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Transportation and Translocation Effects on Leucocytic and Behavioural Responses: A Comparison Between the Red Jungle Fowl and Broiler Chickens

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Abstract: Red Jungle Fowl (RJF) (120 days old; mean body weight 614 g) and Commercial Broiler chickens (CB) (35 days old; mean body weight 1800 g) were used to study the effects of road transportation and translocation on leucocytic and behavioural reactions. The birds were raised in floor pens at a farm in Jenderam Hilir, Selangor. The birds were translocated by road transportation for 60 min to the Poultry Research Unit, University Putra Malaysia and assigned in battery cages with wire floors. Immediately following transportation, the heterophil to lymphocyte ratios in RJF and CB were elevated. The ratios returned to basal level two days following translocation. Translocation to battery cages resulted in higher frequency of standing, pacing and pecking at non-nutritive materials in RJF compared to their CB counterparts. It was concluded that physiologically both RJF and CB were equally stressed following transportation and translocation. However, as measured by stereotypic pacing, RJF were more frustrated than their CB counterparts.

Key words: Red jungle fowl, broiler chickens, translocation, stress, behaviour, Malaysia

INTRODUCTION

There is a wealth of literature suggesting that catching, crating and road transportation of poultry can result in physiological and behavioural alterations indicative of acute stress an extremefearfulness (Zulkifli *et al*., 2000; Zulkifli, 2003; Al-Aqil and Zulkifli, 2009). During transit, 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). Exposing birds to an unfamiliar environment is likely to elicit stress reactions (Jones, 1996). Chicks probably perceive a new unfamiliar environment with a degree of uncertainty that acts as a psychological stimulus. While there has been substantial research on the effect of catching and transportation in commercial broiler chickens and laying hens, there is a dearth of information on the effect of such procedures on red jungle fowl. Most naturalists consider that Red Jungle Fowl (RJF) (*Gallus gallus*) is the direct ancestor of the domestic fowl (Crawford, 1990). However, domestication, genetic selection and environmental manipulations have resulted in vast changes in behavior of the domestic fowl both during development and when birds attained maturity. Siegel *et al*. (1992) and Dunnington *et al*. (1994) compared the domestic and the red jungle fowl by DNA fingerprinting and found considerable degree of genetic divergence between them as measured by band sharing. Studies on underlying fearfulness in Commercial Broiler chickens (CB) and RJF indicated longer tonic immobility fear reactions in the latter (Zulkifli *et al*., 1999).

A free-ranging animal is in a state of stress if it is required to make abnormal or extreme adjustments in its physiology or behaviour in order to cope with novel environment or management (Koolhaas *et al*., 1999). Ursin and Olff (1993) suggested that successful coping depends highly on the controllability and predictability of the stressor. Studies in various species of animals showed that whenever environmental stressors are too demanding and the individual cannot cope, its welfare will be compromised (Verbeek *et al*., 1994; Blokhuis and Metz, 1992; Benus *et al*., 1991). Genotype may have a profound influence on an individual's ability to cope with the environment.

According to Koolhaas *et al*. (1999), coping ability are characterised by consistent neuroendocrine and behavioural characteristics. Several neuroendocrine responses will be triggered once a stressor has been perceived. The hypolthalamic-pituitary-adrenal-axis which is responsible for the release of corticosterone is a major endocrinological pathway associated with the stress response (Zulkifli and Siegel, 1995). Corticosterone can alter circulating population of leucocytes (Maxwell, 1993).

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Gross and Siegel (1983) compared leucocytic and hormonal responses to environmental insults and exogenous corticosterone. They concluded that heterophil to lymphocytes ratios were a more reliable indicator of the perceived magnitude of stressors than plasma corticosterone values in avian species. Animals respond to environmental difficulties by modifying their behavior. The occurrence of so-called abnormal behavior has been associated with impoverished environments and frustrated motivation and is correlated with physiological stress responses (Mench and Mason, 1997). The objective of this study was to compare physiological and behavioural responses to catching, crating, transportation and translocation in CB and RJF.

MATERIALS AND METHODS

Animals and housing: Female RJF and female CB chicks were used in the study. The RJF breeding stock was originally captured from the secondary forests and oil palm plantations in peninsular Malaysia and was assumed to be genetically pure. Purity of the RJF was assessed by gross characteristics, namely the shape and size of the bird, colour of the plumage, colour of the shank and ear lobes, pattern of arrangements of the tail feathers and the size and thickness of the comb (Vidyadaran, 1997). The stocks were maintained as a closed flock at a farm in Jenderam Hilir, Selangor. The CB were obtained from a local hatchery and they were feather sexed. At hatch, chicks of each genotype were raised separately in floor pens with deep litter of wood shavings. The pens were in a conventional open-sided house with cyclic temperatures (minimum, 24°C; maximum, 34°C) and the area of each pen was 10.42 m^2. The feed supply was changed from starter crumble (2950 kcal ME kg^{-1}; 21% crude protein) to finisher pellet (3050 kcal ME kg^{-1}; 19% crude protein) at 21 days of age. Free access to feed and water was provided throughout and photoperiod of 12 h was provided.

Transportation and translocation: On the day of transportation (11:00 h), 20 birds of each genotype were individually, gently removed with minimum disturbance to flock mates. The RJF (not sexually matured) (mean body weight, 614 g) and CB (mean body weight, 1800 g) were 120 days and 35 days of age, respectively. Immediately following capture (T_0), blood samples were collected from each bird (via the wing vein) in tubes containing EDTA as anticoagulant. Blood smears were prepared using May-Grunwald-Giemsa stain and heterophil and lymphocytes counted to a total of 60 cells (Gross and Siegel, 1983). Following blood sampling, birds were placed in plastic crates (0.80×0.60×0.31 m) according to genotype (at 10 birds per crate) and loaded into an open truck and transported to the Poultry Research Unit, University Putra Malaysia. The duration of transit was approximately 1 h (average speed of truck was 70 km h^{-1}). At the time of transporting, the ambient temperature and relative humidity was about 32°C and 80%, respectively. Upon arrival (T_1), the birds were individually removed from the crates and blood samples were collected as described earlier. Following blood sampling, the birds were randomly assigned in groups of 5-20 battery cages with wire floors. The birds were caged according to genotype and floor space allowed was 1107 cm^2 per bird. The cages were in a conventional open-sided house. Feed and water were provided *ad libitum*. Birds were exposed to a constant photoperiod of 24 h. Blood sampling was repeated at 48 (T_2), 96 (T_4) and 168 (T_7) h following caging.

The number of birds standing, pacing, resting, preening, pecking at non-nutritive material, eating and drinking was recorded in each cage at 14:00 h following 1-7 days of caging by scan sampling method (Lehner, 1992). The birds were observed every 1 min for 5 min after a 2 min period for the birds to adjust to the presence of the observer. The observer stood approximately 2 m away from the cage. The observations were conducted by observing each bird in 5 cages at a time. The definition of standing, resting, eating and drinking were as defined by Hurnik (1995). Pacing is defined as stereotyped, short-distance walking back and forth or side to side, typically manifested by animals kept in close confinement (Hurnik, 1995). Preening is defined as an act of integumentary care in birds similar in function to grooming in mammals. Preening is manifested as manipulation of feathers and distribution of secretions from the uropygial gland (preen gland) using the beak and also as scratching of the body surface with claws or beak (Hurnik, 1995).

Statistical analysis: Data were analysed by two-way analysis of variance of SAS software (SAS Institute, 1991). When interactions between main effects were significant, comparisons were made within each experimental variable. The significant differences among means of treatments were compared by Duncan's multiple range test and $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

There was a significant genotype x blood sampling time interaction for HLR (Table 1). The significant effect of genotype was only noted at 0 h where the RJF had significantly higher HLR than CB. For both RJF and CB, there was a dramatic elevation in HLR following 1 h of transportation. The elevated HLR was not sustained at T_2

Table 1: Mean (±SEM) heterophil to lymphocyte ratios genotype x time of blood sampling interactions were significant

	Time of blood sampling				
Genotype	T_0	T_1	T_2	T_4	T_7
CB	0.4457 ± 0.15^{bx}	1.3160 ± 0.18^{a}	0.4642 ± 0.08^{b}	0.3413 ± 0.05^{b}	0.4137 ± 0.06^{b}
RJF	0.7462 ± 0.08^{by}	1.4056 ± 0.53^{a}	0.4191 ± 0.21^{b}	0.3508 ± 0.04^{b}	0.3214 ± 0.04^{b}

$^{a, b}$Means within row-subgroup with no common letters differ significantly ($p<0.05$), $^{x, y}$Means within column-subgroup with no common letters differ significantly ($p<0.05$), CB = Commercial Broiler chicken, RJF = Red Jungle Fowl, T_0 = immediately following capture, T_1= immediately following transportation, T_2 = 48 h following transportation and caging, T_4 = 96 h following transportation and caging, T7 = 168 h following transportation and caging

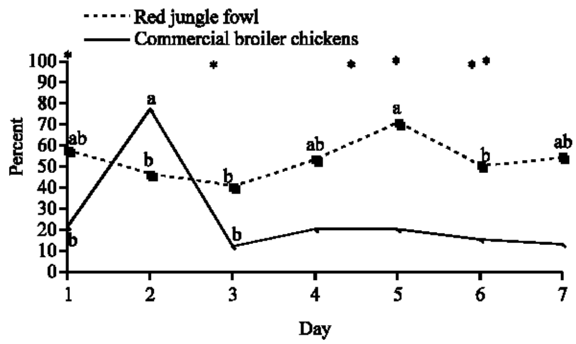

Fig. 1: Mean percentages of chickens standing where genotype (red jungle fowl and commercial broiler chickens) x day of translocation interactions were significant. $^{a, b}$Means within a single line with no common letters differ at $p<0.05$. *Difference between genotypes $p<0.05$

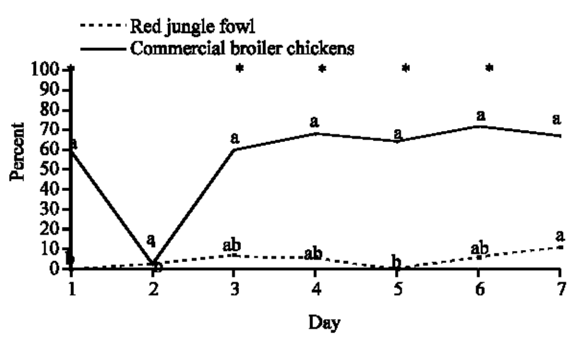

Fig. 2: Mean percentages of chickens resting where genotype (red jungle fowl and commercial broiler chickens) x day of translocation interactions were significant. $^{a, b}$Means within a single line with no common letters differ at $p<0.05$. *Difference between genotypes $p<0.05$

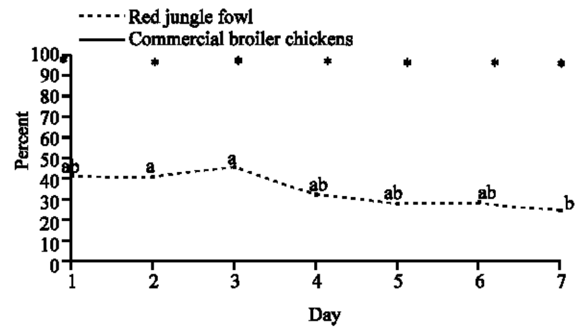

Fig. 3: Mean percentages of chickens pacing where genotype (red jungle fowl and commercial broiler chickens) x day of translocation interactions were significant. $^{a, b}$Means within a single line with no common letters differ at $p<0.05$. *Difference between genotypes $p<0.05$

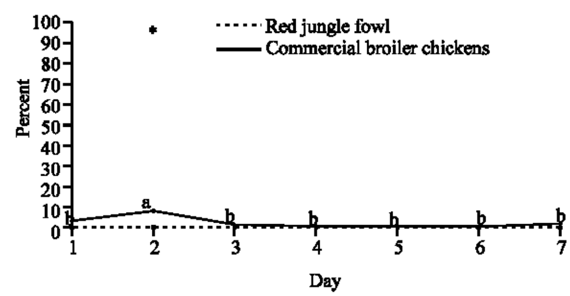

Fig. 4: Mean percentages of chickens pecking on non-nutritive material where genotype (red jungle fowl and commercial broiler chickens) x day of translocation interactions were significant. a,bMeans within a single line with no common letters differ at $p<0.05$. *Difference between genotypes $p<0.05$

and thereafter. The HLR at T_0, T_2, T_4 and T_7 were not significantly different. Significant genotype x h interactions for number of birds standing, resting, pacing, pecking on non-nutritive material, preening, drinking and eating were noted. Except on day 2, CB spent significantly less time standing (Fig. 1) and more time resting (Fig. 2) than those of RJF throughout the period of study. The number of birds pacing was significantly higher in RJF throughout the period of study (Fig. 3). Pacing behaviour was not noted in CB. The RJF showed significantly higher frequency of pecking at non-nutritive material than CB on day 2 (Fig. 4). RJF did not show the behaviour on day 4 and thereafter. The percentages of birds preening on day 1, 2 and 3 were significantly higher for CB compared to RJF (Fig. 5). The RJF did not show any preening until day 6. The number of both CB and RJF drinking remained <10% throughout the study (Fig. 6). Drinking behaviour

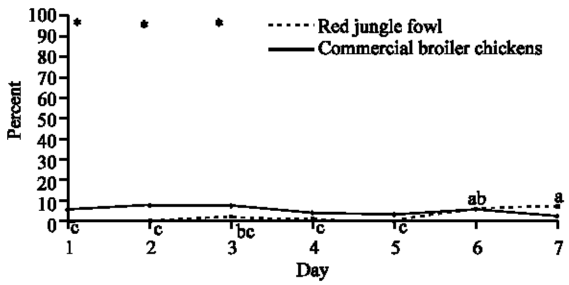

Fig. 5: Mean percentages of chickens preening where genotype (red jungle fowl and commercial broiler chickens) x day of translocation interactions were significant. [a, b]Means within a single line with no common letters differ at $p<0.05$. *Difference between genotypes $p<0.05$

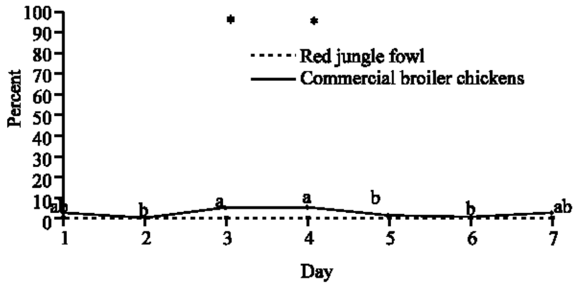

Fig. 6: Mean percentages of chickens drinking where genotype (red jungle fowl and commercial broiler chickens) x day of translocation interactions were significant. [a, b]Means within a single line with no common letters differ at $p<0.05$. *Difference between genotypes $p<0.05$

was significantly more frequent in CB than RJF on day 3 and 4 (Fig. 7). Except for day 4, the CB spent more time eating than RJF. Eating behaviour was not noted in RJF until day 3. The higher basal HLR in RJF than CB at T_0 is consistent with those of Zulkifli (2005). The phenomenon could be attributed to social environment which can have profound impact on many aspects of the birds' biology (Siegel and Gross, 2000). In the present study, unlike the RJF (120 days old), the CB (35 days old) have not established social order and thus they are less susceptible to social stresses. According to Appleby *et al.* (2004), dominance hierarchy in chickens is formed between 6 and 10 weeks of age. The present findings are consistent with those of Zulkifli *et al.* (2000) that road transportation may elevate HLR in poultry, suggestive of stress response. Elicitation of adrenocortical activity is known to precede heterophilia and lymphopenia (Maxwell, 1993). Although, during

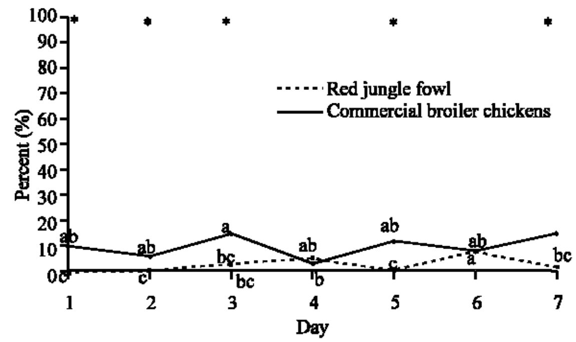

Fig. 7: Mean percentages of chickens drinking where genotype (red jungle fowl and commercial broiler chickens) x day of translocation interactions were significant. a,bMeans within a single line with no common letters differ at $p<0.05$. *Difference between genotypes $p<0.05$

transit birds are exposed to an array of potential stressors, high ambient temperature is a major factor in the elicitation of physiological stress responses (Mitchell and Kettlewell, 1998). The high relative humidity in the tropics may exacerbate the heat stress problems. It is interesting to note that although, CB had greater body weights and thus more likely to be susceptible to heat stress than their RJF counterparts (Zulkifli *et al.*, 1999), both genotypes had similar HLR response to transportation. The phenomenon could be attributed to greater underlying fearfulness in RJF. Craig (1981) indicated that domestication, a continuous genetic process, influences social behaviour and fearfulness, producing more placid and less aggressive animals. It is well documented that road transportation may prolong tonic immobility duration in poultry (Zulkifli *et al.*, 2000; Zulkifli, 2003) indicating heightened fearfulness. Research by Zulkifli *et al.* (1999) and Zulkifli (2005) clearly showed that RJF were more fearful than their CB counterparts and the reaction is closely associated with anti-predator defense behaviour (Rovee *et al.*, 1976, 1977). Because fear is a powerful and potentially damaging stressor (Jones, 1996), it may have induced physiological stress response in RJF following transportation.

Exposing animals to novelty is one of the most potent situations to evoke responses by the hypotlamus-pituitary-adrenal axis (Levine, 1985). Chicks may perceive a new unfamiliar environment with a degree of uncertainty that acts as a psychological stimulus. The similar HLR response of RJF and CB to translocation was unexpected. RJF is known to have more active foraging behavior, higher social motivation than CB. Thus, we hypothesized the former would exhibit a greater magnitude of physiological stress response than the former following caging. There is no clear explanation for the lack of difference in HLR response between the two genotypes.Four months of prior confinement in floor pens may have made the RJF better able to cope with novel environment. We would expect a more dramatic response if the birds were caught form the wild.

In the present study, 2 days following translocation (T_2) from floor pens to battery cages with wire floors, the HLR of both RJF and CB were similar to values at T_0 and suggest habituation to the new environment. The degree of discrepancy between the initial and new environment is critical in determining the magnitude of stress response (Hennessy *et al*., 1979).

There were marked differences in general activity and behavioural responses between CB and RJF. The RJF are more alert and sensitive than the CB, hence the large differences in standing and resting behaviours are expected. The significantly higher percentage of CB birds standing on day 2 than RJF was due to the delay in feeding on that day. Zulkifli *et al*. (2006) showed that feed deprivation resulted in increased levels of alertness and general activity in chickens.

Pacing, a common stereotypic behavior, increases when birds are frustrated (Duncan and Wood-Gush, 1972). Stereotypies are generally defined as unvarying, repetitive behavior patterns that have no obvious goal or function (Fox, 1965). In the present findings, the RJF exhibited significantly more pacing than CB suggesting that caging is more stressful to the former. Duncan and Wood-Gush (1972) reported that stereotyped pacing was common in caged light hybrid strains of hens. It is interesting to note that despite the marked differences in pacing activity, both genotypes had similar HLR in response to caging. The relationship between stereotypies and well-being is not a straight forward one. Gregory (2007) indicated that stereotypic behavior provides some relief form frustration by evoking a surge of β-endorphin, an endogenous opiate, within the brain. Mauldin and Siegel (1979) reported that head shaking in chickens may aid in coping with confinement.

It is known that foraging behaviour, which comprised food searching and food consumption is important to poultry, particularly jungle fowl (Appleby *et al*., 2004). Dawkins (1989) indicated that despite *ad libitum* availability of food, jungle fowl spent a major portion of their time to forage activities such as ground pecking and scratching. In the present study, RJF showed a more frequent non-nutritive pecking than CB only on day 2. Non-nutritive pecking can be considered as a form of exploratory behaviour which is typically performed during the absence of suffering (Duncan, 1998). Preening is a comfort behaviour and it is important for keeping the feathers well groomed (Appleby *et al*., 2004). Preening may vary according to space allowance. It is interesting to note that RJF did preen until day 6. Because preening is a comfort behaviour, it appears that RJF have not coped successfully to the new environment until day 6-7. The frequency of pacing which declined significantly on day 7 supports the notion. As expected the CB exhibited more eating behaviour than RJF. Alterations in appetite due to intense selection for rapid growth in meat-type chickens have been well documented (Dunnington and Siegel, 1996). The absence of eating behaviour in RJF on day 1 and 2 suggests that the translocation to battery cages is stressful to the birds and they take longer time to recover than CB.

CONCLUSION

In general, the HLR data suggest that both RJF and CB are equally stressed following transportation and translocation and the time required by both genotypes to recover from the stressful experience is similar. On the contrary, as measured by stereotypic pacing, preening and eating behaviours, it is evident that RJF are slower to cope with the translocation stress than CB. Although, HLR were considered a reliable indicator of the perceived magnitude of stressors in poultry (Gross and Siegel, 1983; Maxwell, 1993), behaviour has been shown to provide excellent cues about the internal states of animals such fear and frustration (Duncan, 1998). The disparity in behaviour between RJF and CB following translocation may indicate that the novel environment (battery cages) elicits different levels of distress in the birds. Considering the natural behaviour of RJF and the impact of intense artificial selection for economic traits on CB, the noted behavioural responses are not unexpected. Craig (1981) suggested that there has been a selection for more placid and tame behaviour and reduced fear reactions in the domestic fowl.

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