Effect of differently coloured clothes on fear and stress responses, some meat quality traits and performance in broilers

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Abstract

This study was conducted to investigate the effects of regular visual contact by the stockperson wearing clothing of different colour on fear and stress reactions, some meat quality traits and performance parameters in broiler chicks. A total of 192 one day-old male broiler chicks (Ross 308) were randomly assigned to four different colour clothing groups (red, blue, green, and gray), and two visual human contact groups (60 and 300 s durations) were designed. From day 15, the chickens were exposed to regular visual contacts with the stockperson, who wore different colour clothings every day. Tonic immobility (TI) test revealed that visual contact duration increase (300 s) has a positive effect on fear in broilers. The heterophil lymphocyte (H/L) ratio was the highest (0.89) in the green clothing group, but the lowest (0.45) in red clothing group (P<0.001). From meat quality traits in breast muscle (P<0.01) it was determined that clothing colour has statistically significant effects on lightness value. Final body weights were higher in blue coloured chicks (2593.82 g) than the green one (2348.42). The results demonstrate that regular visual contacts with the stockperson, who wore differently coloured clothes every day. The colours chosen differed from the colours usually worn by the stockpersons (red, blue, and green). However, studies showed that visual contact with its socialisation characteristics has significant effects on reducing the fear of human in Red chicks. It is very important to get more information about the relations between spectral traits of the impulse and the colour perception of birds. Indeed, birds have both single and double cones in their eyes. Double cones contain a 565 nm photopigment, while single ones may consist of four different spectral elements such as long (L; red), medium (M; green), short (S; blue) and very short/UV (VS/UV). For coloured visions in birds, the spectral sensitivities of the L, M and S single cones are narrowed by coloured oil droplets. There are lots of studies about the response of poultry to coloured food, feeders, drinkers, lights and stockperson’s clothes (Roper and Marples, 1997; Rozenboim et al., 1999a, 1999b, 2004; Lecuelle et al., 2011).

Introduction

Relations between human and animals are the undeniable part of modern intensive poultry productions. Commonly, there is no fear of humans in poultry (Murphy and Duncan, 1978), but during the routine rearing procedure poultry is repeatedly exposed to different kinds of stressors such as stockpersons, noise, and lighting (Waiblinger et al., 2006). Tonic immobility (TI) is commonly used to measure the level of fear in poultry (Jones and Faure, 1981). Tonic immobility is a typical anti-predator behaviour shown in situations where the chicken has been caught by a predator (Thompson and Liebreich, 1987). Tonic immobility is as effective as physical contact in reducing the fear of humans in poultry (Jones, 1993; Zulkifli and Siti Nor Azah, 2004). Zulkifli et al. (2002) stated that fear and stress reactions to handling broilers could be reduced by standing in the centre of a room (with no physical contact) for 10 min twice a day from 0 to 3 weeks. Eddy and Gallup (1994) also showed that visual contact with its socialisation characteristics has significant effects on reducing the fear of human in Red chicks. It is very important to get more information about the relations between spectral traits of the impulse and the colour perception of birds. Indeed, birds have both single and double cones in their eyes. Double cones contain a 565 nm photopigment, while single ones may consist of four different spectral elements such as long (L; red), medium (M; green), short (S; blue) and very short/UV (VS/UV). For coloured visions in birds, the spectral sensitivities of the L, M and S single cones are narrowed by coloured oil droplets. There are lots of studies about the response of poultry to coloured food, feeders, drinkers, lights and stockperson’s clothes (Roper and Marples, 1997; Rozenboim et al., 1999a, 1999b, 2004; Lecuelle et al., 2011).

Experimental birds and management

This study was conducted in the Poultry Unit of the Faculty of Veterinary Medicine, Adnan Menderes University, Turkey. A total of 192 male broilers (Ross 308) obtained from a commercial hatchery were used in the study. On day 1, the chicks were individually weighed, wing-tagged and housed in groups of 24 chicks in eight treatment pens with deep litter of wood shavings in an experimental barn with controlled light, heating, and hygienic and feeding patterns according to standard breeding requirements for broilers. The feed supply was changed from starter [3100 kcal metabolisable energy (ME)/kg; 22% crude protein] to finisher pellet (3250 kcal ME/kg; 21% crude protein) at 21 days of age. The room barn temperature was gradually decreased from 32±1°C on d 1 to 23±1°C on the last day of fattening (d 42). The relative humidity was varied from 50% to 60%.

Experimental design

Four different colour clothing groups (red, blue, green and gray) and two visual human contact groups (60 and 300 s durations) were designed as to 4×2 experimental design. Starting from day 15, chickens were exposed to regular visual human contact by the same stockperson, who wore differently coloured clothes every day. The colours chosen differed from the colours usually worn by the stockpersons (red, blue, and green). However, studies...
showed that livestock can distinguish these colours from a gray colour of similar luminosity (Rybarczyk et al., 2003). During the visual contact procedure, the stockperson wearing clothes of different colour entered slowly in the pens with the minimal noise and stood in the centre. No attempt was made to have any physical contact with chickens.

**Fear and stress measurements**

On day 41, 13 broilers of each pen (a total of 104 broilers) were randomly selected and TI tests were made. Placing a bird on its back induced TI. The bird was restrained for 10 s by maintaining a light pressure on its sternum. A stopwatch was started to record latencies until the bird righted itself. If the bird righted itself in lower than 10 s, the restraining procedure was repeated. If the bird did not show a righting response over the 10-min test period, a maximum score of 600 s was given (Jones and Faure, 1981). Tonic immobility test from the tact procedure, the stockperson wearing clot

**Meat quality measurements**

A total of 88 birds (11 birds from each pen, i.e. birds that were measured for blood samples) were slaughtered by exsanguination through a neck cut to determine meat quality traits at the age of 42 days. The pH values were determined after 15 min post mortem (initial pH, pH1) and 24 h after slaughter (ultimate pH, pH2), on the left Pectoralis major (breast) muscle of carcasses by using pH meter (Hanna Instrument HI 9124; Hanna Instruments, Woonsocket, RI, USA) with a penetration electrode (Hanna FC-200; Hanna Instruments). Muscle colour was measured at 24 h post mortem on the cranial medial surface (bone side) of breast muscle. A Minolta CR 400 chromameter (Konica Minolta Sensing Inc.; Tokyo, Japan) was used to assess the colour [lightness (L'), redness (a') and yellowness (b')] of breast muscle. Three pH and colour measurements were taken for each sample. The CL was determined with the traditional method (Honikel, 1998). Samples were cooked individually in heat-and-seal bags immersed in 75°C water with an internal temperature of 70°C. Water holding capacity was evaluated 24 h after slaughter, using the methodology described by Hamm (1960). The evaluation is based on measuring water loss when pressure is applied to the muscle (5 g sample of 2250 g in weight by 5 min exposing).

**Performance parameters**

All the birds were individually weighed on day 1, 14 and 42. Total feed consumption (pen basis) on day 14 and 42 was recorded. Feed conversion ratio (feed per gain) was calculated as the ratio of feed consumption to body weight gain. Mortality was also recorded daily.

**Statistical analysis**

SPSS version 15.0 was used for analysis and a general linear model was designed to reveal the effects of the colour of clothing, visual contact duration and their interaction on blood variables, TI duration and body weight. When a significant effect was noted (P<0.05), means were compared by Duncan’s multiple range test (Duncan, 1955). Kruskal-Wallis ANOVA was used for TI induction, feed consumption, and FCR data (Sümbüloğlu and Sümbüloğlu, 1993).

**Results and discussion**

Least square means of pH/L ratio, serum biochemical parameters, meat quality traits, and TI duration of broilers are summarised in Table 1. The induction numbers recorded for each chicken and visual contact had no significant effect

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**Table 1. Least square means for some stress parameters, meat quality traits, and tonic immobility duration in treatment groups.**

| Parameters                        | Red (n=22) | Blue (n=22) | Green (n=22) | Gray (n=22) | 60 s (n=44) | 300 s (n=44) | CCT     | VCT     | CCT×VCT |
|-----------------------------------|------------|------------|-------------|------------|-------------|-------------|---------|---------|---------|
| Haematological                    |            |            |             |            |             |             |         |         |         |
| H/L ratio                         | 0.45a      | 0.47a      | 0.89a       | 0.56a      | 0.61        | 0.57        | 0.03    | 9.725***| 0.268 (ns) | 1.654 (ns) |
| Biochemical                       |            |            |             |            |             |             |         |         |         |
| Glucose, mmol/L                   | 11.49      | 11.09      | 10.65       | 11.21      | 11.05       | 11.47       | 0.25    | 0.825 (ns) | 0.687 (ns) | 0.352 (ns) |
| Cholesterol, mmol/L               | 4.94       | 4.85       | 4.26        | 4.01       | 4.52        | 4.51        | 0.14    | 2.436 (ns) | 0.000 (ns) | 0.553 (ns) |
| Triglyceride, mmol/L              | 0.56       | 0.65       | 0.65        | 0.66       | 0.63        | 0.63        | 0.03    | 0.679 (ns) | 0.002 (ns) | 3.806*    |
| Total protein, g/L                | 38.55      | 37.34      | 37.71       | 41.13      | 39.91       | 37.45       | 0.68    | 1.472 (ns) | 3.254 (ns) | 0.595 (ns) |
| LDH, U/L                          | 625.41     | 551.58     | 557.20      | 686.15     | 634.86      | 575.30      | 20.01   | 2.413 (ns) | 2.216 (ns) | 3.180*    |
| Meat quality traits               |            |            |             |            |             |             |         |         |         |
| pH1                               | 6.33       | 6.36       | 6.38        | 6.37       | 6.39        | 6.32        | 0.02    | 0.295 (ns) | 3.390 (ns) | 0.911 (ns) |
| pH2                               | 5.88       | 5.83       | 5.83        | 5.81       | 5.83        | 5.84        | 0.02    | 1.151 (ns) | 0.068 (ns) | 1.889 (ns) |
| L*                                | 52.88a      | 54.67b      | 50.23c      | 51.97d      | 52.94       | 51.94       | 0.41    | 5.089**   | 1.467 (ns) | 0.687 (ns) |
| a*                                | 2.36       | 2.19       | 2.42        | 2.33       | 2.16        | 2.49        | 0.09    | 0.307 (ns) | 3.706 (ns) | 0.997 (ns) |
| b*                                | 2.44       | 3.29       | 2.09        | 2.39       | 2.62        | 2.46        | 0.19    | 1.795 (ns) | 0.195 (ns) | 0.947 (ns) |
| CL, %                             | 27.12      | 29.78      | 29.37       | 29.93      | 28.82       | 27.79       | 0.65    | 1.295 (ns) | 0.628 (ns) | 0.313 (ns) |
| WHC, %                            | 11.64      | 11.84      | 11.23       | 11.80      | 11.53       | 11.73       | 0.27    | 0.277 (ns) | 0.134 (ns) | 0.577 (ns) |
| TI duration, s                    | 242.81     | 198.32     | 267.04      | 234.35     | 244.10      | 227.16      | 17.99   | 0.641 (ns) | 0.222 (ns) | 0.735 (ns) |

CTT, clothing colours treatment; VCT, visual contact treatment; H/L, heterophil lymphocyte; LDH, lactate dehydrogenase; CL, cooking loss; WHC, water holding capacity; TI, tonic immobility. *Means with different superscript letters in the same row are significantly different (P<0.05). ns, not significant; *P<0.05; **P<0.01; ***P<0.001.
on susceptibility to TI. Despite the lack of statistical differences between visual contact groups, it was determined that TI duration (the level of fear) decreased to 227.16 s from 244.10 s as parallel to an increase in visual contact durations by human. This finding was found to be consistent with other studies (Eddy and Gallup, 1994; Jones 1993, 1995; Zuilkifi et al., 2002; Zuilkifi and Sti Nor Azah, 2004; Türkyılmaz et al., 2010), and it was thought that the statistical difference here may have been caused by factors such as visual contact duration and time of treatment. It was determined that regular visual contact with stockperson for 300 s had led to a decrease in H/L ratio, but this has not reached statistically significance. Similarly, in the studies carried out in broilers, the effect of visual human contact on H/L ratio was found to be statistically non-significant (Zulkifli et al., 2002; Türkyılmaz et al., 2010). On the other hand, in some studies it was determined that regular visual human contact lead to a decrease in stress reaction in broilers (Hemsworth et al., 1994; Zuilkifi and Sti Nor Azah, 2004; Waiblinger et al., 2009). It is known that basal glucose and cholesterol levels in broilers vary from 11.10 to 13.88 mmol/L and from 3.24 to 5.18 mmol/L, respectively (Karagül et al., 2000). In this context, it was thought that serum glucose (11.05 to 11.69 mmol/L) and cholesterol (4.01 to 4.94 mmol/L) were in the range of basal limits. Visual contact was determined not to have statistically significant effects on live weight gain, feed consumption, FCR, and mortality.

Chickens that have been exposed to green stockperson’s clothing colour for once a day showed slightly longer durations of TI than the others (P>0.05). The number of TI inductions in red, blue, green and gray colour groups were found as 1.31, 1.07, 1.15 and 1.15, respectively (P>0.05). However, there are limited numbers of studies about the effects of the colour of the clothing on fear response in broilers. Jones (1995) indicated that the different in the colour of clothing caused to little effect on the approach or avoidance responses of regularly handled chicks. Jones (1987) reported that both young chicks and chickens showed longer TI durations in case of different clothing colours than normally worn. Tallet et al. (2005) stated that some factors such as the colour of clothing, facial features, and spectacles have important role in the definition of the environment in ungulates. Heterophil lymphocyte ratios of 0.2, 0.5, and 0.8 are important indicators for low, medium, and chronic stress (Siegel and Gross, 2000). It can be said that the lowest stress level (0.45) was determined in red coloured (long wavelength of maximum absorption at 565-570 nm) clothing group. Lu et al. (2007), Barbut (2009) and Wang et al. (2009) reported that the percentage of the decrease in pH and the latest pH value were two important factors indicating the quality of meat after slaughter. Fernandez et al. (1994) reported that 24 h after the slaughter, meats with pH<5.7 were accepted as low quality. Van Laack et al. (2006) reported that pale and normal breast meat pH values were 5.70 and 5.96, respectively. It was determined that average pH values in all colour groups varied in acceptable range (from 5.81 to 5.88). Lightness value was found between 50.23 and 54.67 with 52.44 as the mean. As parallel to this result of this experiment suggest that regular

| Parameters          | CCT                  | VCT                  | Pooled SED                     | F value and significancy |
|---------------------|----------------------|----------------------|--------------------------------|--------------------------|
| Body weight, g      |                      |                      |                                |                          |
| Day 1 (n=22)        | 47.10                | 48.35                | 46.67                          | 47.05                    | 47.34                    | 47.24                    | 0.29                     | 1.593 (ns)               |
| Day 14 (n=22)       | 540.88               | 548.49               | 540.84                         | 556.17                   | 555.38                   | 542.92                   | 3.19                     | 2.277 (ns)               |
| Day 42 (n=44)       | 2514.13              | 2593.82              | 2348.42                        | 2548.15                  | 2543.48                  | 2458.77                  | 22.74                    | 5.528***                 |

CCT, clothing colours treatment; VCT, visual contact treatment. * Means with different superscript letters in the same row are significantly different (P<0.05). ns, not significant. ***P<0.001.

Conclusions

To conclude, the effects of humans on fear and stress responses in broilers can be reduced by making regular visual contacts despite lack of significant differences between groups in this study. The regular visual human contact is a more realistic procedure under intensive commercial production conditions than handling. Moreover, this study has shown that visual contact of stockpersons wearing red, blue and grey coloured clothes was less stressful to broilers than green. L* value of broiler breast meat was found to be a result of stress in green colour clothing group. It was concluded that visual contact by people wearing red, blue and grey coloured clothes was effective to increase body weight in broilers. There is little indication that FCR can be improved by stockpersons colour clothing. The results of this experiment suggest that regular
visual contact by humans wearing red, blue and gray exposing broilers to non-stress eye contact at close proximity may improve the body weight of commercial broiler farms. Further studies should be arranged to investigate the aspects of the relations between humans and chickens based on visual contact.

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