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Effect of Human Factor on Fear and Stress Reactions and Some Performance Parameters in Broiler Chickens[1]

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Abstract: The aim of the study was to investigate the effects of visual human contacts on fear and stress reactions and some performance parameters in broiler chickens. A total of 180 (Ross-308), day-old commercial broiler chicks allocated into 3 groups each containing 60 chicks. Birds in Group-I and Group-II were exposed to regular visual contact for 5 min for two and three times a day while control counterparts had no visual contact during the study (1-37 days). Tonic Immobility (TI) duration and Heterophil to Lymphocyte Ratio (HLR) parameters were used to determine the level of fear and stress, respectively. The Newcastle disease antibody titres were analysed by using a commercial ELISA kit. The results revealed that visual human contact has no significant effect on tonic immobility duration and the number of induction in broiler chickens. This study also showed that visual human contact has no effect on parameters such as body weight, feed intake, feed conversion ratio, mortality and immunisation in broilers.

Key words: Broiler chicken, fear, stress, performance, heterophil to lymphocyte ratio (HLR)

INTRODUCTION

A multitude of stressors exist in the rearing of broiler chickens like high temperature, immune challenge, handling, transport and pleasant and unpleasant contacts with humans have more importance regarding animal welfare. There have been considerable studies about altering the physiology and behaviour of farm animals by regular contacts with humans (Hemsworth *et al*., 1994). The detrimental effects of stressors because of proteolysis and gluconeogenesis induced by corticosterone on growth performance and antibody production of broiler chickens have been studied (Lin *et al*., 2006). Gross and Siegel (1983) suggest that regular gentle handling can reduce flightiness and levels of aggression within flocks, improve growth, feed conversion ratio (FCR) and immune response. There is evidence that poultry are sensitive to visual contact with humans (Jones and Faure, 1981). Zulkifli *et al*. (2002) reported that visual contact test involving the experimenter standing in the centre of a pen (with no physical contact) for 10 min twice daily from 0-3 weeks reduced fear and stress reactions to handling and crating and improved immune response in broiler chickens. However, many authors did not observe significant improvement in growing performance of broilers subjected to similar treatment (Zulkifli *et al*., 2002). Jones and Faure (1981) indicated that allowing broiler chickens to observe other chickens being handled was as effective as physical handling in reducing fear of humans. Studies by Zulkifli *et al*. (2002) showed that regular visual contact with humans reduce underlying fearfulness in broiler chickens that were crated for 10 min. Handling of broilers prior to slaughter is a stressor and exposing chickens to an unfamiliar environment is likely to elicit both fear and stress reactions (Nicol and Scott, 1990; Kannan and Mench, 1997; Rushen *et al*., 1999). On the contrary, Keer *et al*. (1996) reported that the application of intuitively unpleasant handling regimen involving brief suspension by the legs reduced the fear of humans in broiler chicks. A method that has been used to determine fear and stress levels is to induce tonic immobility (Benoff and Siegel, 1976; Mills and Faure, 1991; Jones, 1992; Heiblum *et al*., 1998). Tonic immobility is an anti-predator behaviour shown in situations where the chicken has been caught by a predator. By pretending to be dead, there is a better chance to escape in an unguarded moment (Thompson and Liebreich, 1987). The duration of tonic immobility has been shown to correlate positively with fear and stress levels measured by the plasma corticosterone and heterophil to lymphocyte ratio (Lin *et al*., 2006; Zulkifli *et al*., 2003). Zulkifli *et al*. (2002) reported that the heterophil to lymphocyte response to a short duration stress peaked after 20 h. And it's known that the pre-slaughter process may result in heterophilia and lymphopenia in broiler chickens (Heterophil to lymphocyte ratio varies from 0.32-0.48 in case of transport) (Zulkifli and Azah, 2004). The immune response is integrated with other physiological systems and is sensitive to regulation of the brain. Visual human contact may result alterations in brain physiology and morphology (Gross and Siegel, 1983).

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The objective of this study was to investigate the effects of fear and stress reactions caused from visual human contacts such as routine management procedures, handling and treatments on immune response, body weight gain, feed conversion ratio, mortality, tonic immobility duration and heterophil to lymphocyte ratio in broilers.

MATERIALS AND METHODS

This study was carried out in the Poultry Unit of Faculty of Veterinary Medicine, Adnan Menderes University (ADU). All procedures used in the present experiment were approved by ADU Animal Experiments Local Ethics Committee. A total of 180, day-old commercial broiler chicks (Ross 308) were obtained from a local hatchery. On day 1, the chicks were individually weighted; wing-tagged and housed in groups of 60 in 3 rooms with deep litter of wood shavings. Ambient temperature was maintained between 22 and 24°C and a constant photo-period of 24 h was provided. To reduce visual contact with humans and other flocks during feeding and watering opaque partitions were used. The feed supply was changed from starter (3060 kcal ME/kg; 23% crude protein) to finisher pellet (3200 kcal ME/kg; 22% crude protein) at 14 days of age. Chicks were administered live and inactive Newcastle disease vaccine (Hipraviar ND Clone 30) via drinking water on day 7 and 21.

Commencing from day 1, the chickens were exposed visual contact with an experimenter as follows: Group-I visual contact for 5 min twice daily, Group-II visual contact for 5 min for three times daily and no visual contact (Control). During the visual contact procedure no attempt was made to have any physical contact with chickens. On day 37 the following stages were applied.

Stage I: To determine the effects of visual human contacts on TI durations and the number of inductions, 60 chickens (20 chickens from each group) were individually, gently removed with minimum disturbance to flock mates. After capturing the chickens were carried in an inverted manner and placed in plastic transport cages (0.8 m x 0.6 m x 0.3 m) at 10 chickens per cage for 10 min. Then, the chickens were recaptured individually and carried in an inverted manner to a separate room for Tonic Immobility Test (TI). TI induced as soon as the chickens were carried to the test room by restraining them on their right side and wings for 15 seconds (s). The experimenter retreated approximately 1 m away from the test table and waited without noise. A chronometer was used to record latencies until the chicken righted itself. If the chicken righted itself in < 10 s, it was recaptured and the procedure was repeated. If TI was not induced after 3 trials the TI duration was recorded as zero. The maximum duration of TI allowed was 600 s (Benoff and Siegel, 1976).

Stage II: To determine the effect of visual human contacts on HLRs a total of 60 chickens (20 chickens from each group) that were not used for TI were individually, gently removed and blood samples (0.5 ml) for heterophil and lymphocyte counts were taken to the tubes containing EDTA. Blood smears were prepared using May-Grünwald-Giemsa stain; heterophil and lymphocytes were counted to a total of 60 cells (Gross and Siegel, 1983).

Stage III: To determine the effects of caging on HLRs, remained 57 chickens (19 chickens from each group) were captured and caged for 10 min according to the same procedure. Following caging, the birds were returned to their own rooms. After 20 h [The HLR response to a short duration stress peaks after 20 h (Zulkifli *et al*., 2002)] the birds were recaptured gently and blood samples (0.5 ml) were collected immediately for determining heterophil and lymphocyte counts as described earlier.

Newcastle Disease (ND) antibody titres were analysed from blood by using commercial ELISA kit (BioCheck).

All the chickens were individually weighted on day 37. Total feed intake during trial was recorded and Feed Conversion Ratio (FCR) was calculated. Mortality was also recorded daily.

Kruskal-Wallis test was used to analyse the TI duration and Heterophil to Lymphocyte Ratio (HLR). The data for Newcastle disease antibody titres were normalised using logarithmic transformation prior to analyses. Data on percentage of mortality were analysed by Chi-Square test. A one-way analysis of variance was used to test the effects of treatment on body weight (Sümbüloglu and Sümbüloglu, 1993).

RESULTS

TI durations and the number of inductions in Control, Group-I and Group-II were found as 95.72, 85.61 and 77.85; 1.75, 1.65 and 1.44, respectively (Stage I) (Table 1). Chickens that have been exposed to 5 min visual human contact for two and three times daily showed slightly shorter durations of TI than did the controls. A numerical tendency for visual contact exposed to chickens to require fewer induction attempts before adopting TI than controls failed to reach statistically significance ($p>0.05$).

HLRs in Control, Group-I and Group-II for visual human contacts (Stage II) and caging (Stage III) experiments were determined as 0.41, 0.36 and 0.31; 0.51, 0.40 and 0.42, respectively (Table 2). While HLR was not affected by visual contact treatment at T_0 ($p>0.05$), following the caging procedure (20 h later) (T_{20}) ($p>0.05$) chickens showed greater HLR responses but failed to reach statistically significance. In other words, neither visual contact with human nor caging resulted in a significant elevation in HLR in broiler chickens.

Table 1: TI durations and number of inductions

	Control Mean±SEM n = 20	Group I Mean±SEM n = 20	Group II Mean±SEM n = 20	P
Duration (s)	95.72±12.67	85.61±10.14	77.85±10.04	>0.05
Induction number	1.75±0.18	1.65±0.17	1.44±0.12	>0.05

Table 2: The effects of visual human contacts and caging on HLRs

Mean±SEM	Visual human) contact (T_0)	Caging (T_{20})
Control	0.41±0.03 (n = 20)	0.51±0.01 (n = 19)
Group-I	0.36±0.02 (n = 20)	0.40±0.02 (n = 19)
Group-II	0.31±0.02 (n = 20)	0.42±0.03 (n = 19)
P	>0.05	>0.05

T_0: Blood samples taken immediately following the capture (Stage II), T_{20}: Blood samples taken 20 h after the caging (10 min), (Stage III)

Body weights at 37 days of age in Control, Group-I and Group-II were determined as 2084.10, 2107.68 and 2123.57 g, respectively (Table 3). Total feed intake and feed conversion ratio of these groups were calculated as 3402.61, 3405.80 and 3462.32 g; 1.63, 1.62 and 1.63, respectively (Table 3). This study showed that visual human contact has no significant effect on body weight, feed intake, FCR, mortality and immune response.

DISCUSSION

There is a growing body of evidence that chickens are sensitive to visual contact with humans and this may be more effective in reducing the levels of fear of humans than physical contact. Zulkifli and Azah (2004) reported that chickens exposed pleasant physical contact (handling) exhibited shorter TI duration (78 s) than their control counterparts (167 s). Marin *et al.* (2001) indicated that the heightened fear levels induced by a stressor persisted for at least one hour after the event and TI duration of these chickens were greater (1.85 inductions, 327.9 s TI duration) than control (2.60 inductions, 181.0 s TI duration) counterparts. The results of this study showed that there was a tendency for visual contact with humans for 5 min twice daily (Group-I; 1.65 inductions, 85.61 s TI duration) to require fewer inductions and shorter durations than (Group-II; 1.44 inductions, 77.85 s TI duration) 5 min for three times daily and controls (1.75 inductions, 95.72 s TI duration). In other words, chickens in Group-II were less fearful, as measured by TI, than their Group-I and Control counterparts. This finding was consistent with other studies but lack of significant differences between treatment groups can be attributed to differences in various factors such as time and duration of the visual contact (Zulkifli and Sti Nor Azah, 2004; Zulkifli *et al.*, 2002; Kannan and Mench, 1997; Jones, 1992; Bizeray *et al.*, 2002). Nicol (1992) reported that the broilers subjected to visual human contact have longer TI durations after transportation than non-subjected chickens.

In this study, chickens that had been exposed to visual contact with human have also lower HLR (0.36 and 0.31 for Group-I and Group-II, respectively.) despite the lack of statistically significance. Zulkifli *et al.* (2002) reported that simply allowing chickens to see a human being on a regular basis (without physical contact) reduced fear and stress reactions to handling and caging. Elevation of HLR following the transportation confirmed the findings of other authors that handling of chickens was stressful (Zulkifli *et al.*, 2002). However, it's not yet possible to identify what the factors that influence the effect may be. Because, these factors that can modify the relationship between visual contact and stress response need to be investigated (Rushen *et al.*, 1999). It was determined that visual contact with human has no significant effect on body weight, feed intake and FCR. Zulkifli and Azah (2004) reported that positive visual contact has no effect on growth performance in broilers. Regular visual contact is not as effective (FCRs were found as 1.89 and 1.94 for control and visual contact groups, respectively) as handling in improving growing performance of broilers (Zulkifli *et al.*, 2002). On the contrary, Gross and Siegel (1983) reported a significant improvement in weight gain and feed efficiency in broiler chickens. Hemsworth *et al.* (1994) indicated that feed conversion was poor at farms in which chickens avoided the human contact and this was accounted for 28% of the variance in feed conversion at the farms.

Table 3: The effects of visual human contact on some performance parameters and immune response

Parameters		Control Mean±SEM n = 59	Group I Mean±SEM n = 59	Group II Mean±SEM n = 59	P
Body weight (g)	Day 1	43.79±0.41	44.77±0.45	44.07±0.36	>0.05
	Day 37	2084.10±29.70	2107.68±28.11	2123.57±28.89	>0.05
Total feed intake (g) (1-37 days)		3402.61	3405.80	3462.32	
Feed Conversion Ratio (FCR)		1.63	1.62	1.63	
Mortality (%)*		1.67	1.67	1.67	>0.05
Immune response**		3.99±0.02	3.93±0.04	3.99±0.03	>0.05

*Chi-Square test was used for mortality. **Newcastle disease antibody titres (log_{10}) were measured by ELISA

However, the Newcastle antibody production (ND titres were as follows; 3.99, 3.93 and 3.99 for control, Group-I and Group-II, respectively) in this study indicated that visual human contact has no significant effect on immune response in broilers unlike the other visual contact studies (Zulkifli *et al*., 2002; Zulkifli and Azah, 2004). However, Zulkifli *et al*. (2002) reported that ND antibody titres were 3.24, 3.48 for control and visual contact group, respectively. This may be attributing to the lack of significant differences between TI duration and HLR.

Conclusion: The effects of humans on stress and fear reactions in broiler chickens can be reduced by making regular visual contacts despite lack of significant differences between groups in this study. Because, regular visual human contact a realistic procedure under intensive production conditions is only sufficient to reduce fear and stress response to catching and caging in broilers. On the other hand, visual contact has no significant effect on parameters such as body weight, mortality and immune response in broilers.

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