CHAPTER 1: INTRODUCTION

1.1 Background

The broiler industry is regarded as a vital source of animal protein, helping to feed the world's fast-rising population. Genetic advancement had a significant impact on broiler feed conversion efficiency and growth performance in order to meet demand. Due to this, farm environmental factors have recently received a lot of attention in the production of broilers (*Wu et al., 2021*). The lighting period is the most crucial factor among them which has an effect on broiler activities, body development, and overall performance. From placement to market age, broilers have often been exposed to continuous [(24 hours light (L):0 hours darkness (D)] light or almost continuous (23L:1D) light. Its goal is to stimulate feed consumption in broilers in order to accelerate their growth rate. However, the broiler's excessive metabolism and rapid growth result in heavy stress, which in turn leads to health issues like sudden death syndrome (SDS), leg issues, cardiac problems, ascites, and rising mortality, particularly in young broilers (*Freeman et al., 1981; Sanotra et al., 2002*). In an effort to lessen stress and illness prevalence without harming final body weight, shortening the photoperiod is thought to be a remedy for the issues with slowing the early growth rate of modern broilers (*Brown, 2010*).

Darkness is crucial in boiler farming because it can regulate immune system stimulation, enzyme secretion, and hormone secretion (*Olanrewaju et al., 2019; Ozkan et al., 2022*). Data from various photoperiodic regimes that have been utilized and researched over the years indicate an uninterrupted minimum dark period of 4 hours, while the need for sleep may be greater at specific times during the growing season (*Blokhuis, 1983*). Recent research also demonstrates superior characteristics in broiler performance with shorted photoperiod. In addition to promoting relaxation during the dark phase, the presence of a dark period encourages higher feed consumption during the light period, which can lower heat production by roughly 25% (*Rahimi et al., 2005; Malleau et al., 2007*). Besides, melatonin secretion from the pineal gland also increases during dark periods and regulates a variety of daily and seasonal cycles and rhythms in various physiological systems (*Calislar et al., 2018; Farghly et al.; 2019*). Melatonin hormone stimulates the development of bone directly (*Cardinali et al., 2003*) or

indirectly due to hormones such as parathyroid hormone, estradiol or growth hormones, and factors involved in bone development (*Ostrowska et al., 2002*).

Short-day length also improves broiler welfare by reducing physiological stress, boosting immune function, increasing activity levels, and improving leg health, according to recent studies (*Classen et al., 2004; Hassanzadeh et al., 2005; Manfio et al., 2019; Soliman and Hassan, 2019; Baykalir et al., 2020*). In contrast to continuous lighting, broilers exhibit comforting behaviours including preening, dust bathing, and wing flapping more frequently in the dark additional lighting schedule (*Alvino et al., 2009; Schwean-Lardner et al., 2012*). Additionally, the tonic immobility latency time is significantly longer in continuous lighting compared to the reduced lighting duration (*Yang et al., 2022*). It also exhibits less fearfulness in the shorter lighting period. Chickens have been observed to be more stressed out by continuous lighting (24L:0D) than by 12L:12D (*Freeman et al., 1981*). According to *Buckland et al., (1976),* broilers housed under continuous day lengths had higher plasma corticoid concentrations than broilers housed under intermittent 1L:3D light regimes. The ratio of heterophils to lymphocytes (HL), which can be used to assess chronic stress in hens, rises as corticosterone levels do *(Gross and Siegel, 1983).*

According to a Center for Policy Dialogue report in 2018, the poultry sector in Bangladesh was considered as 2nd highest GDP (Gross domestic product) contributor behind only the readymade garment (RMG) industry and is currently contributing 1.5-1.6% GDP. (*Karmoker, 2022).* This sector is holding 14% of the livestock sector and growing rapidly which supplies 37% of the total meat and 22-27% of the total animal protein in Bangladesh (*Hamid et al., 2017).* Currently, there are over 90,000 registered poultry farms where 53,000 are broiler farms which is more than 58.39 percent of the total chickens (*Karmoker, 2022).* The poultry business employs nearly 6 million people, having women accounting for 40% of the workforce. According to Bangladesh Poultry Industry Central Council (BPICC) statistics, the poultry business is developing at a pace of 12 to 15% every year (*Karmoker, 2022*). Although the poultry sector is growing rapidly in Bangladesh, the welfare issue is being heavily neglected and very few initiatives have been taken regarding poultry welfare in the last decades.

1.2 Objectives of this study

In view of the potential improvement in broiler welfare and performance with the reduction in light duration, this study aimed at the following objectives.

1. To investigate the behavioural responses and welfare of broiler chickens exposed to different lighting programs.
2. To assess the stress level caused by different lighting regimes.
3. To evaluate broiler performances (live weight, feed intake, feed efficiency, viability) rearing under different lighting durations.

1.3 Hypothesis of this study

It is hypothesized that including darkness in the lighting program may improve the behaviour and welfare issues (e.g. fearfulness, stress, and leg health) as well as performance parameters (e.g. live-weight, feed intake, FCR, and livability) of the broiler.

1.4 Outline of this thesis

In this thesis paper, Chapter 1 provides background information regarding the research topic, problems, and hypothesis. In Chapter 2, we discuss the previously published relevant literature related to the topics. Chapter 3 explains the methodology used, elaborating on the different test procedures and processes of data collection with a focus on the parameters used. Chapter 4 presents the descriptive statistics and the results of the analysis followed by the discussion in Chapter 5 justifying the research findings. Chapter 6 includes the conclusion. Finally, the limitations and recommendation of this study are discussed in chapter 7.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Introduction

The environment during production plays a fundamental role in modern poultry farming. Temperature, humidity, air velocity, and radiation are the factors that mainly affect animals and can compromise homeothermy (*Amaral et al., 2011*). Broiler chickens show maximum performance when the ambient temperature is between 18 and 26°C during the growth phase (*Gomes and José, 2016*). Lighting may be the most powerful exogenous factor in the control of many physiological and behavioural processes. Light allows the bird to establish rhythmicity and synchronize many essential functions, including body temperature and various metabolic steps that facilitate feeding and digestion. Lighting programs generally consist of three different aspects: intensity, duration, and wavelength. Light intensity, color, and the photoperiodic regime can affect the physical activity of broiler chickens (*Lewis and Morris, 1998*).

Lighting programs are mainly classified as continuous, and intermittent lighting. In the continuous lighting program, the broilers are subjected to a constant photoperiod of 23 to 24 hours. This program provides conditions to maximize feed intake and weight gain due to access to feeders. However, the birds become more susceptible to leg problems and they are more immunologically fragile (*Pandey, 2019*). The intermittent lighting program provides repeated cycles of light and darkness in a 24-hour period. It is thought that the decrease in activity throughout the night may lead to a decrease in heat output, an increase in feed efficiency, or both (*Rahimi et al., 2005*). Different lighting programs have a significant effect on performance, immunity, and stimuli for the secretion of various hormones that control growth and reproduction *(Zheng et al., 2013*).

2.2 Lighting duration effects on broiler performances

To evaluate the performance of broilers against the lighting period many have been conducted in past few decades. Those studies showed both positive and negative relationships between performance and lighting periods. These studies are mainly focused on the performance parameters like body weight (BW), feed efficiency, and livability of broiler.

Studies showed continuous lighting has significantly higher BW for broiler chickens compared to those reared under 8L:16D (*Beane et al., 1962; Weaver and Siegel, 1968*) or 12L:12D (*Freeman et al., 1981*). *Renden et al., (1991)* also reported that broilers had significantly lower body weight (BW) on 42 days (d) when raised under 6L:18D from 1 to 14d followed by either 23L:1D or intermittent system of 6 1L:3D lighting period compared to those reared under 23L:1D for 56d. Broilers were reported to have significantly higher BW when raised under 24L:0D compared to 8L:16D, 20L:4D, or 12L:12D (*Charles et al., 1992). Brickett et al. (2007b)* reported significantly higher BW for broilers raised from 4 to 35d with 20L:4D compared to those under 12L:12D. But studies also reported no significant differences in BW of broilers raised with 23L:1D from 1 to 8d, 18L:6D from 8 to 42d, and 23L:1D from 42 to 49d compared to those under 23L:1D from 1 to 49d (*Lien et al., 2007*) or in between intermittent lighting (1L: 3D) and nearly continuous lighting (23L: 1D) for 42 days (*Rahimi et al., 2005*). *Brown (2010)* reported insignificant differences in BW of broiler raised under the SD/SU (12L:12D), 20L:4D, and 18L:6D treatments for 42 days rearing time. *Khutal et al., (2022)* also reported no significant differences in BW and weight gain in different photoperiods (23L:1D, 18L:6D, 16L:8D, and 14L:10D). However, there was found some significantly lower weight gain in SD/SU (12L:12D) program compared to the birds under either the 20L:4D or 18L:6D treatments at 21 days of rearing. *Olanrewaju et al., (2019a,b)* also reported broilers subjected to the short/non-intermittent (23L:1D) have significantly lower growth performances compared to the regular/intermittent photoperiods (2L:2D).

The average feed efficiency has varied between researches, despite the fact that heavier broilers have often resulted from longer photoperiods. Some studies (*Osei et al., 1989; Charles et al., 1992; Brickett et al., 2007b; Lien et al., 2009*) revealed that shorter photoperiods produced broilers with better feed efficiency when compared to longer photoperiods, whereas other study (*Beane et al., 1962*) had different result between trials. Still, other studies revealed insignificant differences in broiler feed efficiency between light schedules. In the studies that included feed consumption data, it was shown that broilers consumed more feed at longer photoperiods than at shorter photoperiods and results increased BW in birds (*Weaver and Siegel, 1968; Renden et al., 1991; Blair et al., 1993; Lien et al., 2007*). However, according to *Khutal et al., (2022),* there were no significant changes in feed intake (FI) and feed conversion ratio

(FCR) of broilers in different photoperiods (23L:1D, 18L:6D, 16L:8D and 14L:10D) up to six weeks of age.

The excessive and quick growth rate of current broiler strains has been linked to ascites, sudden death syndrome (SDS), and skeletal disorder-related mortality. Previous researches showed no significant differences in broiler mortality for broilers raised on either 1L:3D (*Renden et al., 1991*) or 18L:6D (*Lien et al., 2007*) compared to those raised on 23L:1D. However, broiler birds raised under 23L:1D have been observed to experience higher rates of death and culling compared to birds under an increasing light program and those under 12L:12D (*Charles et al., 1992; Blair et al., 1993, Lewis et al., 2010, Schwean-Lardner et al., 2013*). Mortality rate was also found higher in the 23L:1D lighting schedule compared to the 18L:6D, 16L:8D, and 14L:10D lighting schedules (*Khutal et al., 2022*). In broilers with unlimited access to feed under 24L:0D, ascites was discovered in roughly 77% of congestive heart failure deaths (*Nain et al., 2009*). When the feed was 30% limited, however, the broilers exhibited neither ascites nor congestive heart failure symptoms. In contrast to broilers raised on 23L:1D, *Lott et al., (1996)* found that utilizing 12L:12D reduced feed consumption and the occurrence of ascites. According to *Lewis et, al., (2009)*, the incidence rate of mortality due to SDS is more in the lighting period >10h compared to the <10 h. Mortality related to SDS was also found higher (1.26%) in 20L:4D in contrast to those provided with 12L:12D (0.77%) (*Brickett et al., 2007a*). Thus, it appears from these researches that reducing the photoperiod from 24 or 23h is not harmful to broiler performance and might even increase livability.

2.3 Lighting duration effects on broiler behaviour

Animal behaviour has been regarded as a crucial factor in assessing animal welfare since it may be the best predictor of it. The complex structure of animal behaviour has been thought to be influenced by several variables, many of which are connected to the basic needs of chickens (such as feeding, drinking, preening, dust washing, or sleeping), which are in turn intimately linked to animal welfare (*Duncan, 1998*).

Broilers were found in more inactive during the scotoperiod compared to the photoperiod over 24h day cycle (*Alvino et al., 2009; Blatchford et al., 2009*). However, broilers did not cease activity entirely during the scotoperiod. *Calvet et al., (2009)* reported that lying was the most frequent activity in dark, although other activities like

standing, eating, and drinking occurred sporadically. During the light period, the lying activity was performed on average by only 40% of the birds, although the initial minutes were usually devoted to eating. Other common activities during the photoperiod were scratching and standing, whereas eating, drinking, and walking were less frequent. *Schwean-Lardner et, al., (2012, 2014)* reported a negative linear or quadratic reduction in the percent of time spant by broilers in inactive resting, walking, standing, stretching, dustbathing, and feeding time and behaviour during the photoperiod were frequent in 14L and 17L birds, sporadic in 20L birds and non-existent in 23L birds but litter pecking was found with increasing day length. The running behaviour was also found to be reduced in the photoperiod and 24-h period at 27d, with the behaviour eliminated under 23L. Dustbathing was no longer present in the behavioural repertoire of the older 23L birds. *Bayram and Özkan, (2010)* reported, the number of chicks eating, drinking, walking-standing, and pecking increased under the 16L:8D lighting schedule, whereas resting (sitting and sleeping) decreased compared to the 24h continuous lighting.

Photoperiod is a powerful cue for chickens to develop daily feeding rhythms (*Ballard and Biellier, 1975*). Broilers seemed to eat at lighting hours (6-13%) and liked to eat sporadically in the dark period (1-1.15%) (*Calvet et al., 2009*). Studies showed (*Weaver and Siegel, 1968*) broilers tend to develop daily patterns for feeding at the beginning and the end of the photoperiod, especially 4 h periods immediately following or prior to an 8h scotoperiod (*Siegel et al., 1962*). Other studies had shown that chickens eat the most feed during the 2 h before the lights turn off and for 2h after the lights turn on (*Savory, 1980; Lott and May, 1996*). The results from these studies suggested that chickens are capable of predicting and preparing for the ensuing scotoperiods. Also, when the photoperiod starts, the hens appear to eat more often to make up for the hunger they had during the dark period.

Comfort behaviours like preening, wing flapping, stretching, and dust bathing, are believed to be crucial for the maintenance of feathers by chickens. The rates of comfort behaviours in laying hens are known to be affected by interactions with conspecifics (*Nicol, 1989*) as well as space availability (*Keeling and Duncan, 1991; Keeling, 1994*). Unfortunately, little research has been done on how photoperiod affects how well broilers perform their comfort behaviours. According to *Bayram and Özkan (2010),* broiler rearing under a 16L:8D lighting system showed comfort behaviours, such as preening and wing-shaking, more extensively than 24h continuous lighting.

Alvino et al., (2009) showed broilers hardly ever preened during a one-hour scotoperiod. However, they did show that preening occurred a little less than 5% of the time throughout the 24h lighting period. *Schwean et al., (2012, 2014)* also reported that dustbathing was no longer present in the behavioural repertoire of the older 23h continuous lighting birds.

2.4 Lighting duration effects on broiler fearfulness

In general, fear is characterized as an unpleasant emotional state that an individual could feel as a result of the impression of real danger. This condition encourages individuals to stay away from potentially dangerous circumstances, protecting them from harm and enhancing their fitness (*Hemsworth et al., 1994; Rushen et al., 1999, Forkman et al., 2007).* Prolonged fearful events are considered a detrimental source of stress (*Rushen et al., 1999*) that can lead to increased damage and higher mortality rates during the management, catching, and transport of broiler chicken and reduce productivity and welfare (*Hemsworth and Coleman, 2010; Waiblinger et al., 2006*). According to earlier research, high levels of fear were associated with lower levels of peak hen day production (*Barnett et al., 1992*), egg production and egg shell quality (*Barnett et al., 1993*), feed conversion (*Hemsworth et al., 1994*), first-week mortality, and meat quality (*Cransberg et al., 2000*) in broiler chickens.

A variety of study methods were conducted on chicken to evaluate the fear level which are called fear tests (*Franco et al.,2022, Hakansson, 2015, Giersberg et al., 2020*) i.e. Novel environment (NE) test (*de Haas et al., 2014*), Novel object (NO) test (*Forkman et al., 2007*), Response to the observer (RO) test (*Schwean-Lardner et al., 2012*), Tonic immobility (TI) test (*Jones and Faure, 1981*), Avoidance distance test (ADT), Voluntary approach (VA) test, Stationary person test (SPT) (*Hakansson, 2015*).

Based on previous research on different methods, broilers seemed to have more fearfulness in continuous lighting systems compared to intermittent or increasing lighting systems. Broiler rear under continuous lighting system tents has a significantly greater duration of tonic immobility (*Bayram and Özkan, 2010, Blair et al., 1993, Yang et al., 2022, Onbaşılar et al., 2007, Campo and Devila, 2002*). However, *Fidan et al., (2017b)* showed no significant relationship between lighting period and TI duration. *Wang et al., (2008)* also reported that the increasing lighting program decreased the duration of TI on 10d, had no effect on 22d and increased the duration of TI on 36d.

Blair et al., (1993) reported increasing photoperiod chickens were more susceptible to TI induction than 23h continuous lighting chickens. *Hakansson (2015)* showed no discernible relationship between daytime and AD or VA. Daytime and VA were strongly associated when examined at various ages (6-12d, 21-24d, before slaughter), although the findings were inconsistent. At three weeks, it was discovered that the VA was greater in the morning, while at lower ages and before slaughter, the VA was higher in the evening.

2.5 Lighting duration effects on stress

Stress is one of the affective states in boiler production that may directly impact performance (*Virden and Kidd, 2009; Thaxton et al., 2016*) and welfare. Stress generally occurs when the animal or bird perceives any physical or psychological situation as a threat to its homeostasis (*Goldstein and Kopin, 2007*). Two methods are preferred to evaluate stress in birds *i.e.,* plasma or serum corticosteroid (CORT) hormone level and blood Heterophil -Lymphocyte (HL) ratio.

Stressors activate the hypothalamic-pituitary-adrenocortical (HPA) cascade, resulting in the release of corticosterone (CORT) (*Blas, 2015, Veissier and Boissy, 2007*). According to *Buckland et al., (1976),* continuous lighting had significantly higher plasma corticoid levels than intermittent lighting regimes (1L:3D, 1L: 3D with 13 hours continuous lighting) at 5-10 lux intensity. *Abbas et al., (2008)* reported higher CORT concentration in the non-intermittent restricted light (12L:12D) group compared to continuous (23L:1D) and intermittent light (2L:2D) groups while no significant relationship is found between continuous and intermittent light groups. *Nelson et al., 2020,* also reported higher CORT levels in intermittent, short-dawn/dusk photoperiod (ISD) compare to increasing, long-dawn/dusk photoperiod (ILD). Previous studies also show no significant association between photoperiod and plasma CORT level (*Olanrewaju et al.,2013, 2019*).

However, these CORT changes are short terms and may significantly be changed due to acute stress like the stress of capture, handling, and restraint might confuse as well as the fear of humans at the time of blood drawn from the bird. (*Hemsworth et al., 1994, Kannan and Mench, 1996, Bortolotti et al., 2008; Alm et al., 2014, Blas, 2015*). Blood samples taken more than 2 minutes after the bird is captured show greater CORT concentrations (*Chloupek et al., 2011*). According to *Puvadolpirod and Thaxton*

(2000), heterophil to lymphocyte (HL) ratios in broilers were increased by treatments that stimulate the physiological stress response, e.g. administration of ACTH. Because HL ratios rise in response to prolonged elevations in circulating CORT concentrations, serum or plasma CORT concentrations are frequently evaluated in association with HL ratios. These ratios indicated the immune system's reaction to chronic stress (*Gross and Siegel, 1983; Abbas et al., 2008; Weimer et al., 2018*). Previous studies showed continuous lighting schedules in broilers have a higher value of HL ratio compared to 18L:6D (*Lien et al., 2007*), or 16L:8D (*Coban et al., 2014*) lighting schedules. However, the study also reported no significant difference in the HL ratio in commercial broilers exposed to 23L:1D lighting or an increasing photoperiod program (*Blair et al., 1993*) or among 3 different photoperiod systems (20 h, 18 h, and step-down/step-up 12L:12D) (*Brown, 2010*). According to *Abbas et al., (2008),* non-intermittent restricted light (12L:12D) groups had a higher HL ratio compared to continuous (23L:1D) and intermittent light (2L:2D) groups while no significant relationship was found between continuous and intermittent light groups.

2.6 Lighting duration effect on leg health of broilers

Leg conformation has been a major concern for broiler birds over decades due to their rapid growth rates led to increased prevalence of leg problems (*Buckland et al., 1976; Classen and Riddell, 1989; Renden et al., 1991, Petek et al., 2005; Nelson et al., 2020*), skeletal abnormalities (*Classen et al., 1991*) and tibial dyschondroplasia (TD) (*Renden et al., 1991; Sanotra et al., 2002*). these studies suggested that including darkness in lighting can indirectly help in better leg health by slowing weight gain. Previous studies also showed scotoperiod maximizes melatonin production (*Lynch, 1971; Pang et al., 1983*) which improves birds' bone development directly (*Roth et al., 1999; Cardinali et al., 2003*) and indirectly controls hormones such as parathyroid hormone, estradiol or growth hormones, and factors which involved in bone development (*Ostrowska et al., 2002*). Scotoperiod also increased locomotion during lighting, stimulating long bone development (*Bradshaw et al., 2002; Müller, 2003; Bessei, 2006*). *Classen and Riddell (1989*) found that broilers raised with 6L:18D had fewer leg deformities in comparison to broilers raised with 23L:1D from 3 to 21 days of age. According to *Sorensen et al. (1999),* broilers grown with longer dark periods had a lower frequency of TD than broilers raised with shorter dark periods (8L:16D < 16L:8D, or 16L:8D < 21L:3D, or 16L:8D < 23L:1D).

To evaluate the leg health of broilers, recent experiments are mainly focused on their walking ability (gait score) which can be measured by 2 common methods: the 6-point Kestin system (*Kestin et al., 1992*) and the 3-point system (*Webster et al., 2008*). The effect of photoperiod length had a significant influence on the gait score (*Fidan et al., 2017b). Sanotra et al. (2002)* reported that a step-down/step-up light schedule or a 16L:8D scheduled from 4 to 30 d followed by an abrupt change to 23L:1D resulted in better gait scores than a constant light schedule (24L:0D) for broilers. Also, broilers reared with 20L:4D had worse gait scores compared to those provided 12L:12D (*Brickett et al., 2007a*). *Schwean-Lardner et al., (2013)* also show birds falling in painful gait score categories increased linearly with increasing lighting periods (14L:10D < 17L:7D < 20L:4D < 23L:1D). Based on the results from these studies, it seems that gait scores are better when broilers are raised with shorter photoperiods compared to longer photoperiods. But studies also show no significant relationships between photoperiod and gait score in broilers. No significant association was found between regular/intermittent (2L:2D), and short (8L:16D) lighting systems (*Olanrewaju et al., (2019). Khutal et al., (2022),* also reported no significant difference in gait score among near continuous lighting (23L:1D), 18L:6D, 16L:8D, and 14L:10D lighting schedules.

2.7 Conclusion

In general, previous studies show better production performance of the broiler in continuous lighting than provided darkness as they mainly focused on performance parameters. While recent studies focused not only on performance but also welfare showing better performance and welfare with the addition of darkness in 24 hours day cycle in comparison to continuous lighting. Although these relationships are not well established as very few studies were conducted over the decade and some studies reported a non-significant relationship between lighting duration and broiler behaviour and welfare. Further study should be conducted to assess these characteristics in light of the variations in performance observed and the paucity of knowledge regarding how photoperiod influences broiler behaviour. It is ultimately necessary to link broiler behaviour, performance, and the light environment to create the best lighting program for broiler chickens. Although animal welfare may have started long ago, it is still a new topic and is mostly neglected in Bangladesh livestock farming. There is no study

has been conducted on the photoperiod effect on broiler welfare in Bangladesh. The objective of this study is to understand the effect of lighting duration on broiler performances as well as their welfare with the common management practices of broiler farming in Bangladesh.

CHAPTER 3: MATERIALS AND METHODS

3.1 Statement of the experiment

The study was carried out at Tailardwip village under Anowara Upazilla with the permission from Department of Dairy and Poultry Science (DDPS) of Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. It was conducted in the winter season starting on 12th December 2022 to 10th January 2023 with a total of 30 days, in which data and samples were collected on different parameters based on the objectives. This experiment was also approved by the Ethical Approval Committee (EAC) of CVASU, Bangladesh [Memo no. CVASU/Dir(R&E)EC/2022/435(1)/15] (Appandix 11). This experiment was aimed at focusing on the impact of different lighting duration treatments on the broiler production performance, their behaviour studies and their welfare.

3.2 Broiler housing and management

To conduct the experiment smoothly following actions was run during the experiment period.

3.2.1 Preparation of the experimental house

To prepare house for rearing broiler chicks and running trial, washing, cleaning, painting and disinfection of poultry farm shed is very important. Prior to the 3 days of chick arrival, the shed was cleaned by swiping the floor, removing the spider net from the corners and roof. Then the shed was checked for any holes for potential entry of predators, rodents and feral birds inside. Next, the floor was overlaid by muds (to avoid any sharp end of objects that could be injurious to birds) and calcium carbonate solution with water (500gm in 6 liters water) for disinfecting the floor following day. After that the shed side walls and roof were disinfected by spaying iodine solution (FAM 30®) at recommended dose (5ml/1L water). All the farming utensils like feeder, drinkers, brooder guards were also cleaned and disinfected by rinsing with detergent water followed by dipping into iodine solution. All the utensils were dried before using in the shed to avoid iodine toxicity.

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3.2.2 Collection of day-old chicks (DOC) and experimental design

Day-old broiler chicks (n=160; Lohmann Meat) were collected by purchasing from the local dealer named Aman Chicks Limited, Chattogram to conduct the experiment from d1 to 30 days on a littered floor. A total of 160 day-old broiler chicks of either sex (Lohmann Meat) were randomly distributed into 4 treatments *i.e.* T_1[24 hours light (L): 0 hour darkness (D)], T_2(22L:2D), T_3(20L:4D) and T_4(18L:6D) with 4 replicates, each reapplication had 10 birds in a completely randomized design (CRD), as shown below in Table 3.1. The chicks were weighed on arrival, and then randomly distributed into 16 equal-sized pans to rear them up to 30 days under experimental conditions.

Table 3.1 Lay-out of the experimental design

Treatments	Number of replicates				No. of chicks per treatment
	R_1	R_2	R_3	R_4	
T_1	10	10	10	10	40
T_2	10	10	10	10	40
T_3	10	10	10	10	40
T_4	10	10	10	10	40
Total	40	40	40	40	Grand Total= 160

[T_1 refers to continuous lighting while T_2, T_3, and T_4 refer to treatments which were provided with 2 hours, 4 hours, and 6 hours of darkness, respectively. R_1, R_2, R_3, and R_4 refer to replicates 1, 2, 3, and 4, respectively]

3.2.3 Brooding day-old chicks

The day before the chicks' arrival, a single circularelectric spot brooder with litter flooring was made for first 7 days. As space requirements of 0.2 sq.ft. floor space was given per DOC which was increased up to 0.5 sq.ft. at 7 days of rearing. As the bedding material, 2 inches deep sawdust was given, covered with 2 layers of newspaper. A 3 ft radius hover was placed about 1 ft above the litter materials which was equipped with tungsten bulb. A lab thermometer was also placed to maintain the border temperature. 2 hours prior to the chick arrival, hover lights were turned on keeping the temperature at 99°F and water mixed with glucose and vitamin C was supplied in 3 small drinkers placing under the hover to balance with environment temperature. For the first two days, the birds were brooded at a temperature of 33°C. The temperature then was gradually reduced by 1 or 2°C every 1 or 2 days until the chicks were 19 days old at which point the temperature was maintained at 24°C for the rest of the trial. Total 160 DOCs arrived at the farm at morning 3 am. DOCs then measure for bodyweight and released in the brooder carefully. After 15 minutes later, the feed was provided by spreading on the paper floor. The activity of DOCs was observed carefully over 2 hours

to help chicks with water and feed consumption. After 6 hours the feed was supplied in 2 linear feeders (1 per 100 chicks). The wet papers were removed after 24 hours. The chick was visited 2 hours interval for 2 days to observed and maintain the brooder temperature according to the chick condition. The water and the feeds were supplied 4 times a day up to 7 days of rearing. During this period the shed ventilation also managed carefully to avoid cold environment of winter season.

3.2.4 Floor space

Bird was raised on littered floor by dividing 16 pans of equal sizes. Each pan was constructed with 15 sq. ft. (3 ft x 5 ft) floor space allowing 1.1 sq. ft. area per bird with one drinker and feeder. The pans were divided by a thick polythene sheet with a 1.5 ft height which allowed separate birds confinement as well as good ventilation.

3.2.4 Feeding and water management

Ready-made compounded feeds of broiler pre-starter, starter, and grower were purchased from the local feed dealer of Nourish Feed Mill Ltd. and fed these diets to the birds in *ad-libitum* feeding system from d1- 30 days. Pre-starter, starter and grower diets were provided the chicks in both crumble, crumble-cum pellet and pellet forms respectively, throughout the trial period (Table 3.3). Prior to feeding, proximate analysis of the feeds were conducted in the Nutrition Lab. of Department of Animal Science and Nutrition, CVASU which has been shown in Table 3.2.

Table 3.2 Chemical composition of provided broiler feed

Nutrients	Broiler pre-starter		Broiler starter		Broiler grower	
	Labeled value %	Test value %	Labeled value %	Test value%	Labeled value %	Test value %
DM	88	89.48	88	88.24	88	89.205
Moisture	12	10.52	12	11.76	12	10.795
ME	2950	-	3000	-	3050	-
CP	21	20.3	20	20.065	19	19.6
CF	5	4.505	5	3.455	4	3.355
EE	5	4.96	5	6.17	5	6.555
Ash	-	4.635	-	4.47	-	4.265

Table 3.3 Feeds and feeding time of different types of feeds

Feed	Feed types	Feeding times (day)
Broiler pre-starter	Crumble	1^{st} -12^{th}
Broiler starter	Crumble cum pellet	13^{th} – 22^{nd}
Broiler grower	Pellet	23^{rd} – 30^{th}

Fresh and clean water was supplied a*d-libitum* to the birds from the tube well. Feed and water were provided 3 times a day at 8-hour intervals at 6 AM, 2 PM, and 10 PM regularly from day 1 to 30. Each pan was furnished with a feeder and a drinker to have free access of the broiler to feeder and drinker. Drinkers are washed and dried with detergent water every three days.

3.2.5 Vaccination and Medication

Birds were immunized against New Castle Disease and Gumboro disease in a well-organized schedule and dosages (Table 3.4). Both the live vaccines for New Castle Disease (Bangla BCRDV ®) and Gumboro (Bangla IBD vaccine ®) were purchased from a nearby veterinary pharmacy. On the scheduled immunization day, each individual vaccine was collected in an airtight container with ice carefully to avoid the freezing temperature of vaccine. Each bird received the vaccine within two hours of vaccine collection. To avoid the hot environment temperature, vaccination was done in the early night at 7 PM. Vaccination was performed in two ways *i.e.* eye drops and drinking water. To perform eye- drop method, first freeze-dried live vaccine (300 doses) mixed with 15 ml (1 drop = 0.05 ml) supplied diluent and then provided to the birds carefully to avoid any injuries. To provide vaccines with drinking water, the birds were restricted from drinking water for 1.5 hours to increase their thirst. The live vaccine was mixed with 6-liter fresh water and provided to each pan at the same amount. The water was allowed to be drunk for 1 hour and discarded the remaining. Each pan was observed attentively to ensure every bird was drinking vaccine-mixed water. Following the vaccination, birds were also supplied with immune-stimulator medicine (Pulv. Lisovit ®) at recommended dose mixed with drinking water the next day.

Table 3.4 Vaccination schedule

Date	Vaccine name and type	Pack size	Route	Dosage
4	Bangla BCRDV, live	300 doses	Eye drop	1 drop/bird
12	Bangla IBD vaccine, live	300 doses	Eye drop	1 drop/bird
18	Bangla BCRDV, live	300 doses	Drinking water	200 ml water/pan
21	Bangla IBD vaccine, live	300 doses	Drinking water	200 ml water/pan

The birds were provided with amino acids, multivitamins, and multimineral medicine to avoid nutritional deficiency throughout the rearing period at recommended doses.

3.2.6 Litter management

To rear the broilers in the floor method, sawdust was chosen as litter material due to its easy local availability. In the beginning, 2 inches deep sawdust layer was provided which was increased following days according to the requirement of birds based on their condition. The litter materials were loosened daily to balance litter moisture and temperature. Dumpy litter and litter cake were removed regularly. This was done at noon before changing water and feeds to avoid litter contamination**.**

3.3 Lighting management

As the main purpose of this study is involved with lighting durations/regimes, the pans were designed carefully to meet the objectives. The treatment pans were separated from each other by thick black polythene dividers and a certain distance in between to avoid light emitting from one treatment to another. A reflector was also attached to bulb holder to avoid light emitting by the roof. As the light source a LED light was provided for each treatment. Each treatment area was equipped with an 18-watt white colored LED bulb which can produce a lighting intensity of 22 lux in the treatments. The lighting period was controlled manually by the separate on-off switch. The photoperiod was controlled at night time which generally starts from 6 PM. The 4 hours and 6 hours of intermittent darkness were provided with 1-hour lighting intervals which resulted 2 and 3 periods of 2 hours darkness, respectively (table 3.5).

Table 3.5 Lighting schedule applied in the experiment

Treatment	Light controlling times				
	6pm - 8pm	8pm – 9pm	9pm - 11pm	11pm -12pm	12pm – 2am
T1					
T2					
T3					
T4					

[T1 refers to continuous lighting while T2, T3, and T4 refer to treatments which were provided with 2 hours, 4 hours, and 6 hours darkness, respectively. Here, ▭ means light and ▬ means dark period]

3.4 Sample and data collection

Mortality of bird was recorded as it occurred, while body weight and feed intake were recorded weekly for the calculation of liveweight gain (LWG), and feed conversion ratio (FCR) was corrected for mortality. Livability was calculated from mortality of birds per replicate cage. Behavioural observation, fear tests *i.e.*, response to observer

(RO), novel object (NO) test, novel environment (NE) test, tonic immobility (TI) test, and gait score (GS) index were recorded to evaluate the behaviour and welfare. Again, blood sample was collected and tested to determine the heterophil-lymphocyte ratio and cortisol hormone level to assess the stress of broiler chicken. All these parameters were collected in different time basis which were shown in the following Table (3.6)

Table 3.6 Data and sample collection time and assessed numbers.

Parameters /Tests	Collection time	No. of birds or pans assessed
Performance related data		
Body weight	7 days interval	5 birds per pan
Feed intake	7 days interval	
Mortality	Daily	All pans
Behaviour and welfare related data		
Behavioural observations	11,22,29 d	All birds
Response to observer test	12, 23,30d	All birds
Novel object test	12,23,30d	All birds
Novel environment test	30d	1 birds/pan
Tonic immobility test	24d	2 birds/ pan
Gait score test	30d	2 birds /pan
Stress evaluation tests		
HL (Heterophil – lymphocyte) ratio	27d	2 birds/pan
Serum cortisol level	27d	2 birds/pan

3.4.1 Performance-related data collection

3.4.1a Body weight (BW)

Each treatment's weekly live weight of broilers was measured replication-wise. At the start of the experiment and at the conclusion of each weekend, the average live weight of the broilers was also recorded by the weighing balance.

3.4.1b Liveweight gain (LWG)

The weight increase was estimated by deducting the initial body weight from the end or final body weight.

$$LWG = Final\ body\ weight\ (kg) - Initial\ body\ weight\ (kg)$$

3.4.1c Feed intake

The amount of feed consumed was calculated by subtracting the amount of leftover feed from the total amount of feed provided to birds on each weekend.

$$FI = Total\ feed\ supplied\ (kg) - Leftover\ feed\ (kg)$$

3.4.1d Feed conversion ratio (FCR)

FCR refers to the amount of feed needed per unit of production (meat or egg). Feed efficiency is the efficiency of converting feed to meat or egg or other products. The formula used to compute FCR was as follows:

$$FCR = \frac{Feed\ intake\ (kg)}{Body\ weight\ gain\ (kg)}$$

3.4.1e Mortality and livability

Mortality was measured or recoded as when it occurred. The number of deceased birds during the experimental period divided by the total number of housed birds at the beginning of the experiment served as the foundation for the calculation of bird mortality. The mortality of the birds in each replicate cage was used to calculate livability. This formula was used to determine the fatality percentage.

$$Mortality\ (\%) = \frac{Number\ of\ broiler\ died}{Total\ number\ of\ broiler\ housed} \times 100$$

3.4.2 Behaviour and welfare-related data collection

3.4.2a Behavioural observation

Birds' behaviour was assessed at 3 ages of rearing (11d, 22d, and 29d). The observation test was conducted by direct observation following scan sampling method. Behavioural readings were taken every 30 minutes interval as instantaneous samples to assess the percentage of time for different behaviours described in the ethogram provided in Table 3.7 (*Schwean-Lardneret et al., 2012*). To avoid observer-manipulated behaviour observer was present at the sight of bird 5 minutes prior to recording data. Each behaviour was recorded as the number of broilers engaged in the behaviour divided by the total number of broilers in the pan (n =10) and multiplied by 100.

Table 3.7 Ethograms for behavioural observations

Behaviours	Description
Standing	Bird in an upright position with both feet on the ground (but no other part), idle (not performing any other behaviour).
Walking or running	Bird walking or running for more two seconds or more.
Inactive resting	Bird lying on the straw and not performing any other behaviour − may or may not be sleeping
Foraging	Manipulating litter, feed with beak, previous or after scratching substrate with feet
Feeding	Bird at the feeder, with the head into the lip of the feeder
Drinking	Bird at the drinker, with the head into the lip of the drinker
Preening	Bird manipulating feathers on own body. Maybe lying or standing
Dust bathing	Bird in a sitting position, shaking wings vertically, followed by side or head rubs, involving the motion of the legs
Body shake	Bird shake their body or wing flapping
Wing and leg stretching	Bird stretching leg or wings to the side or behind the body, without taking a step forward, or flapping

3.4.2b Response to Observer (RO)

For this test, an observer walked slowly past each pan while recording the behaviour of the birds counting how many birds moved as a result of the passage of the observer (*Schwean-Lardner et al., 2012*), and results were reported as percentages. The lower percentage indicated lower fearfulness of birds toward the observer. This test was conducted on 12d, 23d, and 30d of rearing broiler chickens.

3.4.2c Novel object (NO) test

In the novel object test (*Forkman et al., 2007*), the observer placed a novel object (light, bright and unique) slowly in the center of each pan, then retreated outside of the pan. The observer then timed (sec) how long it took for the first birds to peck or approach the object by stopwatch. The observation was set for 3 minutes and if no bird approached (<25cm) it, their duration was quantified at 180s. The higher time to peck NO indicated better welfare of birds. This test was conducted 2 times on all replicates on 12d, 23d, and 30d and the time to peck was recorded.

3.4.2d Novel environment (NE) test

A novel environment (NE) test based on the protocol by *de Haas et al., (2014)* was conducted at 30d of age. A bird per location in a pan (i.e. near the feeders, the drinkers,

and the wall) was caught and tested in the central hall (n = 4 birds /treatment). A non-transparent round bucket (1.2 ft in diameter at the bottom and 1.5 ft in height) wrapped with a thick plastic cover which resulted in a dark environment inside the bucket served as NE for the test. After placing a bird in the bucket, the opening was covered with a non-transparent lid. The individual response was recorded for two minutes. The experimenter documented the number of vocalizations, the latency of the first flight attempt, and the number of flight attempts. To determine the lower fearfulness of birds, lower number of vocalizations, higher latency to first flight attempt, and lower number of flight attempts were preferred.

3.4.2e Tonic immobility (TI) test

The tonic immobility test (*Jones and Faure, 1981*) was conducted at 24d with 2 birds per replicate. To test that, birds were placed on their backs and manually restrained for 15s covering their eyes and then releasing the bird slowly. Latency to rise was measured in seconds. If birds stayed in the tonic state for more than 600s, they were raised by providing stimuli, and their tonic immobility duration was quantified at 600s. The lower TI time indicated lower fearfulness of birds. 2 birds per replica were tested and time was recorded.

3.4.2f Gait score (GS) index

Gaits of broilers were rated on 30d of rearing with 2 birds from each replicate. To test that, a 5 ft long flat ramp was constructed outside of the pan and 2 randomly selected birds were placed at the end of the ramp. The broilers were observed walking back toward their home pan. The broilers gate scores were rated on a 3-point system of 0, 1, or 2 (*Webster et al., 2008*) described in table 3.8.

Table 3.8 Gait scoring test criteria

Score	Description
0	No impairment in the gait and can walk 5 ft easily
1	Obvious impairment in the gait but the bird can walk at least 5 ft
2	severe impairment in the gait and the bird cannot walk though there is it may shuffle on shanks or hocks with assistance of wings.

3.4.3 Stress related data collection

Two healthy birds were randomly chosen from each replicate and collected blood from the brachial vein**.** The blood was collected in clot activator vacutainer (n=32) for serum

collection and EDTA vacutainer (n=32) for complete blood cell count (CBC) test. Each vacutainer was filled with 3 ml of fresh blood labeled properly. The samples were immediately transported to the laboratory (Clinical Laboratory of Department of Physiology, Biochemistry and Pharmacology, CVASU) within a cooling box and preserved in a freezer for further tests.

3.4.3a H-L ratio evaluation

First EDTA vacutainers were mixed properly in a roller mixer at 45 rpm. Then the blood samples were tested for red blood cells (RBC) and hemoglobin (Hb) using Celltac ∝® machine (KOHDEN MEK-6550 by NIHON). Then blood smears for each sample were made and allowed to air dry. The smears were stained by the Wright's stain for 5-7 minutes and rinsed in the running water. After that the smears were dried properly. To evaluate the differential leukocytes count (DLC) test the smears were observed under 100x microscope magnification and counted 100 white blood cells (WBCs) distinguishing Monocytes, Eosinophils, Basophils, Heterophils and Lymphocytes, HL ratio (Gross and Siegel, 1983) were calculated by following equation.

$$HL\ ratio = Heterophils\ count \div lymphocytes\ count$$

3.4.3b Serum cortisol (CORT) level evaluation

To separate serum from the blood, blood was collected in clot activator vacutainer and place the stand still for about 15-30 minutes to form a blood clot. Then they transferred to lab where vacutainers were centrifuged at 3000 rpm for 10 minutes. The vacutainers were gently removed not disturbing cell layer. Then the serums were collected in fresh vials by pipetting. The separated serum were labeled according to their blood samples and preserved below 4°C for further testing. Cortisol test was done by serum cortisol ELISA assay kits and results were recorded for each sample.

3.5 Statistical analysis

All collected data were subjected to analyzing by one-way ANOVA procedure using SPSS software (IBM SPSS, Version 26, 2019). The significance of differences between means was tested using Duncan's multiple range tests (DMRT). Statistical significance was considered when $P \leq 0.05$.

Photo gallery of activities:

Figure 3.1: Cleaning and disinfection of shed floor. Preparing shed for experiment.

Figure 3.2: Preparing a brooder before chick arrival.

Figure 3.3: Chick release in the brooder with feed and water supplied.

Figure 3.4: Check up on baby check to ensure proper environment and feeding.

Figure 3.5: BCRDV vaccination of check by eyedrop on 5th day

Figure 3.6: Chick entry in the treatment's replicates. 10 chicks per replicate.

Figure 3.7: Measuring feed to supply broiler chicken - for record keeping.

Figure 3.8: Preparing waterer to supply clean water to the birds.

Figure 3.9: Daily litter manipulation and management

Figure 3.10: Different random broiler behaviours. (a. feeding b. drinking c. foraging d. preening and restring. e. dust bathing f. leg stretching)

Figure 3.11: Random broiler behaviour at different period of a day. a. morning period b. afternoon period and c. night period.

Figure 3.12: Response to observer test. A. bird condition before the appearance of the observer. B. birds' condition after observer passing by (4 birds displaced from their previous position). c. the observer

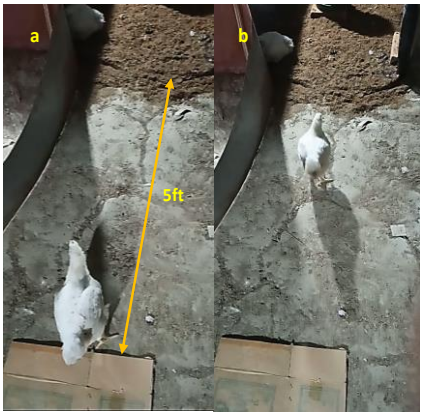

Figure 3.13: Gait score test. a. bird set up 5ft far from pan. b. bird approaching toward the pan

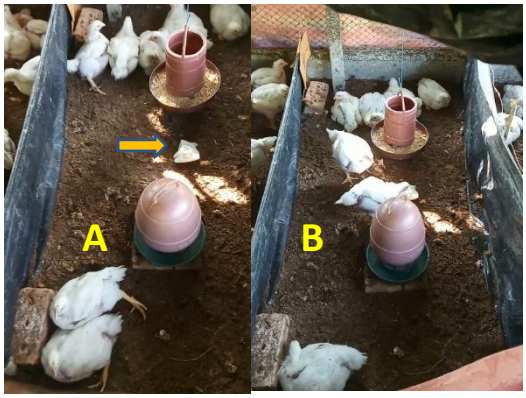

Figure 3.14: Novel object test. A. birds' condition during novel object (arrow) placement. B. first beak to the object by bird.

Figure 3.15: Novel environment test. A. bird placement in dark environment. B. data recording on broiler activities.

Figure 3.16: Tonic immobility test. A. Handling bird for the test. B immobility state of broiler.

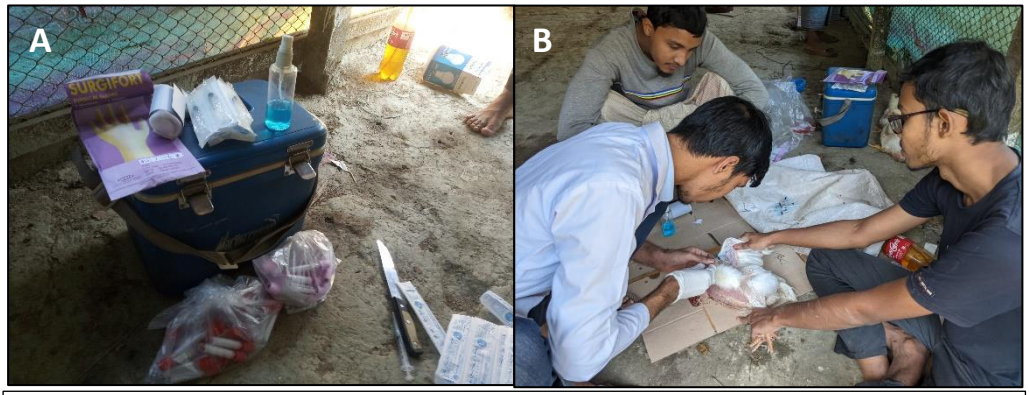

Figure 3.17: Preparation and blood collection from broilers on 27d. A. Equipment for blood sample collection with ice box for sample transportation. B. blood collection from the brachial vein of the left broiler wing.

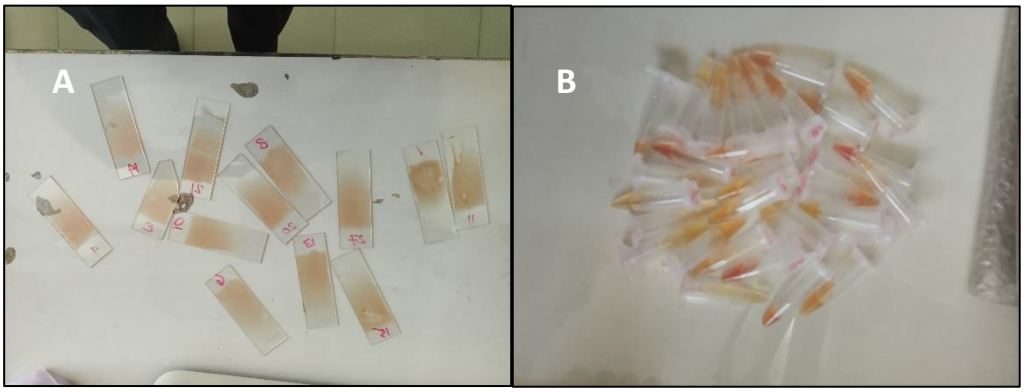

Figure 3.18: Laboratory processing of blood for further tests. A. Slide preparation for DLC test to evaluate the HL ratio. B. Serum separation from the whole blood sample to evaluate serum cortisol level.

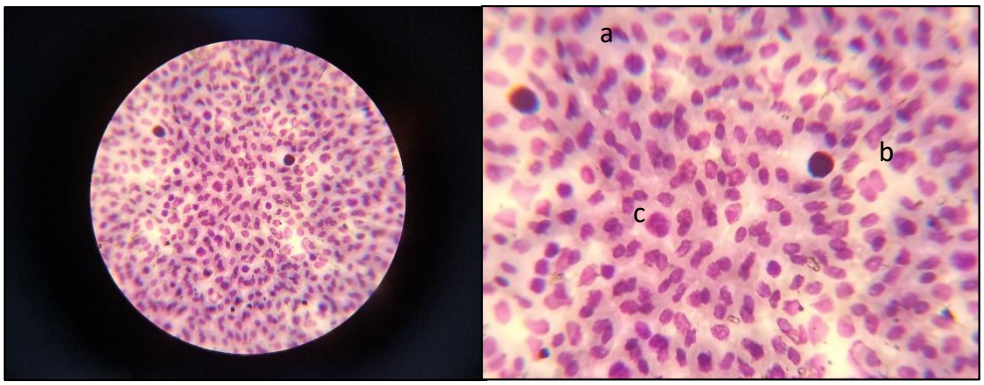

Figure 3.19: Microscope view of chicken blood with Wright's stain for DLC test under 100x magnification. a. heterophil. b. lymphocyte c. monocyte

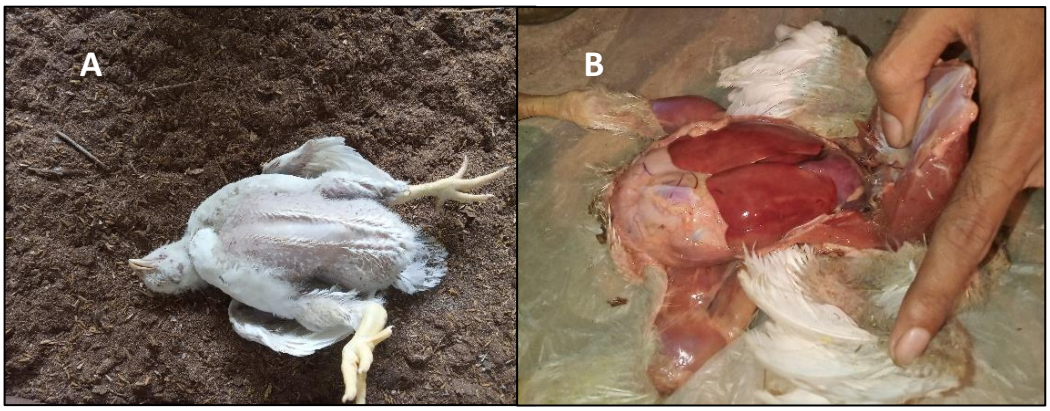

Figure 3.20: Sudden death syndrome (SDS) sign symptoms. A. flop over dead body of broiler chicken. B. Postmortem of SDS with no gross lesion in the bird's internal organ was noticed.

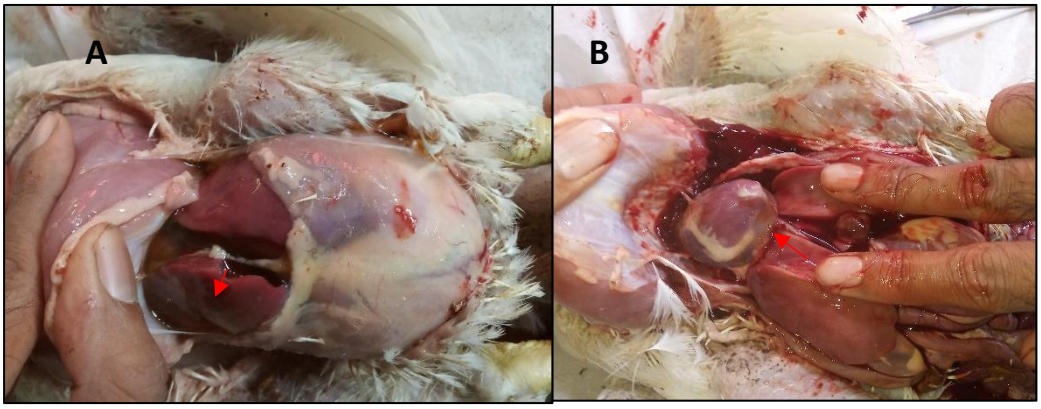

Figure 3.21: Postmortem of Ascites case. A. Yellowish fluid (arrow) inside the body cavity. B. Fluid filled pericardium (arrow) found on postmortem.

CHAPTER 4: RESULTS

The gross responses of broilers in terms of feed intake, live weight, body weight gain, FCR and viability are stated below in a tubular form. Apart from this, parameters of behavioural activities of broiler in terms of standing walking/running, inactive resting, foraging, feeding, drinking, preening, dust bathing, body shake, leg/wing stretching of broiler; novel object, new environment, response to observer, tonic immobility, gait scoring tests, blood heterophil-lymphocytes (HL) ratio and serum cortisol (CORT) level data were also tabulated in this chapter under exposing the birds into different lighting regimes.

4.1 Production performances

4.1.1 Live weight gain

The data of live weight gain (LWG) of broilers exposed to different lighting durations (treatments) are presented in Table 4.1. The data showed that LWG was unaffected ($P>0.05$) from 1d to 28d among the treatments. Significant difference ($P<0.05$) was found in the LWG of birds on 8-14d and 22-28d only except for others. The highest ($P<0.05$) LWG was attained by the birds reared on T_4(0.74kg/b) followed by the birds on T_3(0.72), T_2(0.69), and T_1(0.66), respectively at 22-28 days.

Table 4.1 Live weight gain (LWG) of broilers at different treatments of lighting regimes

Trait	Age	Treatments				SEM	P-values
		T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
LWG (kg/b)	8-14d	0.40[a]	0.37[b]	0.35[b]	0.32[b]	0.015	0.05
	15-21d	0.46	0.48	0.51	0.54	0.029	0.09
	22-28d	0.66[b]	0.69[b]	0.72[a]	0.74[a]	0.017	0.05
	1-28d	1.68	1.70	1.72	1.75	0.031	0.16

[Data refer to mean values of ten birds per replicate from d1-28 days; [a-b] Mean bearing different superscripts in a row differ significantly between treatment at *P<0.05; SEM = Standard error of the mean; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness ;[3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval ;[4]18L:6D: 18 hours of intermittent ;lighting with 6 hours of darkness with 2 x 1-hour lighting interval]

4.1.2 Feed intake

The feed intake (FI) result of broiler chicken up to 28 days of age is shown in Table 4.2. The data shows that the FI of broiler was not influenced (P>0.05) by the dietary treatment except for 8-14d only. The greater (P<0.05) FI was observed in the T_1 followed by T_2, T_3 and T_4, respectively at 8-14d.

Table 4.2 Feed intake (FI) of broilers at different treatments of lighting regimes

Trait	Age	Treatments				SEM	P values
		T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
FI (kg/b)	8- 14d	0.501[a]	0.483[a]	0.453[b]	0.425[b]	0.020	0.012
	15-21d	0.732	0.733	0.744	0.762	0.026	0.658
	22-28d	1.123	1.139	1.146	1.146	0.040	0.931
	1-28d	2.484	2.482	2.470	2.461	0.053	0.966

[Data refer to mean values of ten birds per replicate from d1-28 days; [a-b] Mean bearing different superscripts in a row differ significantly between treatment at *P<0.05; SEM = Standard error of the mean; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness ;[3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval ;[4]18L:6D: 18 hours of intermittent ;lighting with 6 hours of darkness with 2 x 1-hour lighting interval]

4.1.3 Feed conversion ratio:

The data on mean feed conversion ratio (FCR) for the whole experimental period are presented in Table 4.3. There were no significant differences (P>0.05) in total or weekly feed conversion ratio among the different lighting treatments. But at the 15-21d and 22-28d of aged broiler, FCR values were found higher on T_1 (24L:0D) treatment which are gradually reduced with the addition of darkness hours.

Table 4.3: Feed conversion ratio (FCR) of broilers at different treatments of lighting regimes

Trait	Age(day)	Treatment				SEM	P-values
		T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
FCR	8-14d	1.26	1.29	1.31	1.34	0.05	0.407
	15-21d	1.60	1.53	1.47	1.42	0.07	0.145
	22-28d	1.69	1.65	1.59	1.56	0.05	0.081
	1-28d	1.42	1.40	1.38	1.37	0.03	0.248

[Data refer to mean values of ten birds per replicate from d1-28 days; SEM = Standard error of the mean; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness ;[3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval ;[4]18L:6D: 18 hours of intermittent ;lighting with 6 hours of darkness with 2 x 1-hour lighting interval]

4.1.4 Livability

In terms of livability, no significant difference (P= 0.674>0.05) was found between lighting treatments of broilers (Figure 4.1). However, the results showed that intermittent lighting groups T_3 and T_4 had the highest survivability (97.50%) followed by T_2(95%) and T_1(92.50), respectively.

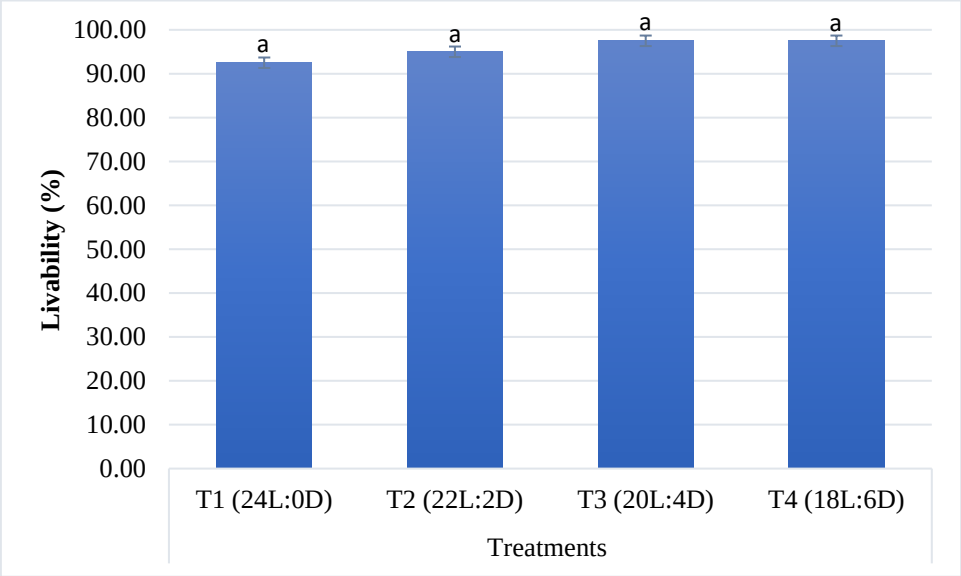

Figure 4.1: Viability (%) of broilers from d1 to 28 days under different lighting regimes; Bar bearing similar superscripts has no significant (P>0.05) difference between treatments

4.2 Behavioural observations

4.2.1 Behavioural observations at 11d

The data on behavioural activities of broiler such as standing, walking/running, inactive resting, foraging, feeding, drinking, preening, dust bathing, body shake, leg/wing stretching of broiler on the 11th day of rearing, are shown in Table 4.4. The results show that only inactive resting, feeding, preening, and leg/wing stretching of birds were affected (P<0.05) by treatments. The highest (P<0.05) resting (73.83%) was found in the T_2 group while lowest resting (70.22%) being in the T_4 group. Significantly greater feeding, preening and leg or wing stretching activities were found in the T_4 and T_3 treatment group of birds than that of other groups.

Table 4.4 Behavioural activities of broiler chicken observed on day 11 under different lighting programs over a period of 24 hours.

Behavioural activities (%)	Lighting treatments				SEM	P-value
	T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
Standing	4.67	4.93	5.41	5.81	0.387	0.052
Walking/running	4.54	3.31	3.93	3.75	0.595	0.268
Inactive resting	73.78[a]	73.83[a]	71.85[b]	70.22[b]	0.991	0.009
Foraging	0.21	0.32	0.37	0.37	0.151	0.686
Feeding	8.92[b]	9.28[b]	10.08[a]	10.02[a]	0.352	0.015
Drinking	5.77	5.75	5.60	6.28	0.501	0.570
Preening	1.09[c]	1.31[b]	1.39[b]	1.76[a]	0.175	0.018
Dust bathing	0.27	0.38	0.43	0.60	0.138	0.176
Body shake	0.39	0.44	0.48	0.48	0.101	0.751
Leg/wing stretching	0.33[c]	0.43[b]	0.43[b]	0.68[a]	0.115	0.048

[Data refer to mean values of ten birds per replicate on 11d of age; [a-c] Mean bearing different superscripts in a row differ significantly between treatment at *P<0.05; SEM = Standard error of the mean; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness;[3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval ;[4]18L:6D: 18 hours of intermittent ;lighting with 6 hours of darkness with 2 x 1-hour lighting interval]

4.2.2 Behavioural observations at 22d

The data on behavioural activities of broiler observed on day 22, are shown in Table 4.5.

Table 4.5 Behavioural activities of broiler chicken observed on day 22 under different lighting programs over a period of 24 hours.

Behavioural activities (%)	Treatments				SEM	P-value
	T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
Standing	4.94	4.60	4.63	4.39	0.329	0.445
Walking/ running	5.04[a]	4.74[b]	4.58[c]	4.33[c]	0.082	0.000
Inactive resting	71.92[a]	71.79[a]	70.65[b]	69.19[b]	0.733	0.010
Foraging	1.11	1.22	1.2	1.29	0.121	0.522
Feeding	9.60[c]	10.34[c]	11.42[b]	12.96[a]	0.759	0.005
Drinking	5.35	5.36	5.75	6.09	0.356	0.165
Preening	1.21	1.31	1.27	1.30	0.084	0.612
Dust bathing	0.22	0.22	0.22	0.23	0.051	0.998
Body shake	0.21	0.19	0.22	0.24	0.071	0.940
Leg/wing stretching	0.19	0.20	0.20	0.20	0.043	0.995

[Data refer to mean values of seven birds per replicate on day22; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness ;[3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval ;[4]18L:6D: 18 hours of intermittent ;lighting with 6 hours of darkness with 2 x 1-hour lighting interval#; [a-c] Values bearing different superscripts in a row differ significantly between treatment at *P<0.05; SEM = Standard error of the mean]

The results reveal that only inactive resting, feeding, and running/walking activities of birds were influenced (P<0.05) by treatments except for other activities of broiler measured in this study. The highest (P<0.01) walking/running (5.04%) and resting activities were found in the T_1 group while lowest resting (4.22%) and walking /running being in theT_4 group. Significantly (P<0.01) greater feeding, activities were found in the T_4 treatment group of birds than that of other groups.

4.2.3 Behavioural observations at 29d

The data on behavioural activities of broiler observed on day 29 are shown in Table 4.6. The result revealed that only inactive resting, drinking and preening activities of birds were influenced (P<0.05) by treatments except for other activities of broiler measured in this study. The highest (P<0.01) resting activities (79.22%) were found in the T_1 group while lowest resting (73.281%) being in the T_4 group. Significantly (P<0.01) greater drinking and preening activities were found in the T_4 treatment group of birds than that of other groups on day 29.

Table 4.6 Behavioural activities of broiler chicken observed on day 29 under different lighting programs over a period of 24 hours.

Behavioural activities (%)	Treatments				SEM	P-value
	T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
Standing	3.31	3.36	3.52	3.73	0.219	0.259
Walking/running	3.04	3.36	3.70	3.52	0.424	0.484
Inactive resting	79.22[a]	77.23[b]	75.93[c]	73.81[d]	0.790	0.000
Foraging	0.38	0.54	0.69	0.76	0.143	0.089
Feeding	7.48	7.63	7.40	8.01	0.392	0.442
Drinking	4.62[c]	5.20[b]	5.74[a]	6.30[a]	0.312	0.001
Preening	1.02[c]	1.32[b]	1.39[b]	1.93[a]	0.221	0.010
Dust bathing	0.22	0.39	0.48	0.60	0.133	0.073
Body shake	0.27	0.44	0.54	0.65	0.131	0.072
Leg/wing stretching	0.46	0.55	0.64	0.70	0.124	0.262

[Data refer to mean values of seven birds per replicate on day29; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness ;[3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval ;[4]18L:6D: 18 hours of intermittent ;lighting with 6 hours of darkness with 2 x 1-hour lighting interval[#]; [a-d] Values bearing different superscripts in a row differ significantly between treatment at *P<0.05; SEM = Standard error of the mean]

4.3 Response to observer test

The results of response to observer test on the 12d, 23d, and 30d of broiler rearing are demonstrated in Table 4.7. The broilers were found to reduce responsiveness toward the observer as the day progressed. The result revealed that responses to observer of birds were influenced (P<0.05) by treatments all the time.

Table 4.7 Broilers' response to the observer under different lighting programs on the 12th, 23th and 30th days only.

Observation time (%)	Treatments				SEM	P values
	T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
12th day	53.75[a]	43.75[b]	30.69[c]	23.75[d]	7.55	0.002
23th day	47.50[a]	38.06[b]	25.28[c]	22.78[d]	6.56	0.002
30th day	32.57[a]	27.50[b]	20.14[c]	19.17[c]	5.21	0.048

[Data refer to mean values of seven birds per replicate on day12, 23th and 30th days; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness ;[3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval ;[4]18L:6D: 18 hours of intermittent ;lighting with 6 hours of darkness with 2 x 1-hour lighting interval[#]; [a-d] Values bearing different superscripts in a row differ significantly between treatment at *P<0.05; SEM = Standard error of the mean]

4.4 Novel object test

The results of the novel object (NO) test on 12d, 23d, and 30d are shown in Table 4.8. The NO test time value of treatment was found to be significant (P<0.01) only on day 30 except for others. No significant (P>0.05) difference was found on the 12d and 23d test results. However, the test time on 30d was found significantly higher in the T_4 treatment (30.13 sec) than that of other treatments in this study.

Table 4.8 Broilers' response to the novel object (NO) under different lighting programs on the 12th, 23th and 30th days only.

Observation time (seconds)	Treatments				SEM	P values
	T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
12th day	7.50	8.25	8.75	10.57	1.94	0.51
23th day	6.75	8.87	10.25	13.87	3.25	0.19
30th day	8.00[c]	8.75[c]	19.63[b]	30.13[a]	5.06	0.01

[Data refer to mean values of seven birds per replicate on day129, 23th and 30th days;; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness ;[3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval ;[4]18L:6D: 18 hours of intermittent ;lighting with 6 hours of darkness with 2 x 1-hour lighting interval[#]; [a-c] Values bearing different superscripts in a row differ significantly between treatment at **P<0.01; SEM = Standard error of the mean]

4.5 Novel environment test

The results of the novel environment (NE) test conducted on 30th day is shown in Table 4.9. There was a significant (P<0.05) difference in latency to the first escape attempt, and no. of escape attempts only. Broiler on T_4(18L:6D) lighting group took a higher (P<0.05) time (66.75 sec) to the latency of first escape than that of other treatment groups. Birds on T_1 group made more (P<0.05) escape attempts (3.25) than those of other treatment groups. The no. of distress noises gradually decreased with the addition of darkness in lighting management but it is not significantly different (P>0.05).

Table 4.9 Broilers' response to the novel environment (NE) under different lighting programs on the 30th days only.

NE Tests	Treatments				SEM	P value
	T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
Latency to first escape (seconds)	15.50[d]	24.00[c]	46.75[b]	66.75[a]	13.96	0.013
No. of escape attempts	3.25[a]	2.00[b]	1.50[c]	0.75[d]	0.72	0.029
No. of distress noise	19.75	10.75	9.75	4.50	5.50	0.096

[Data refer to mean values of seven birds per replicate on day 30; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness ;[3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval ;[4]18L:6D: 18 hours of intermittent ;lighting with 6 hours of darkness with 2 x 1-hour lighting interval[#]; [a-d] Values bearing different superscripts in a row differ significantly between treatment at *P<0.05; SEM = Standard error of the means]

4.6 Tonic immobility and gait score test

The data of the tonic immobility (TI) and gait score (GS) tests conducted on 24th and 30th days are shown in Table 4.10. The results indicate that TI was affected (P<0.01) by treatment except for another parameter (GS). There was no significant (P>0.05) difference found in GS among the treatments, although the scores are gradually decreased with the addition of more darkness hours in lighting programs. Broiler chicken appeared to be spent significantly (P<0.01) more time (223.25 seconds) in T_1 treatment group than that of T_2(214.25 seconds), T_3(169.25 seconds) and T_4(124.38 seconds) group respectively, in TI test.

Table 4.10: Tonic immobility (TI) and gait score (GS) index of broiler under different lighting program on the 24th and 30th days

Tests	Treatments				SEM	P values
	T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
Tonic immobility test (sec) on 24d	223.25[a]	214.25[b]	169.25[c]	124.38[d]	19.32	0.01
Gait score test on 30d	0.38	0.25	0.25	0.13	0.23	0.75

[Data refer to mean values of seven birds per replicate on days 24d and 30d; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness ;[3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval ;[4]18L:6D: 18 hours of intermittent ;lighting with 6 hours of darkness with 2 x 1-hour lighting interval[#]; [a-d] Values bearing different superscripts in a row differ significantly between treatment at **P<0.01]

4.7 Stress level

The stress level of broilers was evaluated by blood heterophil- lymphocytes (HL) ratio and serum cortisol (CORT) level are shown in Table 4.11. The results indicated that HL ratio and CORT level of broiler were unaffected (P>0.05) between treatment.

Table 4.11 Blood heterophil- lymphocytes (HL) ratio and serum cortisol (CORT) test level of broiler under different lighting program on the day 27

Parameters	Treatments				SEM	P-values
	T_1 (24L:0D[1])	T_2 (22L:2D[2])	T_3 (20L:4D[3])	T_4 (18L:6D[4])		
HL ratio	0.3321	0.3203	0.3116	0.3074	0.029	0.846
Serum CORT (ng/ml)	0.1163	0.1178	0.1214	0.1180	0.003	0.354

[Data refer to mean values of seven birds per replicate on days 27d; [1]24L:0D: 24 hours of continuous lighting with no darkness; [2]22L:2D: 22 hours of continuous lighting with 2 hours of darkness; [3]20L:4D: 20 hours of intermittent lighting with 4 hours of darkness with 1 hour of lighting interval; [4]18L:6D: 18 hours of intermittent lighting with 6 hours of darkness with 2 x 1-hour lighting interval[#];]

CHAPTER 5: DISCUSSION

4.1 Effect of lighting duration on broiler performances

4.1.1 Live weight gain (LWG)

Our study result reveals that there was increased final body weight of the broiler with the addition of dark hours but was not significant among the lighting treatments on day 1-28. Our result is supported by previous investigators (Rahim *et al.*, 2005, *Abbas et al., 2008; Lien et al., 2007, Coban et al., 2014, Fidan et al., 2017a, Khutal et al., 2022*), who did not find any association in between 1L: 3D intermittent lighting system and nearly continuous lighting 23L:1D. However, we also observed significantly increased LWG in the broiler chickens on day 8-14 and day 22-28, respectively, when the birds were exposed to different lighting program. The result is also supported by previous researchers (*Freeman et al., 1981; Charles et al., 1992, Brickett et al., 2007b, Das and Lacin, 2014).* A report showed that significantly higher body weight was achieved in continuous lighting (23L:1D) compared to constant lighting (18L:6D) and intermittent lighting (4L:2D) (*Das and Lacin, 2014*). The results suggest that lighting hours might influence the body weight of broilers, because broilers have a good chance to consume more feed in the increased lighting hour compared to shorter exposure of lighting program under good management condition.

4.1.2 Feed consumption and feed conversion ratio (FCR)

In our study it is obvious from the data that significantly increased feed intake was found in the continuous lighting program T_1(24L:0D) compared to other programs during the early period or day 8-14 only. The reason for this is likely to be increased exposure to lighting hour or photoperiod which stimulated the birds to consume more feed than that of other groups. The result is consistent with the report of previous investigators (*Das and Lacin, 2014*), who also reported significantly higher feed intake in the continuous lighting (23L:1D) compare to the constant lighting (18L:6D) and intermittent lighting (4L:2D). But birds' feed intake was similar in the later period of growth, which is also consistent with many other previous investigators (*Coban et al., 2014, Zhao et al., 2019, Khutal et al., 2022*). Apart from lighting, many other factors such as feed color, palatability, type, odor, smell, requirement, feed composition, fiber level, foreign particles, and so on could also affect the feed choice and consumption of broiler chicken (*Farghly, 2017*; *Abdollahi et al., 2018*).

It is also clear from the data that, the FCR of broilers to different lighting hours or photoperiod was not influenced in this current study. A similar FCR of broiler might occur due to supplying the same feed to all birds of all dietary treatment groups. However, it is noteworthy that the final FCR was gradually improved with the addition of extra-darkness in lighting schedules, even though the difference between treatments was insignificant on day 1-28. The FCR was found slightly poorer in continuous lighting treatments (24L:0D and 22L:2D) than that of intermittent lighting systems (20L:4D and 18L:6D). The result is also supported by previous researchers (*Coban et al., 2014, Fidan et al., 2017a, Zhao et al., 2019, Khutal et al., 2022),* who found similar results when the broiler was exposed to different lighting regimes.

4.1.3 Livability

The data on viability of the broiler show that applying different photoperiods of lighting hour to birds had no significant effect on the livability or death of birds. It is interesting to note that birds of the T_3(20L:4D) and T_4(18L:6D) treatment groups got the highest (97.50%) viability amongst others in this study, which implies that the different photoperiods of lighting are not detrimental for the broiler chicken at these levels. Though the livability did not differ significantly, the mortality was comparatively a bit increased in the control or continuous group T_1(24L:0D). Our findings agreed with previous researchers who showed similar results when broilers exposed to different photoperiods of lighting program (*Lien et al., 2007, Schwean-Lardner et al., 2013, Coban et al., 2014*). Our result is contradicted by the report of *Khutal et al., (2022)* who got a significantly higher mortality rate in 23L:1D compared to the 18L:6D, 16L:8D, and 14L:10D lighting schedules in 6 weeks. The result is contradicted by *Julian (2000)* and *Hassanzadeh et al., (2005),* who suggested that the reduction in lighting hours can decrease mortality specially ascites syndrome significantly. Apart from lighting, other factors say disease incidences, seasonal impact, heat stress, and feed might influence this mortality of birds. The early case fatality could also be caused by the extreme winter season with low temperatures as the lighting system was the only source of heat (*Kalmar et al., 2013*)

4.2 Effect of lighting duration on broiler behaviour and welfare

4.2.1 Behavioural Observations

The data on behavioural activities (*e.g*, standing, walking/running, inactive resting, foraging, feeding, drinking, preening, dust bathing, body shake, leg/wing stretching) of broiler chicken observed on 11d, 22d, and 29 days, respectively, were measured in this study. It is evinced from the data that only inactive resting, feeding, preening, and leg/wing stretching on day 11; inactive resting, feeding, and running/walking activities on day 22; and inactive resting, drinking, and preening activities on day 29 were found to be significantly influenced by lighting regimes. It is interesting to note that inactive resting activities of broilers were found to be significantly improved in the continuous lighting group T_1 (24L:0D) of birds compared to others during entire the trial period. Our result agrees with the report of previous investigators (*Bayram and Özkan, 2010; Schwean-Lardner et, al., 2012, 2014*), who stated a negative relation between resting time and dark hours meaning inactive resting was significantly reduced with the addition of darkness. But our result contradicts their result also as they found more frequent standing, and walking behaviour in darkness involved lighting compare to the continuous lighting.

Feeding, preening, and leg/wing stretching activities were increased significantly in the T_4, T_3, and T_2 groups as the darkness ameliorated in the lighting programs on day 11. Running or walking activities of birds on day 22 were seen to be increased in T_1 group. It may be due to continuous photoperiod as long lighting exposure could stimulate/excite the birds to move to and fro. Significantly greater drinking and preening activities were found in the T_4 treatment group of birds than that of other groups on day 29. Feeding behaviour supports the results of *Schwean-lardner et al., (2012, 2014)* and *Bayram and Özkan, (2010)* as they also reported increased feeding time with the addition of darkness. *Brown, (2010)* reported insignificant differences in standing, walking, running, foraging, and resting at 9d, 22d, 29d, and 43d over 24h observation. Preening was found significantly more frequent in 18L:6D than in 24L:0D treatment on 22d and 29d. *Bayram and Özkan (2010)* reported broiler rearing under a 16L:8D lighting system shows comfort behaviours, such as preening and wing-shaking, more extensively than 24 h continuous lighting. But *Brown (2010)* reported no significant difference in comport behaviours like preening and wing flapping behaviour among different lighting periods.

4.2.2 Fear test

The fearfulness of broilers was assessed in our study using the novel environment (NE) test, the novel object (NO) test, the response to observer (RO) test, and the tonic immobility (TI) test.

It is evident from the results of RO that the bird's reaction to observer was affected significantly during 12^{th}, 23^{rd} and 30^{th} days of observation times. The percentage of birds responding to the observer was significantly greater in the T_1(24L:0D) treatment than that of others in this study. On the day30, the NO test data revealed that birds in T_4 group spant significantly more time choosing a novel object than that of others. In NE test we see that birds in T_4 group delayed more time to the first escape attempt than those of other treatments. All these results indicate that broilers showed boldness and bravery art or less fearfulness when they were exposed to extended darkness period of time.

The result of TI test showed that birds in T_1 group demonstrated significantly higher latency to rise in the continuous lighting program than that of other treatment groups. It implies that broilers exposed to extended darkness period spent less time in TI test. The results of TI test indicate that birds feel less stress when they expose to higher darkness period. Our results agree with the report of previous workers who reported a negative relationship between TI and lighting time (*Blair et al., 1993; Bayram and Özkan, 2010, Yang et al., 2022*). This means that TI time reduces with increasing dark period and lessen the fearful stress in broilers. But the result contradicts the report of *Fidan et al., (2017b)* who showed no significant relationship between lighting period and TI duration. *Wang et al., (2008)* also reported increasing the lighting period decreased the duration of TI on 10d, had no effect on 22d, and increased the duration of TI on 36d.

4.2.3 Stress control

Blood heterophil-lymphocytes (HL) ratio and serum cortisol (CORT) was measured to assess the stress level of broiler chicken. There was no significant difference found among the treatments for HL ratio and CORT levels. Our results agree with the findings of previous investigators who found similar results, when the birds were exposed to different lighting regimes (*Abbas et al., 2008; Olanrewaju et al., 2013, 2019*). Despite the insignificance, the serum CORT level was found slightly lower in continuous

lighting (24L:0D) following 22L:2D, 18L:6D, and 20L:4D, respectively. This may be due to the acute stress of the blood collection pattern of birds (20L:4D, 18L:6D, 20L:2D, and 24L:0D) *(Hemsworth et al., 1994; Alm et al., 2014; Blas, 2015*). According to *Lien et al., (2007)* and *Coban et al., (2014),* continuous lighting schedules result in much higher HL ratios than 18L:6D or 16L:8D lighting schedules, which indicates greater stress.

4.2.4 Gait score

In our study, the gait score (GS) test was conducted on 30d using a 3-point GS system (*Webster et al., 2008*), which showed an insignificant relationship between the lighting period and GS. It indicates that GS of the broilers were identical between the treatments on 30d with no affecting in leg health issues. The result agrees with the report of previous investigators (*Olanrewaju et al.,2019, Khutal et al.,2022*), who also found insignificant GS among various lighting periods. But our result is in contrast to the reports of *Sanotra et al., (2002), Brickett et al., (2007a)* and S*chwean-Lardner et al., (2013),* who found significantly decreased GS of broilers with the addition of darkness in lighting schedules indicating better leg health in reduced lighting periods. *Fidan et al., (2017b)* also reported significantly higher GS in continuous lighting periods compared to increasing lighting periods for 42d.

CHAPTER 6: CONCLUSION

From an overview of the results obtained in this study revealed that the live weight gain (LWG) on 2nd and 4th week and feed intake (FI) on 2nd week were influenced by lighting treatments without affecting overall weight gain, FI, FCR, and viability of broiler chickens. However, the reduced lighting treatment slowed growth and feed intake significantly during the second week of grow-out, which could potentially reduce mortality. So, there are no significant changes in overall boiler performances among these lighting durations.

However, there were significant differences found in broiler behaviour and welfare matters. It is evinced from the data that only inactive resting, feeding, preening, leg/wing stretching, running/walking and drinking activities were found to be significantly influenced by lighting regimes. Broiler performed inactive resting activities significantly or commonly in continuous lighting program, this behaviour was less responsive when the birds were exposed to increased darkness period. Besides, increased activity of feeding and drinking time were found in reduced lighting periods. Among the comfort behaviour, preening behaviour seems to be more frequent with the addition of darkness in the lighting schedule while others like, dust bathing, body shaking and leg/wing stretch were insignificant among the lighting treatments. Broilers in short lighting durations also showed low fearfulness compared to the continuous lighting in fear test (*i.e.* NO, NE, RO, and TI test), indicating improved welfare for broilers. The gait scores of the broilers were similar between the treatments with no changes in leg health issues. The H:L ratio and serum CORT level suggested similar responses and no changes in stress management by altering lighting duration. It can be concluded from the result that actually not so many variations were noticed among the different lighting durations (24L:0D, 22L:2D, and 20L:4D), but 18L:6D lighting program could provide some extra attributes to broilers, especially in welfare issues. In short it can be inferred that, reducing lighting hours or providing increased darkness in the lighting regimes can improve broiler welfare and potentially boost broiler performances.

CHAPTER 7: LIMITATIONS AND RECOMMENDATIONS

There are, however, a number of limitations to this research. The first is that the study was only done throughout the winter for a single flock, which means that results may vary at other times of the year, especially during the summer. The second limitation concerns the population and the sample size of the study was small, which may have produced more accurate and consistent analytical results with a larger sample size. Thirdly, there is a dearth of adequate data because, with the exception of the TI test, no previous information is available on the fearful tests. Last but not least, no information on carcass and meat quality was gathered for this investigation, which would have revealed additional variations in the lighting strategies.

This study only focuses on the lighting duration which is one of the factors in lighting programs including lighting intensity and light color. Recent studies are also conducted on the remaining factors but mostly on a single factor. So, there is a potential scope of developing better lighting programs combining all three lighting factors which ensure high quality broiler farming.

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Appendix 1: Weekly recorded data of broiler on performance parameters.

TR	Rep.	1st week		2nd week				3rd week				4th week				Final FCR
		DOC BW = 0.042 kg **1st week:** CBW = 0.154 kg WG = 0.112 kg FI = 0.128 kg FCR= 1.143	BW1 (kg)	CBW (kg)	WG (kg)	FI (kg)	FCR	CBW (kg)	WG (kg)	FI (kg)	FCR	CBW (kg)	WG (kg)	FI (kg)	FCR	
T1	11		0.156	0.573	0.417	0.560	1.343	1.063	0.490	0.724	1.478	1.737	0.674	1.126	1.671	1.408
	12		0.155	0.554	0.399	0.489	1.226	0.987	0.433	0.678	1.566	1.643	0.656	1.103	1.681	1.404
	13		0.148	0.536	0.388	0.492	1.268	0.963	0.427	0.782	1.831	1.616	0.653	1.206	1.847	1.522
	14		0.161	0.542	0.381	0.461	1.210	1.036	0.494	0.745	1.508	1.712	0.676	1.058	1.565	1.357
T2	21		0.16	0.532	0.372	0.471	1.266	1.010	0.478	0.752	1.573	1.736	0.726	1.188	1.636	1.405
	22		0.151	0.482	0.331	0.462	1.396	1.012	0.530	0.799	1.508	1.690	0.678	1.102	1.625	1.418
	23		0.150	0.547	0.397	0.521	1.312	1.008	0.461	0.683	1.482	1.710	0.702	1.152	1.641	1.394
	24		0.155	0.552	0.397	0.477	1.202	1.005	0.453	0.697	1.539	1.663	0.658	1.112	1.690	1.393
T3	31		0.143	0.495	0.352	0.458	1.301	0.993	0.498	0.746	1.498	1.740	0.747	1.186	1.588	1.382
	32		0.156	0.513	0.357	0.482	1.350	0.996	0.483	0.723	1.497	1.675	0.679	1.086	1.599	1.397
	33		0.143	0.470	0.327	0.442	1.352	1.043	0.573	0.775	1.353	1.707	0.664	1.067	1.607	1.363
	34		0.152	0.502	0.350	0.428	1.223	0.976	0.474	0.731	1.542	1.707	0.731	1.143	1.564	1.368
T4	41		0.161	0.510	0.349	0.440	1.261	1.002	0.492	0.742	1.508	1.754	0.752	1.234	1.641	1.388
	42		0.159	0.458	0.299	0.410	1.371	1.055	0.597	0.784	1.313	1.741	0.686	1.052	1.534	1.340
	43		0.152	0.461	0.309	0.416	1.346	1.006	0.545	0.765	1.404	1.720	0.714	1.055	1.478	1.343
	44		0.167	0.477	0.310	0.432	1.394	0.995	0.518	0.756	1.459	1.692	0.697	1.103	1.582	1.395

TR= Treatments (T1: 24L:02D, T2: 22L:2D, T3: 20L:4D and T4: 18L:6D)
Rep.= Replicates
DOC = Day old chick
CBW = Cumulative body weight
BW1= Body weight before replicates entry
WG = Weight gain
FI = Feed intake
FCR = Feed conversion ratio

Appendix 2: Behavioural observation record on 11d

TR	Rep.	Behaviour observed (%)									
		1	2	3	4	5	6	7	8	9	10
T1	11	4.86	6.02	71.06	0.23	8.80	6.71	1.16	0.46	0.46	0.23
	12	5.00	3.33	73.13	0.42	9.79	6.25	1.04	0.21	0.42	0.42
	13	5.09	5.09	74.07	0.00	8.33	5.56	0.93	0.00	0.46	0.46
	14	3.75	3.75	76.88	0.21	8.75	4.58	1.25	0.42	0.21	0.21
T2	21	5.00	3.33	73.75	0.42	8.96	5.83	1.46	0.42	0.42	0.42
	22	4.63	3.94	72.92	0.23	9.49	6.25	1.16	0.46	0.69	0.23
	23	5.09	3.47	74.07	0.23	9.72	5.09	1.16	0.23	0.46	0.46
	24	5.00	2.50	74.58	0.42	8.96	5.83	1.46	0.42	0.21	0.63
T3	31	5.39	3.73	72.20	0.62	9.96	5.60	1.24	0.41	0.41	0.41
	32	6.25	3.47	70.83	0.46	9.95	5.56	1.85	0.69	0.46	0.46
	33	5.21	3.54	73.33	0.42	9.79	5.21	1.46	0.42	0.42	0.21
	34	4.79	5.00	71.04	0.00	10.63	6.04	1.04	0.21	0.63	0.63
T4	41	6.67	2.92	69.79	0.63	10.00	6.46	1.88	0.42	0.42	0.83
	42	5.83	3.54	70.00	0.42	10.83	5.63	2.08	0.63	0.42	0.63
	43	5.56	4.17	70.83	0.46	9.72	5.56	1.62	0.93	0.69	0.46
	44	5.20	4.37	70.27	0.00	9.56	7.48	1.46	0.42	0.42	0.83

1: Standing, 2: Walking/ running, 3: Resting, 4: Foraging, 5: Feeding, 6: Drinking, 7: Preening, 8: Dust bathing, 9: Body shake 10: Leg / wing stretch
TR= Treatments (T1: 24L:02D, T2: 22L:2D, T3: 20L:4D and T4: 18L:6D)
Rep.= Replicates

Appendix 3: Behavioural observation record on 22d

TR	Rep.	Behaviour observed (%)									
		1	2	3	4	5	6	7	8	9	10
T1	11	5.14	5.18	71.24	1.31	10.51	5.34	0.98	0.12	0.12	0.16
	12	4.94	4.86	72.16	0.86	9.35	~~6.08~~	1.24	0.3	0.23	0.22
	13	4.2	4.98	72.62	1.06	8.67	5.1	1.32	0.24	0.43	0.26
	14	5.48	5.14	71.67	1.19	9.87	4.86	1.28	0.21	0.06	0.14
T2	21	4.78	4.88	71.24	1.33	10.62	5.24	1.23	0.25	0.23	0.28
	22	4.87	4.57	72.3	1.21	9.24	5.67	1.32	0.23	0.25	0.24
	23	4.08	4.81	72.91	1.18	9.72	5.59	1.34	0.14	0.12	0.15
	24	4.68	4.69	70.72	1.16	11.79	4.93	1.35	0.26	0.17	0.14
T3	31	5.23	4.67	71.57	1.26	10.64	5.48	1.23	0.12	0.27	0.27
	32	4.37	4.55	70.35	0.86	12.49	5.33	1.45	0.21	0.17	0.19
	33	4.36	4.62	71.47	1.19	10.31	6.06	1.15	0.21	0.32	0.24
	34	4.55	4.43	69.19	1.49	12.25	6.14	1.24	0.32	0.13	0.12
T4	41	4.06	4.29	70.54	1.35	11.26	6.47	1.34	0.14	0.25	0.2
	42	4.23	4.38	68.75	1.25	13.63	5.86	1.43	0.21	0.18	0.18
	43	5.17	4.36	69.88	1.37	12.88	5.26	1.16	0.32	0.21	0.28
	44	4.09	4.27	67.57	1.19	14.06	6.78	1.26	0.23	0.3	0.16

1: Standing, 2: Walking/ running, 3: Resting, 4: Foraging, 5: Feeding, 6: Drinking, 7: Preening, 8: Dust bathing, 9: Body shake 10: Leg / wing stretch
TR= Treatments (T1: 24L:02D, T2: 22L:2D, T3: 20L:4D and T4: 18L:6D)
Rep.= Replicates

Appendix 4: Behavioural observation record on 29d

TR	Rep.	Behaviour observed (%)									
		1	2	3	4	5	6	7	8	9	10
T1	11	3.24	3.94	78.24	0.46	7.41	4.40	1.16	0.23	0.46	0.46
	12	3.54	3.13	78.13	0.63	7.92	4.79	1.25	0.21	0.21	0.21
	13	3.13	2.60	80.73	0.00	7.29	4.69	1.04	0.00	0.00	0.52
	14	3.33	2.50	79.79	0.42	7.29	4.58	0.63	0.42	0.42	0.63
T2	21	2.92	3.54	77.29	0.42	7.08	5.83	1.46	0.42	0.42	0.63
	22	3.70	4.17	76.85	0.46	7.18	5.09	1.39	0.46	0.23	0.46
	23	3.47	3.01	76.85	0.46	8.33	4.86	1.16	0.46	0.69	0.69
	24	3.33	2.71	77.92	0.83	7.92	5.00	1.25	0.21	0.42	0.42
T3	31	3.75	2.71	77.29	0.63	6.88	5.63	1.67	0.21	0.63	0.63
	32	3.54	4.38	76.67	0.83	6.67	4.79	1.25	0.63	0.63	0.63
	33	3.47	4.17	74.54	0.46	8.33	6.48	1.16	0.46	0.46	0.46
	34	3.33	3.54	75.21	0.83	7.71	6.04	1.46	0.63	0.42	0.83
T4	41	4.38	3.33	72.92	0.83	8.54	6.46	1.88	0.42	0.63	0.63
	42	3.75	3.54	75.63	0.63	7.50	6.25	1.25	0.63	0.42	0.42
	43	3.47	3.47	73.15	0.93	7.87	6.02	2.31	0.93	0.93	0.93
	44	3.33	3.75	73.54	0.63	8.13	6.46	2.29	0.42	0.63	0.83

1: Standing, 2: Walking/ running, 3: Resting, 4: Foraging, 5: Feeding, 6: Drinking, 7: Preening, 8: Dust bathing, 9: Body shake 10: Leg / wing stretch
TR= Treatments (T1: 24L:02D, T2: 22L:2D, T3: 20L:4D and T4: 18L:6D)
Rep.= Replicates

Appendix 5: Weekly broiler livability and mortality rate record

TR	Rep.	Fetal cases (no.)			Mortality rate (%)	Livability (%)	Diseases-related case (no.)	
		2^{nd} week	3^{rd} week	4^{th} week			SDS	Ascites
T1	11	1	0	0	10	90	1	0
	12	0	0	0	0	100	0	0
	13	0	1	1	20	80	1	1
	14	0	0	0	0	100	0	0
T2	21	0	0	0	0	100	0	0
	22	0	1	0	10	90	1	0
	23	1	0	0	10	90	1	0
	24	0	0	0	0	100	0	0
T3	31	0	0	0	0	100	0	0
	32	1	0	0	10	90	1	0
	33	0	0	0	0	100	0	0
	34	0	0	0	0	100	0	0
T4	41	0	0	0	0	100	0	0
	42	0	0	0	0	100	0	0
	43	1	0	0	10	90	0	1
	44	0	0	0	0	100	0	0

SDS: Sudden death syndrome
TR= Treatments (T1: 24L:02D, T2: 22L:2D, T3: 20L:4D and T4: 18L:6D)
Rep.= Replicates (each replica holds 10 birds)

Appandix 6: Response to observer test at different ages

TR	REP	RO on 12d (%)		RO on 23d (%)		RO on 30d (%)	
		OB1	OB2	OB1	OB2	OB1	OB2
T1	11	60	40	66.66667	44.44444	33.33333	22.22222
	12	60	50	40	30	40	40
	13	60	40	33.33333	55.55556	50	25
	14	70	50	70	40	20	30
T2	21	70	50	30	40	20	30
	22	50	40	33.33333	33.33333	33.33333	22.22222
	23	44.4	55.55556	44.44444	33.33333	22.22222	22.22222
	24	10	30	50	40	40	30
T3	31	40	40	30	40	30	20
	32	22.22222	33.33333	22.22222	0	0	11.11111
	33	30	40	40	20	40	30
	34	0	40	30	20	20	10
T4	41	40	30	40	40	20	20
	42	0	40	0	30	30	10
	43	20	40	22.22222	0	0	33.33333
	44	0	20	30	20	20	20

RO= response to observer (%)
TR= Treatments (T1: 24L:02D, T2: 22L:2D, T3: 20L:4D and T4: 18L:6D)
OB= Observation no.
Rep.= Replicates

Appandix 7: Novel object test at different ages

TR	REP	NO on 12d		NO on 23d		NO on 30d	
		OB1	OB2	OB1	OB2	OB1	OB2
T1	11	3	13	4	6	8	7
	12	4	10	6	10	4	16
	13	12	7	3	7	5	12
	14	5	6	4	14	4	8
T2	21	4	8	6	25	5	9
	22	8	6	7	6	7	13
	23	4	15	10	8	7	15
	24	13	8	5	11	6	8
T3	31	5	6	5	11	27	15
	32	10	14	7	8	8	24
	33	4	16	9	18	28	7
	34	6	9	12	26	36	12
T4	41	12	16	13	11	42	17
	42	9	11	24	14	57	9
	43	5	9	5	15	24	45
	44	13	8	23	6	19	28

NO= Novel object test (sec.)
TR= Treatments (T1: 24L:02D, T2: 22L:2D, T3: 20L:4D and T4: 18L:6D)
OB= Observation no.
Rep.= Replicates

Appendix 8: Novel environment test of broiler on 30d

Treatment	Replicates	Latency to first escape (sec)	No. of escape attempts	No. of distress noise
T1	11	18	4	11
	12	16	5	40
	13	16	3	15
	14	12	1	13
T2	21	35	2	15
	22	11	2	9
	23	18	2	11
	24	32	2	8
T3	31	47	2	7
	32	52	0	6
	33	46	2	6
	34	42	2	20
T4	41	58	1	4
	42	120	0	5
	43	32	1	5
	44	57	1	4

Appendix 9: Tonic immobility test and Gait score test of broiler

TR	REP	TI on 24d		GS on 30d	
		OB1	OB2	OB1	OB2
T1	11	247	233	1	0
	12	342	218	1	0
	13	195	228	0	0
	14	207	186	0	1
T2	21	227	255	0	0
	22	243	194	1	0
	23	192	162	0	0
	24	245	196	0	1
T3	31	173	157	0	0
	32	186	198	0	0
	33	126	167	0	0
	34	192	155	1	1
T4	41	162	117	0	0
	42	73	192	1	0
	43	111	124	0	0
	44	98	118	0	0

TI= Tonic immobility test (sec.)
GS= gate score test.
TR= Treatments (T1: 24L:02D, T2: 22L:2D, T3: 20L:4D and T4: 18L:6D)
OB= Observation no.
Rep.= Replicates

Appendix 10: CBC and CORT level of blood collected on 27d

TR	Hb (g/dl)	RBCs ($x10^6$/µl)	Mono %	Eosino. %	Baso. %	Hetero. %	Lympho. %	H:L ratio	CORT (ng/ml)
T1	12.30	3.71	7	4	1	24	65	0.369	0.104
	8.90	3.05	14	4	1	23	58	0.397	0.121
	12.60	3.77	11	4	3	15	67	0.224	0.110
	9.60	3.13	8	4	1	21	66	0.318	0.115
	11.30	3.49	3	2	1	22	72	0.306	0.124
	11.60	3.43	5	2	1	23	65	0.354	0.116
	12.60	3.12	13	4	3	21	59	0.356	0.124
	9.70	3.04	9	2	1	22	66	0.333	0.116
T2	9.00	2.96	10	6	3	22	59	0.373	0.117
	10.10	2.82	8	3	1	23	65	0.354	0.112
	13.00	3.16	9	4	2	17	68	0.250	0.112
	9.60	3.13	9	3	2	15	69	0.217	0.122
	9.90	2.88	11	5	2	19	63	0.302	0.118
	12.80	3.28	6	3	1	21	69	0.304	0.122
	10.20	3.08	10	5	3	24	58	0.414	0.118
	11.60	3.58	5	5	1	23	66	0.348	0.121
T3	15.10	3.67	7	3	1	19	69	0.275	0.120
	11.80	2.90	11	6	2	21	62	0.339	0.121
	11.70	3.59	10	5	3	21	61	0.344	0.123
	12.10	3.66	7	6	3	15	70	0.214	0.127
	11.50	3.08	9	4	3	22	62	0.355	0.123
	9.70	3.00	6	3	3	20	68	0.294	0.118
	9.70	3.25	9	2	1	18	70	0.257	0.123
	12.80	3.31	8	5	2	29	70	0.414	0.115
T4	11.90	3.04	10	4	2	19	63	0.302	0.124
	9.90	3.22	10	4	2	21	62	0.339	0.129
	14.10	3.29	7	4	0	25	65	0.385	0.116
	13.90	3.52	9	3	2	19	67	0.284	0.111
	11.80	3.50	12	3	2	15	68	0.221	0.109
	9.60	3.15	7	4	3	21	65	0.323	0.122
	10.60	3.34	6	3	1	22	68	0.324	0.109
	12.10	3.51	8	4	2	19	67	0.284	0.124

TR= Treatments
CBC= complete blood cell count
CORT= corticosteroid
Hb= hemoglobin, RBC= red blood cell, Mono. = monocytes, Eosino = eosinophils, Baso = basophils, Hetero = heterophils, Lympho= lymphocytes, H:L = heterophil – lymphocytes ratio

Appendix 11: Ethical Approval Certificate (EAC) from the Ethics Committee (EC) of CVASU

Directorate of Research and Extension

Chattogram Veterinary and Animal Sciences University
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E-mail : drecvasu@gmail.com, Website: www.cvasu.ac.bd

Memo no.- CVASU/Dir(R&E)EC/2022/435(1)/15 Date: 15/12/2022

Ethics Committee (EC) of CVASU

This is to certify that, the project "Effect of Lighting Duration on the Behaviour, Welfare and Performance of Broiler chicken" being investigated by Prof. Dr. Marjina Akter, Dept. of Dairy and Poultry Science, CVASU has met the necessary requirements of it's Chattogram Veterinary and Animal Sciences University Ethics Committee to carry out the project activities. The CVASU Ethics Committee approval number for the project is Memo no.- CVASU/Dir(R&E)EC/2022/435(1)/15 Date: 15/12/2022

Member Secretary
CVASU-EC

Director (Research & Extension)
Chattogram Veterinary and Animal
Sciences University, Khulshi, Chattogram.

BIOGRAPHY

Abdullah Al Masud, the author of this research paper graduated in Doctor of Veterinary Medicine (DVM) from the renowned Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh in 2019. He passed Secondary School Certificate (SSC) in the Science group in 2012 from Natmura Pukuria High School, Banskhali, Chattogram. He completed Higher Secondary School Certificate (HSC) in Science group in 2014 from Govt. City College, Chttogram. Now he is a candidate for Master's degree in Poultry Science at the Department of Dairy and Poultry Science, Faculty of Veterinary Medicine, CVASU. He has an immense interest to work in the field of Poultry science.