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Influence of Intermittent Lighting on Broiler Performance, Incidence of Tibial Dyschondroplasia, Tonic Immobility, Some Blood Parameters and Antibody Production

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ABSTRACT: The aim of this study was to determine the effect of two lighting programs (continuous lighting (CL) 24L:0D and intermittent lighting (IL) 1L:3D) on the broiler performance, carcass traits, incidence of tibial dyschondroplasia (TD), relative asymmetry (RA), duration of induced tonic immobility (TI), heterophils-lymphocytes ratio (H/L), serum glucose, cholesterol and triglyceride levels. The chicks were randomly divided into 2 treatment groups consisting of 100 chicks per treatment, continuous lighting (CL) 24L:0D or intermittent lighting (IL) 1L:3D. Each treatment consists of 5 replicates of 20 chicks. The experimental period was 6 weeks. Use of IL decreased feed to gain ratio, improved immune response and reduced fearful. Body weight, carcass traits, TD and stress parameters (organ weights, RA, H/L, glucose, cholesterol and triglyceride levels) were not significant in different lighting groups. As a result IL was beneficial for producers and chickens than CL. (**Key Words:** Lighting, Broiler, Performance, Tibial Dyschondroplasia, Tonic Immobility, Relative Asymmetry, Blood Parameters, Antibody Production)

INTRODUCTION

Broilers are commonly reared from hatch to slaughter under constant photoperiod of 23 to 24 h. Long photoperiods (24L:0D, 23L:1D) allow continuous feed access, whereas intermittent lighting (IL) provides a similar opportunity for feeding and an improvement in feed efficiency due to the presence of dark periods (Classen and Riddell, 1989). Some researchers showed that continuous lighting (CL) increased leg problems like tibial dyschondroplasia (TD) (Manser, 1996; Sanotra et al., 2002) as well as suppression of developmental stability (Moller et al., 1995, 1999). TD continues to be of major concern to the broiler industry. It is characterized by unvascularised cartilage extending distally from the proximal tibia tarsal metaphysis (Yalçın et al., 2000).

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Broiler welfare has been assessed from performance, relative asymmetry (RA), tonic immobility (TI), heterophils-lymphocytes ratio (H/L), some blood parameters (serum glucose, cholesterol and triglyceride levels) and immune response. RA provides information on the level of responses of chickens to suboptimal environmental conditions. Genetic and environmental stressors may increase asymmetry of bilateral traits in poultry (Yalçın et al., 2003). Tonic immobility (TI) is an adaptive psycho-physiological response characterized by reduced responsiveness induced by physical restraint and has been widely used a measure of fearfulness in poultry (Jones and Faure, 1981; Sanotra et al., 2002).

The aim of the experiment was to estimate the effect of IL on broiler performance, carcass traits, incidence of TD, RA, duration of induced TI, H/L, some blood parameters (serum glucose, cholesterol and triglyceride levels) and antibody production.

MATERIALS AND METHODS

Animals and diets

This experiment was carried out with 200 one-day old male broiler chicks (Ross PM3). The chicks were randomly distributed into 2 light-proof controlled rooms. In one room

Table 1. Composition of the diets (g/kg)

	Starter diet (0-21 d)	Grower diet (22-42 d)
Corn	420.0	518.5
Wheat	44.5	47.7
Soyabean meal	260.0	188.3
Full-fat soya	170.0	168.0
Fish meal	30.0	20.0
Soyabean oil	40.0	23.0
Limestone	15.0	15.0
Dicalcium phosphate	13.0	13.0
Salt	2.5	2.5
Vitamin premix[a]	1.0	1.0
Mineral premix[b]	1.5	1.5
DL-methionine	2.5	1.5

[a] Composition per kg: 12.000.000 IU vitamin A, 3.000.000 IU vitamin D_3, 30 g vitamin E, 3 g vitamin K_3, 2 g vitamin B_1, 5 g vitamin B_2, 5 g vitamin B_6, 15 mg vitamin B_{12}, 40 g niacin,12 g panthotenic acid, 0.75 g folic acid, 50 g vitamin C, 50 mg D-biotin.

[b] Composition per kg: 160 g Mn, 120 g Fe, 120 g Zn, 10 g Cu, 0.4 g Co, 2 g I, 0.3 g Se.

the lighting schedule remained at continuous lighting (24L:0D) during the whole experiment (CL), while in the other room the schedule was changed to an intermittent cycle of 1 h of light and 3 h of darkness (IL). Using black cartoon on the surface of the windows provided darkness. An automatic timer was used for intermittent lighting. The intermittent cycle started at 9 am. A clear incandescent light bulb was used and light intensity was provided as 1.5 watt/m^2. Each room contained 5 floor pens (20 chicks per pen) at a density of 12.7 bird/m^2. Each pen had wood shavings litter, an electrical heater with thermostat, one hanging suspended feeder and two nipples. Birds were fed (mash form) with a starter diet from 1 to 21 d of age (ME = 13.39 MJ/kg, 230 g/kg crude protein) and grower diet from 22 to 42 d of age (ME = 13.39 MJ/kg, 200 g/kg crude protein). Composition of diets in starter and grower periods of the experiment were presented in Table 1. Feed and water were available *ad libitum* during the experiment. All chicks were reared at 30 to 33°C in week 1, 27 to 30°C in week 2, 24 to 27°C in week 3, 21 to 24°C in week 4 and 18 to 21°C in weeks 5 to 6. This condition maintained was by thermostat of electrical heaters. During the experiment room temperature was measured three times a day. All chicks were immunized of NDV on days 10 and 27. The experimental period was 6 weeks.

Traits measured

Every week all chickens were individually weighed. Feed intake and feed to gain ratio were measured weekly too. Dead chickens were removed daily and recorded.

At d 40, for each sub group, 4 randomly selected broilers were assessed for TI and RA. TI was measured as described by Jones and Faure (1980). Broiler was carried to a separate and quiet room, to avoid unnecessary cumulative stress, which might be associated with catching, handling and moving the birds. Each chicken was carefully restrained for 15 s by covering the head with one hand, while placing the other hand on the sternum. Latency to self-righting was used as the measure of TI. If this had not happened after 10 min, this session was terminated and the individual was assigned a value of 600 s. If the chicken terminated the state of immobility before 10 s, the trial was repeated. Observations were performed at a distance of 2-2.5 m without making unnecessary noise and movements.

After the TI test, RA was measured as the length of the right and left tarsometatarsus, the width of the tarsometatarsus at the spur and the width of the upper joint of the tarsometatarsus were recorded to the nearest 0.1 mm using digital calibers. Trait size was the mean of the left and right sides. RA was defined as the ratio of the absolute value of the left minus right divided by the value for the size of the trait. Mean RA defined as the mean RA of the different traits.

At d 41, four broilers from each sub group were randomly selected and bled from the brachial vein. Blood samples were taken in two tubes, one contained EDTA for estimating the H–L ratio, and the other had no anticoagulant for estimating cholesterol, glucose, triglyceride levels and anti-NDV titer. The bleeding procedure was limited to 1 min or less to minimize the influence of handling stress. All blood samples were collected at the same time in the morning and centrifuged.

Blood samples were smeared on to a glass slide for the determination of the H-L ratio. After drying, the smears were stained with May-Grünwald-Giemsa stain (Gross and Siegel, 1983). The total leukocyte count includes heterophils, lymphocytes, monocytes, basophils and eosinophils. One hundred leucocytes were counted, once on each slide, using a light microscope at ×1,000 magnification. The H-L ratios were determined by dividing the number of heterophils by the number of lymphocytes.

Serum cholesterol, glucose and triglyceride levels were determined using a Hitachi auto-analyzer (Hitachi, Tokyo; Serial Number 1238-23) and its accompanying commercial kits.

Serum anti-NDV titer was assayed using the haemagglutination inhibition method (Allan and Gough, 1974).

At 42 d of age, 4 birds of each sub group (20 birds of each group) were randomly selected for processing. Feed was removed 12 h prior to slaughter. Slaughtering is conducted by cutting the jugular veins and carotid arteries. Carcass, abdominal fat, bursa of Fabricius, heart, liver and spleen were weighed. These weights were expressed as percentage of slaughter weight. The carcasses were stored at 4°C for 20 h by hanging. Cold carcass weights were recorded and were expressed as percentage of slaughter

Table 2. Effect of lighting on growth performance of broiler*

	Groups		p
	CL	IL	
Initial weight (g)[1]	46.02±0.33	46.14±0.33	0.805
Final weight (g)	2,465±23[1]	2,523±22[2]	0.073
Body weight gain (g)			
Weeks from 1 to 3	677±9[1]	713±8[1]	0.002
Weeks from 4 to 6	1,742±24[1]	1,763±23[2]	0.536
Weeks from 1 to 6	2,419±23[1]	2,477±22[2]	0.073
Feed consumption (g)[3]			
Weeks from 1 to 3	1,072±18	1,007±8	0.011
Weeks from 4 to 6	3,385±45	3,255±14	0.040
Weeks from 1 to 6	4,457±49	4,261±14	0.014
Feed to gain ratio (g/g)[3]			
Weeks from 1 to 3	1.58±0.02	1.41±0.02	0.001
Weeks from 4 to 6	1.94±0.02	1.85±0.03	0.034
Weeks from 1 to 6	1.84±0.02	1.72±0.03	0.005

* Values are means±SEM, [1] n = 100, [2] n = 99, [3] n = 5.

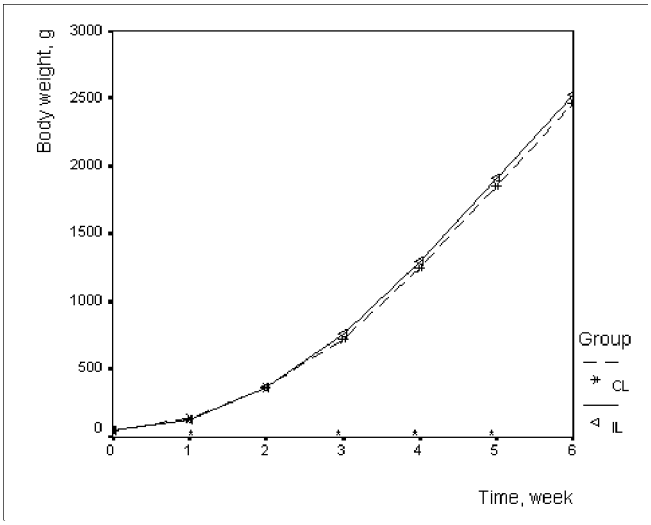

Figure 1. Body weight of broilers in the CL and IL groups.

weight as dressing yield. The carcasses were cut into wings, legs, breast and weighed. The carcass part weights were expressed as a percentage of cold carcass weight. Both left and right tibiotarsal bones were dissected from surrounding tissues and they were examined for TD. The head of the tibiotarsal bone was cut with a sharp knife on proximal tibiotarsus and scoring the amount of cartilage tissue on the cut (0 = no cartilage, 1 = some to one third, 2 = one third to two thirds and 3 = more than two thirds).

Statistical analyses

Statistical analyses were performed by using software package SPSS for Windows (SPSS Inc. Chicago, IL, USA). Data were tested for distribution normality and homogeneity of variance. The differences in parameters between groups were compared with Student-t test. Geometric means of anti-NDV titers were calculated. A repeated-measures ANOVA was conducted on body weight to examine the time-effect with different lighting.

Table 3. Effect of lighting on carcass characteristics and organ weights of broilers*

	Groups		p
	CL	IL	
Hot dressing yield (%)	72.3±0.3	72.6±0.3	0.410
Cold dressing yield (%)	70.7±0.3	71.0±0.3	0.340
Breast** (%)	40.87±0.41	41.63±0.43	0.211
Legs** (%)	14.81±0.14	14.59±0.15	0.269
Wings** (%)	5.62±0.06	5.75±0.04	0.082
Heart*** (%)	0.50±0.02	0.52±0.02	0.247
Gizzard*** (%)	1.36±0.04	1.35±0.03	0.844
Liver*** (%)	2.08±0.04	2.11±0.05	0.680
Spleen*** (%)	0.19±0.01	0.16±0.01	0.077
Bursa of Fabricius*** (%)	0.23±0.01	0.22±0.02	0.468
Abdominal fat*** (%)	1.89±0.11	1.89±0.12	0.976

* n = 20, Values are means±SEM.
** Percentage of cold carcass weight.
*** Percentage of body weight.

RESULTS

Initial and final body weights, body weight gain, feed consumption and feed to gain ratio of chickens reared under different lighting programs are shown in Table 2. Body weights of chickens reared under IL were significantly ($p<0.05$) lower than for chickens reared under CL in the first week (Figure 1). After this week IL chickens showed superior body weight but only 3, 4 and 5 weeks were significant ($p<0.05$). Body weight gains of chickens reared under the IL were significantly higher than the CL chickens in 0-3 weeks of age. Feed to gain ratio of chickens reared under the IL was significantly ($p<0.05$) better than the CL chickens. During the experiment only one broiler died in IL group. Therefore mortality was not significant in each

Table 4. Effect of lighting on right and left tibial dyschondroplasia (TD) score (%) and TD (%) incidence of broilers*

Groups	Distribution of birds according to right TD score				Right TD incidence as sample birds	Distribution of birds according to left TD score				Left TD incidence as sample birds
	0	1	2	3	1+2+3	0	1	2	3	1+2+3
CL	45	30	20	5	55	45	30	25	-	55
IL	60	35	5	-	40	65	30	5	-	35
p	0.347				0.342	0.183				0.204

* n = 20.

Table 5. Effect of lighting on relative asymmetries (RA) of tarsometatarsus length, tarsometatarsus thickness and joint thickness and mean relative asymmetry for broilers*

	Groups		p
	CL	IL	
RA of tarsometatarsus length	2.35±0.48	3.63±0.77	0.149
RA of tarsometatarsus thickness	2.92±0.64	3.90±1.13	0.565
RA of joint thickness	1.67±0.42	1.76±0.22	0.341
Mean relative asymmetry	2.31±0.39	3.10±0.48	0.096

* n = 20. Values are means±SEM.

Table 6. Effect of lighting on glucose, cholesterol, triglyceride and H/L levels of broilers*

	Groups		p
	CL	IL	
Serum glucose (mg/dl)	220.55±2.36	219.60±2.07	0.764
Serum cholesterol (mg/dl)	112.55±2.15	116.40±2.10	0.208
Serum triglyceride (mg/dl)	900.50±48.91	974.50±37.82	0.239
H/L	0.70±0.07	0.59±0.06	0.218

* n = 20. Values are means±SEM.

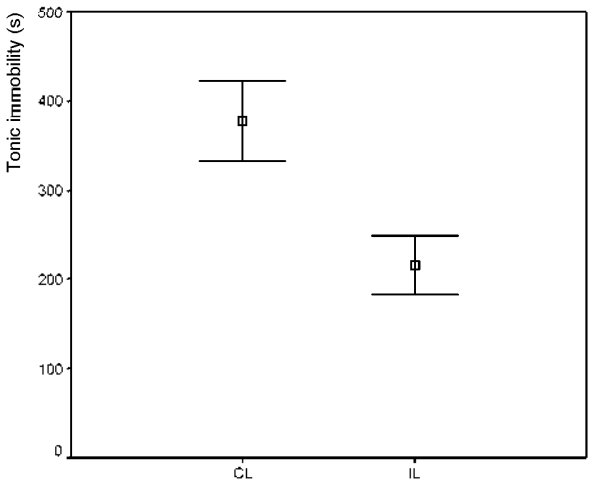

Figure 2. Duration of TI reactions in the CL and IL broilers.

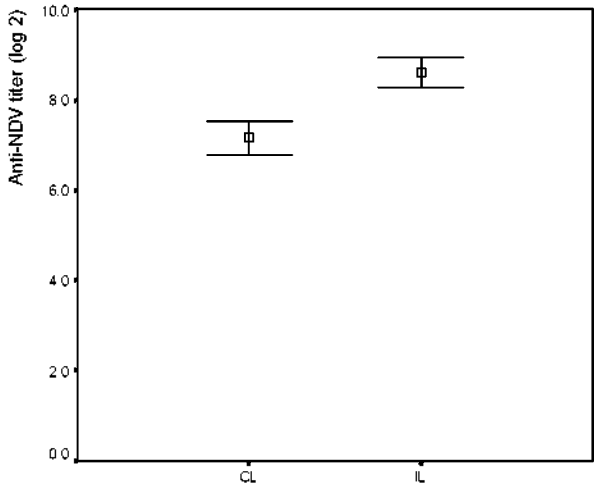

Figure 3. Anti-NDV titers in the CL and IL groups of broilers.

treatment group (data not shown).

IL did not influence the carcass characteristics, organ weights (Table 3), TD incidence (Table 4), mean RA (Table 5), H-L ratio, serum glucose, cholesterol and triglyceride levels (Table 6).

Broilers in IL displayed significantly ($p<0.01$) shorter duration of TI (Figure 2) and significantly ($p<0.01$) higher anti-NDV titers than those in CL (Figure 3).

DISCUSSION

In the experiment body weight of chickens reared under IL to be lower during the early stage and manifested catch-up growth during the subsequent period. The difference in final body weights between groups were not statistically significant. Similar initial depressions in the body weight by chickens subjected to IL schedules have been reported by Ohtani and Leeson (2000). Feed to gain ratio of chickens reared under IL was better than the CL chickens because of

the short meal feeding period, followed by a larger period for digesting the meal. As chickens under IL will be essentially quiet during a dark period, it is assumed that the reduction of activity during darkness may result in lower heat production and higher feed efficiency. Buyse and Decuypere (1988) reported that under IL, chickens eat about 80% of their total feed intake during the light period and eat little during the dark period. This rhythm might exert some influence on intake and digestibility of feed in chickens subjected to IL. This result was similar to the previous studies (Ohtani and Tanaka, 1998; Petek et al., 2005; Rahimi et al., 2005).

None of the carcass traits varied significantly due to the different lighting groups in the present experiment (Table 3). On the contrary some researchers (Buyse et al., 1996; Rahimi et al., 2005) found that the percentage of abdominal fat of IL chickens was lower than the CL chickens.

Liver, spleen and bursa of Fabricius are used for anatomical indicators of stress (Freire et al., 2003). In the present experiment these indicators were not statistically significant in groups. H-L ratio, serum glucose, cholesterol and triglyceride levels are also as indicators of stress. Some researchers (Mahmoud and Yaseen, 2005; Dozier et al., 2006; Mumma et al., 2006) reported that H-L ratio increased under the stressful conditions. However in the present experiment IL didn't affect the H-L ratio, glucose, cholesterol and triglyceride levels. These results showed that IL did not occur the stress for chickens.

IL did not significantly affect the RA of the morphological characters. Moller et al. (1999) found that mean RA was significantly ($p<0.05$) larger in CL chickens than the chickens in groups of 16L:8D. Some of these differences may be due to the using of different L:D treatment and slaughter age of chickens.

A leg problem such as TD is serious consequences for welfare as they have difficulty for reaching food-water and may suffer pain (McGeown et al., 1999; Danbury et al., 2000). Although in the present experiment IL chickens had numerically lower TD values, the light treatment had not significant effect on TD incidence and lesion scores of 0, 1, 2 and 3. Petek et al. (2005) have reported similar results. Contrary some researchers (Wilson et al., 1984; Laster et al., 1999; Ingram et al., 2000) showed that IL had significantly fewer and less severe skeletal diseases. This may be explained due to the positive correlation between TD and body weight (Su et al., 1999) and also may be due to the different slaughter ages.

Duration of TI has been used as a measurement for evaluating fearful behaviour and may be used as a criteria for measuring well-being and levels of stress of chickens (Yalçın et al., 2003). CL chickens showed prolonged TI duration therefore these chickens more fearful than the IL chickens. Long dark periods might reduce the fear because of resting. This result was similar to the findings of some researchers (Zulkifli et al., 1998; Moller et al., 1999; Sanotra et al., 2002).

IL enhanced the anti-NDV titer. Antibody production of broilers is affected by different factors such as stocking density (Onbaşılar and Aksoy, 2005) and taurine supplementation (Lee et al., 2004). Also this experiment showed that antibody production positively was influenced by IL. Lack of dark period might reduce the antibody production in CL chickens.

CONCLUSION

As a result of this study IL decreased feed to gain ratio, improved immune response, reduced fearful and also may provide economic benefit due to the cost of electricity to the producer. Body weight, carcass traits, TD and stress parameters (organ weights, RA and some blood levels) were not statistically significant in CL and IL groups. Therefore IL was beneficial for producers and chickens than CL.

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