*, 2008). Melatonin can enhance peripheral blood and splenic lymphocyte proliferation, therefore accelerating development of cellular and humoral immune responses (Moore *et al.*, 2000). While Blatchford *et al.* (2009) and Olanrewaju *et al.* (2014 a, b; 2016) found no difference in immune parameters in broilers reared under different light intensities.

In conclusion, low light intensity can improve anti-stress ability and immune function without significant effects on growth performance of the yellow feathered broilers, which is therefore more appropriate for keeping the chicken's health and improving the efficiency of energy usage.

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REFERENCES

- Abbas A, El-Dein AKA, Desoky AA, Galal MAA. The effects of photoperiod programs on broiler chicken performance and immune response. *International Journal of Poultry Science* 2008;7:665-671.
- Abbas AO, Gehad AE, Hendricks GL, Gharib HBA, Mashaly MM. The effect of lighting program and melatonin on the alleviation of the negative impact of heat stress on the immune response in broiler chickens. *International Journal of Poultry Science* 2007;9:651-660.
- Ahmad F, Haq AU, Shraf MA, Abbas G, Siddiqui MZ. Effect of different light intensities on the production performance of broiler chickens. *The Pakistan Veterinary Journal* 2011;3:203-206.
- Ali MB, Hahn EJ, Paek KY. Effects of light intensities on antioxidant enzymes and malondialdehyde content during short-term acclimatization on micropropagated *Phalaenopsis* plantlet. *Environmental and Experimental Botany* 2005;54:109-120.
- Alvino GM, Archer GS, Mench JA. Behavioural time budgets of broiler chickens reared in varying light intensities. *Applied Animal Behaviour Science* 2009;118:54-61.
- Blatchford RA, Acher GS, Mench JA. Contrast in light intensity, rather than day length, influences the behavior and health of broiler chickens. *Poultry Science* 2012;91:1768-1774.
- Blatchford RA, Klasing KC, Shivaprasad HL, Wakenell PS, Archerand GS, Mench JA. The effect of light intensity on the behavior, eye and leg health, and immune function of broiler chickens. *Poultry Science* 2009;88:20-28.
- Chen ZL. Separation and purification of cells. In: Xue, Q, editor. *Principles and technologies of in vitro culture*. Beijing: China, Science Press; 2000. p.176-177.
- Deep A, Schwean-Larder K, Crowe TG, Fancher BI, Classen HL. Effect of light intensity on broiler production, processing characteristics, and welfare. *Poultry Science* 2010;89:2326-2333.
- Deep A, Schwean-Lardner K, Crowe TG. Effect of light intensity on broiler behavior and diurnal rhythms. *Applied Animal Behaviour Science* 2012;136:50-56. Available from: http://apps.webofknowledge.com/full_record.do?product=WOS&search_mode=GeneralSearch&qid=1&ID=Q1SYnThcHGhx9kcj6NW&page=1&doc=2
- Deep A, Raginski C, Schwean-Lardner K. Minimum light intensity threshold to prevent negative effects on broiler production and welfare. *British Poultry Science* 2013;54:686-694.
- Evrin DF, Ahmet N, Mehmet KT, Solmaz K, Mehmet K. Effects of photoperiod length and light intensity on performance, carcass characteristics and heterophil to lymphocyte ratio in broilers. *Kafkas University Veteriner Fak Ültesi Dergisi* 2017;23:39-45.
- Fidan, ED, Nazligul, A, Turkyilmaz, MK, Aypak, SU, Kilimci, FS, Karaarslan, S, *et al.* Effect of photoperiod length and light intensity on some welfare criteria, carcass, and meat quality characteristics in broilers. *Revista Brasileira de Zootecnia - Brazilian Journal of Animal Science* 2017;46:202-210.
- Fidan ED, Turkyilmaz MK, Nazligul A. Effects of noise and light intensities on stress and fear reactions in broilers. *Indian Journal of Animal Sciences*. 2015;85(12):1375-1378.
- Grune T, Berger MM. Markers of oxidative stress in ICU clinical settings: present and future. *Current Opinion Clinical Nutrition and Metabolic Care* 2007;10:712-717.
- Kjaer JB, Vestergaard KS. Development of feather pecking in relation to light intensity. *Applied Animal Behaviour Science* 1999;62:243-254.
- Lien RJ, Hess JB, Mckee SR, Bilgili SF. Effect of light intensity on line performance and processing characteristics of broilers. *Poultry Science* 2008;87:853-857.
- Lin QH. A-naphthyl acetate esterase (ANAE) tests. In: Lin, QH, editor. *Methods of immune research*. Wuhan: Wuhan University Press; 1999. p.204.
- Moore CB, Siopes TD. Effects of lighting conditions and melatonin supplementation on the cellular and humoral immune responses in Japanese quail *Coturnix coturnix japonica*. *General and Comparative Endocrinology* 2000;119:95-104.
- Olanrewaju HA, Miller WW, Maslin WR, Collier SD, Purswell JL, Branton SL. Effects of light sources and intensity on broilers grown to heavy weights. Part 1: Growth performance, carcass characteristics, and welfare indices. *Poultry Science* 2016;95:727-735.



- Olanrewaju HA, Miller WW, Maslin WR, Collier SD, Purswell JL, Branton SL. Effects of strain and light intensity on growth performance and carcass characteristics of broilers grown to heavy weights. *Poultry Science* 2014a;93:1890-1899.
- Olanrewaju HA, Purswell JL, Collier SD, Ranton SL. Effects of genetic strain and light intensity on blood physiological variables of broilers grown to heavy weights. *Poultry Science* 2014b;93:970-978.
- Pan J, Lu M, Lin W, Lu Z, Yu Y, Yue Y, *et al.* The behavioral preferences and performance of female broilers under unevenly distributed yellow led lights with various intensities. *Transactions of the Asabe* 2014;57:1245-1254.
- Senaratna D, Samarakone TS, Gunawardena WWDA. Red color light at different intensities affects the performance, behavioral activities and welfare of broilers. *Asian-Australasian Journal of Animal Sciences* 2016;29:1052-1059.
- SPSS - Statistical Packages for the Social Sciences. SPSS base 22.0 user's guide. Madison: IBM United States Software Announcement; 2013. p.213-309.
- Rault JL, Clark K, Groves PJ, Cronin,GM. Light intensity of 5 or 20lux on broiler behavior, welfare and productivity. *Poultry Science* 2017;96(4):779-787.
- Rodriguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, *et al.* Regulation of antioxidant enzymes: significant role for melatonin, *Journal of Pineal Research* 2004;36:1-9.
- Siegel PB, Gross WB. General principles of stress and well-being. In: Grandin T, editor. *Livestock handling and transport*. Wallingford: CABI; 2000. p.27-41.
- Wang YZ, Xu CL, An ZH, Liu JX, Feng J. Effect of dietary bovine lactoferrin on performance and antioxidant status of piglets. *Animal Feed Science and Technology* 2008;140:326-336.
- Yadav GP, Srivastava K, Singh VP, Prasad SM. Light intensity alters the extent of arsenic toxicity in *helianthus annuus L.* seedlings. *Biological Trace Element Research* 2014;58:410-421.
- Zhang XX, Wang HH, Li M, Wu N, Xu XL. Near-freezing temperature storage(-2C) for extension of shelf life of chilled yellow-feather broiler meat:a special breed in Asia. *Journal of Food Processing and Preservation* 2016;40:340-347.