INTRODUCTION

The quality of human-animal interaction can have a profound impact on many facets of an animal’s physiology and behavior. Regular gentle handling of chicks has been shown to reduce aggression within flocks (Collins and Siegel, 1987; Zulkifli, 2008); improve growth, feeding efficiency, and egg production (Barnett et al., 1994; Zulkifli et al., 2002b; Zulkifli and Siti Nor Azah, 2004); and dampen physiological stress reactions (Zulkifli et al., 2002b; Zulkifli and Siti Nor Azah, 2004). Similarly, several handling studies implicate stress in the deleterious effects of negative handling on animal welfare and productivity. Observations in the pig industry indicated that regular negative tactile interaction can result in high levels of fear of humans. Hemsworth and Gonyou (1997) indicated that pigs exhibited marked avoidance of humans following imposition of daily negative interactions as little as 15 to 30 s. Conversely, however, it has been reported that regular aversive handling failed to affect growth in young pigs (Paterson and Pearce, 1989, 1992; Pearce et al., 1989). In chickens, Jones (1993) reported that application of an intuitively unpleasant handling regimen can reduce underlying fearfulness, but Zulkifli and Siti Nor Azah (2004) reported otherwise. A better understanding of the nature of handling procedures and their effect on fear and stress reactions could be useful in explaining these contradicting results.

Changes in heat shock protein 70, blood parameters, and fear-related behavior in broiler chickens as affected by pleasant and unpleasant human contact

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ABSTRACT An experiment was conducted to determine the effects of combining both pleasant and unpleasant contacts with human beings on physiology and behavior of broiler chickens. Birds were subjected to the following treatments: (i) received no physical or visual contact with humans (control); (ii) from d 1 to 28, chicks were individually stroked gently for 30 s once daily (PL); (iii) from d 1 to 28, chicks were picked up individually, suspended by both legs, exposed to recorded noise, and swung gently for 15 s once daily (UNPL); (iv) from d 1 to 14 and from d 15 to 28, chicks were subjected to PL and UNPL, respectively (PL-UNPL); and (v) from d 1 to 14 and from d 15 to 28, chicks were subjected to UNPL and PL, respectively (UNPL-PL). On d 42, birds from each treatment group were road-transported for 3 h. Heat shock protein (hsp) 70 expression, plasma levels of corticosterone, serum creatine kinase concentration, heterophil/lymphocyte ratios (HLR), and tonic immobility duration were determined pre- and posttransit. There were significant ($P < 0.05$) duration of transportation × human contact treatment interactions for HLR and hsp 70 density. Following transit, the PL chicks had significantly ($P < 0.05$) lower HLR and greater hsp 70 density than the other groups. The corticosterone of PL and UNPL chicks were lower than their control, PL-UNPL, and UNPL-PL counterparts. The PL and PL-UNPL treatments were effective in shortening tonic immobility duration significantly ($P < 0.05$). Except for UNPL-PL, the serum creatine kinase activity of PL was significantly lower than the other groups. In conclusion, subjecting birds to pleasant human contact reduced stress and fear reactions to transportation by enhancing the ability to express hsp 70 in the brain. Unpleasant human contact had adverse effect on the birds’ response to transportation. Early age pleasant experience with humans failed to negate the adverse effects of subsequent unpleasant contact.

Key words: human contact, heat shock protein 70, stress, fear, broiler chicken

2013 Poultry Science 92:33–40
http://dx.doi.org/10.3382/ps.2012-02446

INTRODUCTION

The quality of human-animal interaction can have a profound impact on many facets of an animal’s physiology and behavior. Regular gentle handling of chicks has been shown to reduce aggression within flocks (Collins and Siegel, 1987; Zulkifli, 2008); improve growth, feeding efficiency, and egg production (Barnett et al., 1994; Zulkifli et al., 2002b; Zulkifli and Siti Nor Azah, 2004); and dampen physiological stress reactions (Zulkifli et al., 2002b; Zulkifli and Siti Nor Azah, 2004). Similarly,
In general, farm animals are particularly sensitive to human stimulation that occurs early in life, while many systems of the chicks are still developing. This may have long-lasting impact and could possibly modify their genetic potential. Zulkifli et al. (2002b) reported that regular visual contact seems to be less effective in altering the physiology and behavior of chickens when evoked after the first 3 wk of life. Studies in pigs suggested that early handling during the first 8 wk of life increased the approach behavior of pigs to the experimenter in a standard test from 10 to 24 wk of age (Hemsworth et al., 1986). It is not clear whether the quality of human contact experienced by chicks during an early age can be modified by subsequent pleasant or unpleasant interaction with human beings. This is critical because under commercial settings it is likely that there will be variation both between and within stockpersons in their behavior toward chickens.

The question as to how human contact can have a profound impact on stress responsiveness at a molecular level has yet to be answered. Recent studies have demonstrated that improved tolerance to transportation stress in chickens could be attributed to enhanced heat shock protein \( (hsp) \) 70 response. Heat shock proteins are a group of highly conserved proteins rapidly synthesizing in response to a wide range of stressors such as feed restriction, social isolation, crating, and transportation (Al-Aqil and Zulkifli, 2009; Soleimani et al., 2011, 2012). The expression of hsp 70 in response to stress serves to protect against the initial insult, augment recovery, and produce a state of resistance to subsequent stresses (Kregel, 2002). Because positive human contact may attenuate physiological stress responses, it was our hypothesis that the practice would improve hsp 70 expression. The objective of the present study was to determine the effects of combining both pleasant and unpleasant experiences with human being on hsp 70 expression, circulating levels of corticosterone, heterophil/lymphocyte ratios \( (HLR) \), serum creatine kinase \( (CK) \) activity, and tonic immobility \( (TI) \) duration in broiler chickens.

**MATERIALS AND METHODS**

**Birds, Housing, and Diet**

The study was undertaken following the guidelines of the Research Policy on animal ethics of the Universiti Putra Malaysia. A total of 750 one-day-old female broiler chicks (Cobb × Cobb) were obtained from a local commercial hatchery. On d 1, the chicks were wing-banded, individually weighed, and housed in groups of 25 in 30 floor pens with wood shavings deep litter and cyclic temperatures (minimum, 24°C; maximum, 34°C). The RH was between 80 and 90%. The area of each pen was 10.42 m². The birds were fed a standard broiler starter crumble (2,950 kcal of ME/kg; 21% CP) and finisher pellet (3,100 kcal of ME/kg; 19.5% CP) from d 1 to 20 and d 21 to 42, respectively. Feed and water were provided ad libitum. Chicks were vaccinated against Newcastle disease via drinking water on d 7 and 21.

**Experimental Procedures**

Commencing from d 1, an equal number of chicks (6 pens per treatment) was randomly assigned to one of the following treatments. First, from d 1 to 28, non-handled controls \( (n = 150) \) received no physical or visual contact with humans other than the routine husbandry (control). Second, from d 1 to 28, chicks \( (n = 150) \) in the pleasant contact group were caught with both hands, held in an upright position, placed in plastic crates, and moved to a separate room. The chicks were picked up individually and stroked gently for 30 s once daily. Following the human contact procedure, the birds were returned to their home pens (PL). Third, from d 1 to 28, chicks \( (n = 150) \) in the unpleasant contact group were caught with both hands, held in an inverted position, placed in plastic crates, and moved to a separate room. Subsequently, chicks were picked up individually, suspended by both legs, exposed to recorded noise (alarm clock plus car engine noise), and swung gently for 15 s once daily \( (UNPL) \). To broadcast the sounds (97 dB), a compact stereo component system with 2 speakers was used. Following to the human contact procedures, the birds were returned to their home pens. Fourth, from d 1 to 14, the chicks \( (n = 150) \) in the pleasant-unpleasant contact group were subjected to the pleasant human contact procedure described earlier. Subsequently from d 15 to d 28, the birds were subjected to the unpleasant human contact procedure described earlier \( (PL-UNPL) \). Fifth, from d 1 to 14, the chicks \( (n = 150) \) in the unpleasant-pleasant contact group were subjected to the unpleasant human contact procedure described earlier \( (UNPL-PL) \). The same experimenter who always wore a white laboratory coat conducted the entire human contact procedure. The PL and UNPL procedures were adapted from Jones (1993), Zulkifli and Siti Nor Azah (2004), and Campo et al. (2005).

**Road Transportation**

On d 42, at 0800 h, 10 birds from each pen were randomly selected and placed in plastic crates \( (0.80 \times 0.60 \times 0.31 \text{ m}) \) at 9 birds to each crate. The crates were loaded to an open truck and transported for 3 h at an average speed of 80 km/h. The journey covered highways, roads with heavy traffic, and traffic lights. At the time of transportation, the ambient temperature was 33 to 35°C.
Blood and Brain Samples

Prior to (0 h) and following 3 h of transit, 10 birds from each human contact treatment group were randomly selected and blood samples (3 mL) were obtained via the wing vein for plasma corticosterone concentration (CORT) assay, heterophil and lymphocyte counts (EDTA, anticoagulant), and serum levels of CK. Each bird was caught and sampled, one immediately after another. Time elapsed from catching to obtaining blood sample was less than 50 s. This procedure should not influence circulating levels of corticosterone (Craig, 1985; Lagadic et al., 1990; Romero and Reed, 2005). Blood samples for hormone assay were centrifuged and stored at −20°C until assayed. The CORT was measured by radioimmunoassay using the ImmuChem Double Antibody 125/I-RIA kit (M P Biomedical, Irvine, CA). Blood smears were prepared using May-Grunwald-Giemsa stain, and heterophils and lymphocytes were counted to a total of 60 cells (Gross and Siegel, 1983). Analysis for CK was conducted on an automated spectrophotometer (Ultraspec 300, Cobas-Mira, Roche Diagnostic System, Basel, Switzerland) using a standard diagnostic kit. Immediately after blood collection, 5 birds (those that were used for blood sampling) from each treatment group were randomly chosen, killed by cervical dislocation, and the entire brain samples were removed, frozen quickly in liquid nitrogen, and stored at −70°C until further analysis for hsp 70 density (Zulkifli et al., 2002a).

SDS-PAGE and Immunoblot Analysis

Brain samples (0.5 g) were homogenized in an Ultra-Turrax homogenizer, using 5 mL of chilled Tris-HCl buffer (20 mM Tris pH 7.5, 0.75 M NaCl, 2 mM 2-mercaptoethanol) and centrifuged at 23,000 × g for 30 min at 4°C. The protein concentration of the supernatants were quantified by the Bicinchoninic Acid Protein Assay Kit Procedure No. TRPO-562 (Sigma Chemical Co., St. Louis, MO) with bovine serum albumin as the standard. Thirty micrograms of total protein were loaded and separated on 1.5 × 80 × 100 mm 12% polyacrylamide gels containing SDS (Laemmli, 1970) using the Hoefer Mini Gel apparatus. Gels were electrophoresed at 150 V until the tracking dye reached the base of the gel. The fractionated proteins were visualized by Coomassie Blue staining or transferred to polyvinylidene difluoride (PVDF) membranes (MSI, Westborough, MA; Towbin et al., 1979). After electrophoretic transfer, the PVDF membranes were stained with 0.5 g/L of Ponceau S in 10 g/L of acetic acid solution to visualize and mark the positions of the proteins used as molecular weight standards. After washing the Ponceau S with distilled water, the nonspecific binding sites were blocked using 10 mL of cold blocking buffer containing 10% nonfat milk and 0.05% sodium azide for 30 min. The membranes were incubated overnight (4°C) with 5 mL of blocking buffer containing antiserum (mouse anti-chicken hsp 70; Sigma Chemical Co., St. Louis, MO) against hsp 70 in a 1:1,000 dilution. Following overnight incubation, the blots were washed 4 times (5 min each) with 10 mL of cold blocking buffer. The blots were then reacted with goat anti-mouse secondary antibody conjugated to alkaline phosphatase (Sigma Chemical Co.) for 1 h. After rinsing with cold PBS, the color reaction on the PVDF membrane was developed using commercially prepared BCIP/NBT (Sigma Chemical Co.). Relative density of the hsp 70 was determined using a densitometer (UVP, Cambridge, UK) with UVP Gel Base Pro program.

TI Test

Prior to transportation (0 h) and immediately following transit, 15 birds (those that were not blood sampled) from each human contact treatment group were chosen at random and individually tested for duration of TI in a separate room (no visual contact with other birds). Birds were carried by both legs in an inverted manner to the room. A modification of the procedure described by Benoff and Siegel (1976) was used. Tonic immobility was induced as soon as the birds were carried to a separate room by gently restraining them on their right side and wings for 15 s. The experimenter then retreated approximately 1 m and remained within the sight of the bird but made no unnecessary noise or movement. Direct eye contact between the observer and the bird was avoided because it may prolong TI duration (Jones, 1986). A stopwatch was started to record latencies until the bird righted itself. If the bird righted itself in less than 10 s, it was recaptured and the restraining procedure was repeated. If TI was not induced after 3 attempts, the duration of TI was considered 0 s. The maximum duration of TI allowed was 600 s.

Statistical Analyses

Data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute Inc., 1991). All data were analyzed using human contact, duration of transportation, and their interactions as main effects. When interactions between main effects were significant, comparisons were made within each experimental variable. When significant effects were found, comparisons among multiple means were modeled by Duncan’s multiple range test.

RESULTS

HLR

There was an interaction (P = 0.0051) between the duration of transportation and human contact treatment for HLR (Table 1). The interaction was observed because effect of human contact treatment was only noted after 3 h of transportation. The PL birds had...
lower \((P < 0.05)\) HLR compared with other groups following 3 h of transportation. The HLR of all birds were not different \((P = 0.9722)\) before transportation. Three hours of transit increased \((control, P < 0.0001; UNPL, P < 0.0001; PL-UNPL, P = 0.0410; UNPL-PL, < 0.0001; PL-UNPL, P < 0.0001)\) the HLR of all groups except PL \((P = 0.1644)\).

**hsp 70**

There was a duration of transportation \(\times\) human contact treatment interaction \((P = 0.0007)\) for hsp 70 (Table 1). Prior to transportation, the mean hsp 70 densities of PL birds were not different \((P > 0.05)\) from the other groups. However, following 3 h of transportation, the PL birds showed greater \((P < 0.05)\) hsp 70 expression than their control, UNPL, PL-UNPL, and UNPL-PL counterparts. Irrespective of human contact treatment, 3 h of transportation increased \((control, P = 0.0006; PL, P < 0.0001; UNPL, P = 0.0001; PL-UNPL, P < 0.0001; UNPL-PL, P < 0.0001)\) hsp 70 expression of all the chickens.

**CORT Concentrations**

There was no duration of transportation \(\times\) human contact treatment interactions for CORT \((P > 0.05)\). Irrespective of human contact treatment, transportation of birds for 3 h elevated CORT \((P < 0.0001; Table 2)\). Human contact had a significant \((P < 0.0001)\) effect on CORT response to transportation. The PL and UNPL birds had lower \((P < 0.05)\) CORT than those of control, PL-UNPL, and UNPL-PL birds.

**TI Test**

There was no duration of transportation by the human contact treatment interactions for TI duration \((P = 0.717)\) and number of attempts to induce TI \((P = 0.2631; Table 3)\). Birds exhibited longer \((P = 0.0169)\) TI durations after transportation. The TI reactions were shorter \((P < 0.05)\) among PL and PL-UNPL birds than their UNPL and UNPL-PL counterparts. Number of inductions to induce TI was affected by human contact treatment \((P = 0.0304)\) but not transportation \((P = 0.313)\). The UNPL birds were more susceptible \((P < 0.05)\) to TI compared with the control and UNPL-PL birds.

**Serum CK**

Duration of transportation had no significant \((P = 0.1275)\) effect on CK levels (Table 4). However, serum CK response was influenced \((P = 0.0348)\) by human contact treatment. Irrespective of duration of transportation, levels of CK was lower \((P < 0.05)\) in PL than control, PL-UNPL, and UNPL counterparts.

**DISCUSSION**

The noted elevations in HLR and CORT following transportation are expected. Confirming earlier studies that broiler chickens subjected to transportation showed marked physiological changes indicative of acute stress (Mitchell et al., 1990; Zulkifli et al., 2001, 2003). There were significant human contact treatment \(\times\) duration of transportation interactions for HLR, TI duration, and hsp 70 density, suggesting that the response of chickens to the stress of transportation was

### Table 1. Mean (±SEM) heterophil/lymphocyte ratios Nicol, C. J. (HLR) and heat shock protein (hsp) 70 densities where duration of transportation \(\times\) human contact treatment interactions were significant

<table>
<thead>
<tr>
<th>Item1</th>
<th>0 h</th>
<th>3 h</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.35 ± 0.04&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.77 ± 0.05&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.56 ± 0.06&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PL</td>
<td>0.40 ± 0.05</td>
<td>0.49 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.44 ± 0.03&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>UNPL</td>
<td>0.45 ± 0.06&lt;sup&gt;y&lt;/sup&gt;</td>
<td>1.06 ± 0.09&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.74 ± 0.09&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>PL-UNPL</td>
<td>0.54 ± 0.45&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.81 ± 0.12&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.67 ± 0.07&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>UNPL-PL</td>
<td>0.39 ± 0.03&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.72 ± 0.09&lt;sup&gt;y&lt;/sup&gt;&lt;&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.55 ± 0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean</td>
<td>0.43 ± 0.02&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.76 ± 0.04&lt;sup&gt;x&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Means within a column-subgroup with no common letters differ at \(P < 0.05\).

<sup>y</sup>Means within a row-subgroup with no common letters differ at \(P < 0.05\).

<sup>1</sup>PL: pleasant contact from d 1 to 14; UNPL: unpleasant contact from d 1 to 14; PL-UNPL: pleasant contact from d 1 to 14 and unpleasant contact from d 15 to 28; UNPL-PL: unpleasant contact from d 1 to 14 and pleasant contact from d 15 to 28.

### Table 2. Mean (±SEM) plasma corticosterone (CORT) concentrations (ng/mL) by duration of transportation and human contact treatment in broiler chickens

<table>
<thead>
<tr>
<th>Item</th>
<th>CORT (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>2.26 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 h</td>
<td>5.70 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a column with no common letters differ at \(P < 0.05\).

<sup>1</sup>PL: pleasant contact from d 1 to 14; UNPL: unpleasant contact from d 1 to 14; PL-UNPL: pleasant contact from d 1 to 14 and unpleasant contact from d 15 to 28; UNPL-PL: unpleasant contact from d 1 to 14 and pleasant contact from d 15 to 28.
influenced by their experience with human beings. The results of this study, as measured by both HLR and CORT, support the hypothesis that positive handling treatment reduces chickens’ reaction to stressors. Thus, pleasant human contact may improve the ability of birds to cope with the stress of road transportation. In the present study, the HLR of UNPL birds were significantly higher than the other groups following transportation. On the contrary, the CORT of both PL and UNPL birds were not significantly different, suggesting that the latter can also dampen response to transportation stress. There is no clear explanation to the contradicting findings. Zulkifli and Siti Nor Azah (2004) found that both pleasant and unpleasant contacts with human beings dampened HLR reaction to the stress of translocation. Work on rodents suggested that early age intuitively traumatic treatments such as stroking, slapping, pinching, tossing in the air, suspension by the tail, partial starvation, and electric shocks improved learning, reduced emotionality, and susceptibility to stress (Jones, 1996). In pigs, however, there is considerable evidence that unpleasant handling treatments can produce chronic stress response (Hemsworth et al., 1981, 1987). The effectiveness of HLR and CORT as biological indicators of stress response may also have accounted for the discrepancies. The CORT is considered as an effective biological indicator of acute stress response, whereas HLR has been suggested to be a reliable bioassay for chronic stress (Siegel, 1995). Gross and Siegel (1983) compared leukocytic and hormonal responses to environmental insults and exogenous corticosterone. They concluded that HLR was a more reliable indicator of the perceived magnitude of stress than CORT in avian species.

Hemsworth et al. (1987) subjected pigs to a combination of pleasant and unpleasant treatments (imposed at a ratio of 1:5) and found that the procedure has resulted in a chronic stress response with consequent adverse effects on growth performance. The present study was designed to evaluate whether early age (d 1 to 14) experience with human beings can modify the response to subsequent human contact. Both PL-UNPL and UNPL-PL failed to attenuate HLR and CORT reactions following transportation. Based on the HLR and CORT, birds subjected to PL-UNPL were more stressed following transportation compared with their PL counterparts. Thus, it appears that the benefits of early age positive human contact, which was reported to have a long-term impact (Zulkifli et al., 2002b; Zulkifli and Siti Nor Azah, 2004), can be modified by subsequent unpleasant experience with human beings. These findings have important implications for large poultry operations where birds are likely to be handled by several stockpersons. It is likely that aversive handling by one stockperson, even though others are treating the birds pleasantly, may heighten stress responses.

It is interesting to note that the UNPL birds had lower CORT than those of PL-UNPL or UNPL-PL. The higher CORT of PL-UNPL chicks could be associated with hope-disappointment syndrome (Harvey et al., 1983). Birds that have experienced positive human earlier contact may perceive the presence of human beings with experience with humans other than the routine husbandry; PL: pleasant contact from d 1 to 14; UNPL: unpleasant contact from d 1 to 14; PL-UNPL: pleasant contact from d 1 to 14 and unpleasant contact from d 15 to 28; UNPL-PL: unpleasant contact from d 1 to 14 and pleasant contact from d 15 to 28.

### Table 3. Mean (±SEM) tonic immobility (TI) durations (s) and number of attempts to induce TI by duration of transportation and human contact treatment in broiler chickens

<table>
<thead>
<tr>
<th>Item</th>
<th>Duration (s)</th>
<th>Induction attempts (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of transportation (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>124.35 ± 11.60b</td>
<td>1.50 ± 0.08</td>
</tr>
<tr>
<td>3</td>
<td>174.23 ± 17.08a</td>
<td>1.41 ± 0.06</td>
</tr>
<tr>
<td>Human contact treatment1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>164.67 ± 21.82a</td>
<td>1.30 ± 0.11b</td>
</tr>
<tr>
<td>PL</td>
<td>84.83 ± 12.20b</td>
<td>1.45 ± 0.13b</td>
</tr>
<tr>
<td>UNPL</td>
<td>182.87 ± 18.03a</td>
<td>1.50 ± 0.11b</td>
</tr>
<tr>
<td>PL-UNPL</td>
<td>101.73 ± 14.31b</td>
<td>1.75 ± 0.11b</td>
</tr>
<tr>
<td>UNPL-PL</td>
<td>220.50 ± 37.49a</td>
<td>1.27 ± 0.09b</td>
</tr>
</tbody>
</table>

a,bMeans with no common letters differ at P < 0.05.

### Table 4. Mean (±SEM) serum levels of creatine kinase (CK; IU/L) by duration of transportation and human contact treatment in broiler chickens

<table>
<thead>
<tr>
<th>Item</th>
<th>CK (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of transportation (h)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>520.13 ± 251.36</td>
</tr>
<tr>
<td>3</td>
<td>6,050.42 ± 300.78</td>
</tr>
<tr>
<td>Human contact treatment1</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6,380.41 ± 370.98a</td>
</tr>
<tr>
<td>PL</td>
<td>4,963.19 ± 472.46b</td>
</tr>
<tr>
<td>UNPL</td>
<td>5,026.76 ± 398.03a</td>
</tr>
<tr>
<td>PL-UNPL</td>
<td>6,322.31 ± 410.72a</td>
</tr>
<tr>
<td>UNPL-PL</td>
<td>6,086.90 ± 409.86ab</td>
</tr>
</tbody>
</table>

a,bMeans with no common letters differ at P < 0.05.

### References
- Zulkifli and Siti Nor Azah (2004)
- Jones, 1996
- Hemsworth et al., 1981, 1987
- Siegel, 1995
- Gross and Siegel (1983)
- Harvey et al., 1983
ings as a signal for continuous pleasant treatment. The subsequent exposure to unpleasant human contact may result in disappointment with consequent elicitation of the stress responses. The increase in CORT among the UNPL-PL birds suggests that although the birds were subjected to a more pleasant human contact from d 15 to 28, the change from an unpleasant treatment, to which the birds may have habituated, could be stressful. Zulkifli et al. (1993) reported that releasing chicks from a more severe feed restriction to a less severe practice elevated HLR. This phenomenon could be linked with exposure to novelty and uncertainty that involves underlying psychological process (Levine, 2000).

The results of the pleasant contact treatment on fear reaction in the present study were similar to those found in previous experiments (Barnett et al., 1992; Jones and Waddington, 1993). There is considerable report to suggest that regular positive human contact is a powerful and reliable method of reducing the birds’ specific fear of humans, presumably through a process of habituation (Jones, 1996). For example, handling treatment reduced the incidence of orientation away from the experimenter and reduced the withdrawal from the experimenter in several standard tests measuring the avoidance of humans. Jones (1996) suggested that regular human contact exerted its effect by specifically reducing the birds’ fear of humans rather than through any effect on their nonspecific underlying fearfulness. In the present study, irrespective of duration of transportation (0 vs. 3 h), the PL birds were less fearful than controls. Although crating and transportation involves handling by human beings, birds are also exposed to an array of traumatic events such as feed withdrawal, noise, vibration, thermal extremes, social disruption, crowding, and restriction of movement. It appears that PL may attenuate nonspecific underlying fearfulness. Similarly, Zulkifli et al. (2002b) showed that regular visual contact with human beings attenuated TI reaction to crating which involved exposure to novelty and restriction of movement.

Unlike in pigs, there is little information on the consequences of negative interactions with human beings in poultry. Jones (1993) and Zulkifli and Siti Nor Azah (2004) showed that application of an intuitively unpleasant handling regimen in chickens can either reduce or have a negligible effect on underlying fearfulness. In the present study, however, the UNPL chicks exhibited longer TI durations than those of PL. The discrepancies could stem from the differences in the protocol of human contact. Nicol (1992) reported that different handling treatments may have different effects on fearfulness in chickens. The author indicated that birds that were stroked avoided the experimenter in the home pen and showed no attenuation of their TI response after transportation. Birds which were picked up showed less avoidance of the experimenter in the home pen and did have an attenuated TI response after transportation. Birds which were stroked while they were fed from an open hand actively approached the experimenter in the home pen but, unexpectedly, showed no attenuation of their TI response after transportation. In the present study, the birds were not only handled in an unpleasant manner but were also exposed to noise. Although the TI durations of UNPL-PL birds were longer than PL, the PL-UNPL chicks and the latter were not significantly different. There is no clear explanation for the phenomenon.

It is well documented that exposure of cells and organisms to elevated temperatures and other stressors triggers the synthesis of a small group of proteins called hsp (Morimoto, 1993; Zulkifli et al., 2002a). In the present study, irrespective of human contact treatment, 3 h of road transportation significantly increased hsp 70 expression. It appears that the hsp 70 density data correlate well with HLR and CORT. Hence, hsp 70 density may be used as a biological index of stress attributed to transportation, which is consistent with the report where road transportation increased hsp 70 expression in heart and kidney tissues of pigs (Yu et al., 2007).

The induction of hsp is often associated with increased tolerance, both to the intermediate inducing agent and to other types of stress. For example, early age feed restriction improved heat tolerance and disease resistance in chickens later in life (Liew et al., 2003). Similarly, mild heat treatment induced tolerance to both higher temperature and to high concentration of ethanol (Plesset et al., 1982; Hahn and Li, 1990). It has been shown that the hsp are responsible for the phenomenon (Zulkifli et al., 2002a; Liew et al., 2003). Certain hsp participate in protein assembly and folding pathways (Rothman, 1989). It has been proposed that the common signal for hsp induction is protein denaturation and that one function of the induced proteins is to prevent or repair denaturation damage (Craig and Gross, 1991). Thus, manipulating the expression of hsp offers a prospect for altering the tolerance of chickens to transportation stress.

There is the question whether the increase in hsp 70 among PL birds following transportation resulted in improved tolerance to the stress of transportation or high ambient temperature. It is known that during transportation chickens may be exposed to numerous potential stressors including handling by humans, feed withdrawal, noise, vibration, thermal extremes, social disruption, crowding, and restriction of movement (Nicol and Scott, 1990). Al-Aqil (2009) transported chickens at 25°C and demonstrated that those with greater hsp 70 had improved tolerance to transportation stress. Thus, it can be concluded that hsp is also associated with improved tolerance to the nonthermal stressors during transportation.

One of the earliest studies on the effects of neonatal stress on adrenocortical function was by Levine (1962), who reported that infantile stimulation through handling evoked long-lasting alterations of the hypothalamic-pituitary-adrenal axis in rats. When adults, these rats had lower CORT, both basally and during recovery from stress, than animals that were not handled dur-
ing the neonatal stage. The physiological mechanisms underpinning the adrenocortical function alteration following neonatal handling have been associated with concentration of hypothalamic corticosteroid receptors (Meaney et al., 1985). The most striking finding of the current work is the observation that the PL treatment, enhanced hsp 70 expression in the brain following transportation. As measured by HLR and CORT reaction, it appears that hsp 70 played a key role in improving tolerance to transportation stress by pleasant human contact.

Creatine kinase is released into the blood when there is muscle damage. Thus, CK could be considered, in conjunction with other indicators as a welfare measure following transportation (Broom, 2000). However, the present findings concur with those of Deleze et al. (2007) that transportation had negligible effect on blood CK activity in chickens. In the present study, irrespective of duration of transportation, the PL birds had significantly lower blood CK levels than those of the control, PL-UNPL, and UNPL. It is reasonable to suppose that the lower CK activity of the PL chicks may reflect the lower magnitude of stress experienced by them.

In conclusion, this study demonstrated that subjecting birds to pleasant contact with human beings leads to reduced stress and fear reactions to transportation, by enhancing the ability to express hsp 70 in the brain. Despite the beneficial effect of regular pleasant physical contact, the procedure is obviously not feasible and practical in commercial flocks. This experiment also showed that unpleasant contact with human beings can adversely modify the birds’ response to transportation stress. There is little indication that early age pleasant experience with humans can mask or negate the adverse effects of subsequent unpleasant human contact.

ACKNOWLEDGMENTS

The research was partially supported by King Faisal University (Al-Hassa, Saudi Arabia).

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