Daylength Effects on Stress and Fear Responses in Broiler Chickens

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ABSTRACT : Heterophil (H) to lymphocyte (L) ratios and durations of tonic immobility (TI) were measured to assess stress and fear responses, respectively, in broiler chickens provided either 12 h of natural lighting (12L) or 12 h natural lighting and 12 h of supplementary lighting (24L). Birds illuminated 24L had greater H/L ratios and TI durations than their 12L counterparts. Neither age, sex nor cage level had significant effect on TI reactions.
(**Key Words** : Broilers, Daylength, Stress, Fear)

INTRODUCTION

There is a substantial body of literature on the welfare of broilers raised under various day lengths, light intensities and sources. Many of these studies have been reviewed recently (Gordon, 1994; Buyse et al., 1996). Although continuous or near continuous illumination is the common husbandry practice to raise broilers as it allows uniform access to food throughout the day (Gordon, 1994), it has been associated with immunosuppresion (Kirby and Froman, 1991), and greater incidence of ocular (Whitley et al., 1984) and leg (Buckland et al., 1976; Classen and Riddel, 1989) deformities. Furthermore, there is evidence to show that extended daylength reduced the opportunity for birds to sleep and rest (Yano et al., 1974; Murphy and Preston, 1988) thereby compromising the birds' welfare.

Literature regarding the influence of light on stress responses is conflicting. Buckland et al. (1976) observed higher plasma corticosterone in broilers raised under continuous light compared with intermittent lighting programme. Based on adrenal hypertrophy and elevated free fatty acid concentrations, Freeman et al. (1981) indicated that continuous lighting was stressful to chicks. On the other hand, Freeman et al. (1981) and Renden et al. (1994) reported that extended daylength had negligible effects on concentration of corticosterone in chicks. There is, however, a paucity of information on the effects of daylength on heterophil (H) to lymphocyte (L) ratio response, a more reliable indicator of the perceived magnitude of stressors in avian species (Gross and Siegel, 1983).

Work carried out with both meat- and egg-type chickens suggest that stress responses attributable to beak trimming (Lee and Craig, 1991), transportation (Cashman et al., 1989; Mills and Nicol, 1990), forced molting (Campo and Alvarez, 1991), and social disruption (Jones and Faure, 1982) may augment fear-related behaviour. Craig and Swanson (1994) indicated that information on fear response, one of the behavioural indices of internal states, is important in the assessment of poultry welfare. The present study investigates physiological stress and fear responses to two lighting programmes in broiler chickens.

MATERIALS AND METHODS

Ninety six day-old straight run broiler chicks (AVIAN) were housed in a conventional open-sided house with cemented flooring and wood shavings as litter. The chicks were vaccinated against Newcastle Disease (days 7 and 21) and Fowl Pox (day 21). Starter (crumble form; 210 g protein/kg and 12.3 MJ AME/kg) and finisher (pellet form; 190 g protein/kg and 12.8 MJ AMF/kg) diets were provided from days 1 to 20 and 21 onwards, respectively. Water and feed were available *ad libitum*. Birds were under continuous fluorescent illumination.

Commencing from day 14, chicks were wingbanded and housed in 12 groups of 8 in two blocks of three-tiered (2 cages per tier) battery cages. Each cage comprised of rectangular wire mesh. Equal number of chicks were provided either 12 h natural lighting (12L) (of dawn and dusk were about 06:00 h and 19:20 h, respectively) or 12 h natural lighting and 12 h supplementary lighting (fluorescent lighting) (24L). The mean intensities of the supplementary lighting measured at birds' level was 6 lux. The batteries for each lighting regimen were in separate houses. Annual ambient

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temperature and humidity respectively range from 25℃ to 34℃ and 80% to 90%.

On days 31 and 38 (between 08:00 h and 11:00 h), 12 males (2 birds per cage) and 12 females (2 birds per cage) from each lighting regimen were selected for tonic immobility (TI) measurements. Equal number of chicks were selected from each cage tier for the TI test. Birds that have been used for TI measurement in the first occasion were not used in the subsequent measurement. A modification of the procedure described by Benoff and Siegel (1976) was used. Tonic immobility was induced as soon as the birds were caught by gently restraining their left side by the legs and the wings for 15 second. The experimenter then retreated approximately 1 m and direct eye contact between the observer and the bird was avoided as it may prolong TI durations (Jones, 1986). A stopwatch was started to record latencies until the bird righted itself. If the bird righted in less than 10 s, it was captured again and the restraint procedure was repeated. The number of inductions required to obtain TI was recorded (up to three attempts). The maximum duration allowed was 600 s.

On days 20, 27, 34 and 41 (between 08:00 h and 09:00 h), six chicks (one bird per cage and those that were not used for TI measurement) from each lighting regimen were chosen and blood samples (0.3 mL) were obtained from the wing vein with EDTA as the anticoagulant. The same six birds per lighting regimem were bled on two occasions, namely days 20 and 34. Similarly, blood samples were collected twice from another six birds per lighting group on days 27 and 41. Blood smears were prepared using May-Grunwald-Giemsa stain and were used to determine number of H and L in the first 60 cells counted and an H/L ratio was calculated (Gross and Siegel, 1983).

Lighting regimen, sex, age and cage tier were considered as main effects for the analyses of TI durations and number of induction data. Durations of TI were transformed to square roots prior to analysis. Counts of H and L were converted to a ratio of H/L and analyzed with lighting regimen and age as main effects. All analyses were conducted with aid of the General Linear Models (GLM) procedure of SAS® software (SAS® Institute, 1985). When significant effects were found, comparisons among multiple means were made by Duncan's multiple test. Statistical significance is considered as $p \leq 0.05$ throughout the paper.

RESULTS

Birds provided 24L showed significantly longer TI reactions than their 12L counterparts (table 1). Neither age, sex nor cage tier had significant effects on TI durations. Regardless of lighting regimen, age, sex or cage level number of inductions required to achieve TI was similar (table 2).

Table 1. Mean (± SEM) tonic immobility durations by lighting regimen, age, sex and cage level

Variable	Duration (s)
Lighting regimen	
12L	84 ± 20.6[a]
24L	130 ± 27.6[b]
Age	
Day 31	90 ± 16.9
Day 38	123 ± 30.2
Sex	
Male	87 ± 15.6
Female	127 ± 30.8
Cage level	
Top	107 ± 39.5
Middle	90 ± 17.1
Bottom	123 ± 34.9

Means within a column-subgroup with no common letters differ significantly ($p \leq 0.05$).

Table 2. Mean (±SEM) number of inductions required to achieve tonic immobility by lighting regimen, age, sex and cage level

Variable	Number
Lighting regimen	
12L	2.0 ± 0.15
24L	1.8 ± 0.14
Age	
Day 31	1.8 ± 0.14
Day 38	2.0 ± 0.15
Sex	
Male	1.8 ± 0.14
Female	2.0 ± 0.15
Cage level	
Top	2.0 ± 0.17
Middle	1.7 ± 0.15
Bottom	2.1 ± 0.25

Means are not significantly ($p > 0.05$) different.

Heterophil to lymphocyte ratios were significantly higher for birds provided 24L than 12L (table 3). Significant effect of age on H/L ratios was evident with (day 27 = day 34 = day 41) > day 20.

Table 3. Mean (± SEM) heterophil to lymphocyte ratios by lighting regimen and age

Variable	Ratio
Lighting regimen	
12L	0.43 ± 0.02^{a}
24L	0.53 ± 0.02^{b}
Age	
Day 20	0.40 ± 0.02^{a}
Day 27	0.48 ± 0.02^{b}
Day 34	0.49 ± 0.03^{b}
Day 41	0.54 ± 0.03^{b}

Means within a column-subgroup with no common letters differ significantly ($p \leq 0.05$).

DISCUSSION

Results of this experiment are consistent with earlier findings concerning extended daylength and elicitation of stress responses in chickens (Buckland et al., 1976; Freeman et al., 1981). As measured by H/L ratios, birds subjected to 24L were more stressful than their 12L counterparts. Deprivation of adequate sleep and rest due to continuous illumination may have accounted for the phenomenon (Gordon, 1994).

Growing concern for animal well being is partly responsible for interest to evaluate the impact of intensive husbandry practice on fear response in poultry. Deleterious consequences of heightened fearfulness in broilers include injuries such as bruises, thigh hemorrhages and bone fractures attributable to violent wing flapping and struggling during handling (Jones, 1996). Newberry and Blair (1993) studied TI durations in broilers provided either a constant 23 h light or increasing photoperiod. The authors reported that the effect of lighting on fear in broilers was inconclusive. The results of the present study demonstrated significant differences in durations of TI between 24L and 12L broilers. Tonic immobility was prolonged when birds were illuminated for 24L. It is interesting to speculate that this phenomenon could be associated with the natural environment of the red jungle fowl, the ancestor of the domestic fowl (Crawford, 1990), and antipredator defense behaviour (Rovee et al., 1976; 1977; Rovee-Collier et al., 1983). Grigor (1993; as cited by Jones, 1996) suggested that because the natural habitat of the jungle fowl is in dense forest, birds may feel exposed to attack by predators in open areas. In view of that, the present authors hypothesized that longer durations of TI in 24L birds is related with the natural habitat of the jungle fowl, which is illuminated by natural day length (12 h). There is a possibility that 24 L birds felt exposed and vulnerable to predation during night, which was continuously illuminated in the present study, and thus, had augmented TI response. There is considerable evidence that TI or death feigning is an important predation defense in avian species (e.g. Rovee et al., 1976; 1977; Rovee-Collier et al., 1983).

The data presented here confirmed previous observations (Gallup, 1974; Jones and Faure, 1981a) that TI durations were not affected by sex. The lack of cage level effect on TI reactions observed here for broiler chickens is in contrast with that reported in laying hens (Jones, 1985a,b). Genetic factors (e.g. Jones and Mills, 1983; Campo and Carnicer, 1994) may have accounted for the discrepancies.

The present findings add to the growing body of evidence (e.g. Jones and Faure, 1981b; Craig et al., 1986; Duncan et al., 1986; Lee and Craig, 1991) that stress-provoking stimuli may heighten fear response in chickens. Although the neurochemistry of fear response has not been clearly elucidated, it encompasses adrenergic, dopamergic and cholinergic systems (Jones, 1986), which also play a pivotal role in physiological stress responses (Fillenz, 1993; Stanford, 1993). Prolonged tonic immobility durations following treatment with exogenous corticosterone (Jones et al., 1988; Campo and Carnicer, 1994) clearly demonstrate the cardinal impact of stress hormones on fear reaction in chickens. Hence, results of this experiment underpinned the notion that although fear and stress are not synonymous, fear, considered as an adaptive psychophysiological response to perceived danger, is a vital component of stress response (Jones et al., 1988). In conclusion, the current report indicates that continuous daylength appears to augment both stress and fear responses in broiler chickens.

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