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Fear-Related Behaviour, Muscle Glycogen Stores and Serum Creatine Kinase Activity in Transported Broiler Chickens as Affected by Housing and Early Age Feed Restriction

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Abstract: An experiment was conducted to determine the effects of two types of housing systems and early age feed restriction on tonic immobility reaction, muscle glycogen content and serum creatine kinase activity in broiler chickens subjected to road transportation. On day 1, chicks were housed either in windowless environmentally controlled chambers (temperature was set at 32°C on day 1 and gradually reduced to 23°C by day 21) (CH) or in conventional Open-sided Houses (OH) with cyclic temperatures (minimum, 24°C; maximum, 34°C). Equal number of chicks from each housing system were subjected to either *Ad libitum* feeding (AL) or 60% Feed Restriction on day 4-6 (FR). On day 42, all the birds were crated and transported for 6 h. Birds raised in OH had shorter TI duration and higher muscle glycogen content than those of CH. Subjecting birds to FR shortened TI duration following transportation. Tonic immobility duration increased with duration of transportation while the converse was noted for muscle glycogen content. Serum creatine kinase was not affected throughout the period of study. Collectively, the results suggested that raising birds in OH dampened fear-related behavior and improved muscle glycogen content in response to road transportation under the hot and humid tropical climate.

Key words: Transportation, tonic immobility, muscle glycogen, housing, broilers, Malaysia

INTRODUCTION

The act of being crated and transported involved exposure to various nocuous stimuli such as noise, motion and social disruption which are likely to be novel and thus may heighten fear-related behaviors (Weeks and Nicol, 2000).

Exposing birds to an unfamiliar environment is likely to elicit both fear and stress reactions (Jones, 1996; Zulkifli and Azah, 2004). The most robust measures of fearfulness in poultry are the parameters associated with an induced state of tonic immobility (Jones, 1986). Duration of Tonic Immobility (TI) fear response was augmented by road transportation (Zulkifli, 2003) and the duration of TI was positively correlated to journey time (Cashman *et al*., 1989). Cashman (1987) found a highly significant linear relationship between journey times from 10-120 min and the duration of TI in broiler chickens. A similar investigation has been carried out by Mills and Nicol (1990) with spent hens. The researchers reported that the duration of TI was greatly elevated by road transportation and remained constant after journey lengths of up to 5 h. Closed-house system that provides manageable environment and prevents the contact with external wild birds and/or other pathogens carriers is becoming increasingly popular in the Malaysian poultry industry.

Closed-house system offers a solution to heat stress related problems in broiler chicken production in the hot humid tropical conditions. However, there is little information on the effect of such housing system on the behavioural and physiological reactions of chickens to road transportation. In an experiment conducted concurrently with this study, Al-Aqil and Zulkifli (2009) reported broiler chickens raised in conventional open-sided houses were better able to cope with the stresses associated with road transportation in hot, humid climate than those under environmentally controlled closed house. The researchers suggested that chickens raised in open-sided houses had experienced a greater variety of visual and auditory stimuli than those raised in closed house system. The visual and auditory stimuli can be considered as a form of environmental enrichment. Environmental enrichment provides extra stimulation in

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home environment that may affect birds' expectations about environmental complexity and enhance their ability to adapt to novelty. Birds housed in different systems have been shown to have different levels of fear when placed into TI (Jones and Faure, 1981; Hansen *et al*., 1993) and in general, the durations of TI are shorter for birds housed in pens than in cages (Jones and Faure, 1981; Kujiyat *et al*., 1983). Birds which were frequently observed in the free range area were less fearful than those which were never seen to venture out of the poultry house (Grigor *et al*., 1995). Nicol (1992) reported reduced levels of fear after transportation in broiler chickens that had been reared in an enriched environment. However, Scott *et al*. (1998) found no effect of enrichment when TI durations were measured after a 74 min journey. Handling and transport can affect the rate of post-mortem glycolysis in the meat and its subsequent quality. Warriss *et al*. (1993) reported that longer journeys progressively depleted liver and muscle glycogen content in broiler chickens. In pigs, lower muscle glycogen concentration was associated with high pH values, dark colour and low expressible water ratios (Briskey *et al*., 1959). Elevation in plasma glucocorticoids in response to stress may stimulate cathecolamines release (Scanes, 1986) and consequently, glycogenolysis in the muscle (Lehninger *et al*., 1993). Although, stress is known to deplete muscle glycogen, the potential relationships between underlying fearfulness and muscle glycogen reserves has not been examined in chickens. Remignon *et al*. (1998) selected Japanese quail for short or long tonic immobility reactions and found that the latter had higher drip loss values for breast meat. In pigs, nervousness is positively related to the incidence of pale soft exudative meat (Grandin, 2000).

In addition to effects of housing systems, a great deal of the literature on farm and laboratory animals suggests that stressful experiences during the neonatal stage can have profound influence on their ability to cope with stressors later in life (Meaney *et al*., 1987; Hilakivi-Clarke *et al*., 1991; Zulkifli and Siegel, 1995). Zulkifli (2003) indicated that early age feed restriction alleviated physiological stress reaction in 42 days old broiler chickens transported for 2 h but had negligible effect on underlying fearfulness.

Therefore, the objectives of this study were to determine the influence of housing systems (Conventional open-sided house system versus Environmentally controlled chamber house system) and early age feed restriction on fear reactions, muscle glycogen concentration and serum creatine kinase activity of broilers subjected to 6 h transit under hot and humid tropical environment.

MATERIALS AND METHODS

The study was conducted at the Poultry Research Unit, Department of Animal Science, Universiti Putra Malaysia during the period of March to May 2008.

Animals and experimental design: A total of 432 days old female commercial broiler chicks (Cobb) were obtained from a local hatchery. On day 1, 216 chicks were placed at random in groups of 6 into 36 cages in 3-tiered batteries with wire floors in windowless environmentally controlled chambers (12 cages per chamber) (2.3×9.1×3.8 m) (CH). Floor space allowed was 923 cm^2 per bird. Ambient temperature on day 1 was set at 32°C and gradually reduced to 23°C by day 21. The relative humidity ranged between 65-75%. The remaining 216 chicks were placed in similar battery cages and housed in conventional open-sided houses (12 cages per house) (OH) with cyclic temperatures (minimum, 24°C; maximum, 34°C). The relative humidity ranged between 80-90%. Equal number of chicks for each housing system was subjected to either *Ad libitum* feeding (AL) or 60% Feed Restriction on days 4-6 (FR). Food restriction was 60% of food consumption of the *ad libitum* fed group on the previous day. All birds were fed a standard broiler starter crumble (2,950 kcal ME kg^{-1}; 21% crude protein) finisher pellet (3,050 kcal; 19% crude protein) diets from age 1-21 and 22-42 days, respectively. Water was available at all times. The chicks were reared under continuous lighting. Chicks were vaccinated against Newcastle disease via intraocular route on day 7 and 21st.

Road transportation: On day 42 (mean body weights = 2, 224±14.48 g), all the birds from each housing system-feeding regimen subgroup were road transported at 12:00 h. The birds were individually, gently removed with minimum disturbance to flock mates held in an inverted manner and placed in plastic crates (0.80×0.60×0.31 m) at 10 birds to each crate. The crates were loaded to an open truck and transported for 6 h with an average speed of 80 km h^{-1}. The journey covered highways, roads with heavy traffic and traffic lights. At the time of transportation, the ambient temperature was 34-36°C.

Mortality: The number of broilers that died during transport was determined.

Tonic immobility tests: Prior to transportation (0 h) and following 2-6 h of transit, 15 birds of each housing system-feeding regimen subgroup 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. TI 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 sec. The experimenter then retreated approximately 1 m and remained of within the sight of the bird but made no unnecessary noise or movement. Direct eye contact between the observer and the bird was avoided as it may prolong TI duration (Jones, 1986). A stopwatch was started to recorded latencies until the bird righted itself. If the bird righted itself in <10 sec, it was recaptured and the restraining procedure was repeated. If TI is not inducing after three attempts, the duration of TI was considered 0 sec. The maximum duration of TI allowed was 600 sec (Zulkifli *et al*., 2000a, c).

Blood samples: Immediately following TI test, 10 birds from each housing system-feeding regimen subgroup were randomly selected and their blood samples were collected for the determination of serum levels of creatine kinase. The blood samples were serum separated and stored at -20°C. Analyses for creatine kinase were conducted spectrophtometrically with an automated apparatus (Hitachi 902) and by using a standard diagnostic kit (Roche Diagnostic GmbH, Mannheim).

Muscle glycogen concentration: After blood sampling, all the birds were slaughtered according to the Halal Method for measurement of glycogen content. Immediately after death, approximately, 5 g of muscle tissue was dissected out from pectoralis major muscles for enzymatic determination of glycogen content (Dreiling *et al*., 1987). Once dissected, the muscle tissue was immediately frozen in liquid nitrogen, labeled and stored at -80°C until enzymatic determination of glycogen analysis. Approximately, 1 g of crushed muscle tissue was initially homogenized in 8.25% perchloric acid and centrifuged for 15 min at 15,000×g at 4°C. The resulted supernatant containing solubilized glycogen was neutralized with saturated potassium bicarbonate and acetate buffer (0.2 M; pH 4.8) and added with amyloglucosidase (Roche Applied Science) at a concentration of 80 units mL^{-1} for 30 min incubation at 37°C. After centrifugation (1,200×g, 10 min), the supernatants were allowed to react with the glucose oxidase perid (Roche Applied Science) solution before spectrophotometrically analysed at 600 nm for glucose content.

Statistical analyses: Data were analyzed as a 2×2×4 factorial design using the general Linear Models procedure of SAS® software and multiple means were separated by Duncan's multiple range test (SAS Institute, 1991). The statistical model included the effects of housing system (OH and CH), feeding regimen (AL and FR), transportation time (0, 2, 4 and 6 h) and their interactions. Data for TI duration were normalized using logarithm transformation. Results were considered statistically significance at $p \leq 0.05$.

RESULTS AND DISCUSSION

The TI, muscle glycogen concentration and CK activity data are shown in Table 1. There was no significant main effects interaction for all the parameters measured. TI durations increased significantly with transit time. The CH birds showed significantly longer TI durations than their OH counterparts. Birds subjected to AL had significantly longer TI duration than those of FR. Longer duration of transportation resulted in a significant reduction in muscle glycogen content. Irrespective of duration of transportation, birds reared in OH had significantly greater muscle glycogen content than their CH counterparts. Feeding regimen had no significant effect on glycogen content. Serum CK activity was not significantly affected by duration of transportation, housing system or feeding regimen. No mortality was noted throughout the study.

Warriss *et al*. (1992) showed that mean dead-on-arrival percentage was 1.81 times higher when broilers were transported for >4 h compared with transports of shorter duration. In the present study, despite 6 h of transportation under high ambient temperature there was no mortality throughout the experiment. Other than transport time and ambient temperature, Catching Method

Table 1: Mean (±SEM) Tonic Immobility (TI) durations, muscle glycogen contents and serum creatine kinase concentration of broiler chickens by transit time, housing system and feeding regimen

CK activity	TI duration (sec)	Muscle glycogen content (mg kg^{-1})	Serum creatine kinase concentration (IU L^{-1})
Duration of transportation (h)			
0	175±22^{c}	51.537±1.08^{a}	6461±225
2	249±35^{b}	39.133±1.27^{b}	6261±254
4	358±30^{a}	29.067±1.02^{c}	5830±195
6	398±31^{a}	10.712±0.48^{d}	6467±246
Housing system			
OH	245±21^{b}	33.858±2.49^{a}	6041±198
CH	328±23^{a}	30.387±2.45^{b}	6423±265
Feeding regimen			
AL	334±23^{a}	32.275±2.25	6409±245
FR	240±22^{b}	30.933±2.68	6035±220

$^{a-b}$Means within a column with no common superscripts differ at $p<0.05$; OH: Conventional open-sided house with cyclic temperature (minimum 24°C; maximum 34°C); CH: Environmentally controlled chamber (day 0 was set at 32±1°C and then gradually decreased until 24±1°C was reached by day 21); AL: *Ad libitum* feeding, FR: 60% Feed Restriction on days 4-6

and stocking density per crate (Bayliss and Hinton, 1990) have been reported to influence mortality. Hence, it appears that broilers can be transported for 6 h under ambient temperatures of 34-36°C without resulting in any mortality provided they are carefully caught and allowed sufficient space during crating. As expected, broilers subjected to road transportation showed longer TI duration than those that were not transported. Earlier researches involving measurement of TI showed that transport per se may greatly increase underlying fearfulness in poultry (Cashman *et al*., 1989; Nicol and Scott, 1990; Zulkifli *et al*., 2000a, c; Zulkifli, 2003). Cashman *et al*. (1989) suggested that harvesting, loading and unloading may have contributed to the overall fear following transit as exposure to human beings, social disruption and crating are all capable of eliciting fear reaction in the domestic fowl. The present findings and those of Zulkifli (2003) and Cashman *et al*. (1989) suggested that journey time and duration of TI are positively related. The lack of significant differences in TI duration between 4 and 6 h of transportation suggested that fear levels have reached a plateau or has decreased through familiarization with the progressively longer journeys. However, Mills and Nicol (1990) reported that transportation increased fear in spent hens and there was no evidence that the chickens became habituated to transport during journeys of up to 5 h.

Birds housed in different systems have been shown to have different levels of fear when placed into TI. Grigor *et al*. (1995) reported that previous regular response to complex environment reduced fearfulness and enriched bird's ability to adapt to a novel environment. Birds exposed to various enrichment stimuli showed reduced freezing and avoidance of novel objects introduced into the home cages, accelerated emergence from a sheltered area into an exposed, unfamiliar one and increased vocalization, ambulation and pecking in an open field or novel environment (Jones and Waddington, 1993). This study showed that the OH birds which were exposed to a wide variety of stimuli (viewing the outside area and hearing the surrounding noises) had reduced underlying fearfulness in response to crating and transportation when compared to their CH counterparts. Al-Aqil and Zulkifli (2009) demonstrated that raising birds in conventional open-sided houses with cyclic ambient temperatures improved heat shock protein (hsp) 70 expression and may lead to better ability to cope with the stresses associated with road transportation in hot and humid climate than those under environmentally controlled closed house. In a heat shocked cell, the hsp may bind to heat sensitive proteins and protect them from degradation or may prevent damaged proteins from immediately precipitating and permanently affecting cell viability (Etches *et al*., 1995). Hence, there is a possibility that heat shock protein 70 expression and underlying fearfulness are interrelated. Zulkifli *et al*. (2009) classified broiler chickens as Low Fear (LF) or High Fear (HF) responders based on TI duration. The researchers showed that following 3 h of heat exposure, the hsp 70 response was greater for the HF than the LF group. However, both groups had similar hsp 70 reactions to crating.

It has been shown that stresses which occur early in life while many systems of the chicks are still developing, may have a long-lasting impact and could improve their ability to cope with stressors later in life (Meaney *et al*., 1987; Hilakivi-Clarke *et al*., 1991; Zulkifli *et al*., 2000b). In the present study, the researchers noted significantly shorter TI duration among FR chicks than those of AL in response to transportation. Because early age fasting improved hsp 70 expression (Zulkifli *et al*., 2002; Liew *et al*., 2003) and the proteins are associated with underlying fearfulness (Zulkifli *et al*., 2009), the reduced fear reactions among FR chicks is not unexpected.

The results of this study confirm earlier findings that longer transport may deplete muscle glycogen content. The loss of muscle glycogen could be attributed to stress of handling and transport and feed deprivation (Lambooij, 2000). The present experiment clearly shows that housing condition may have profound influence on muscle glycogen contents following transportation. The CH birds which were more fearful had significantly greater depletion of pectoral muscle glycogen than their OH counterparts following 4 and 6 h of transportation. Research on pigs suggested that nervousness is closely associated with pale soft exudative meat (Grandin, 2000). Similarly, Voisinet *et al*. (1997) reported that cattle with excitable temperament produced carcasses with tougher meat. Both fear and excitement may elevate circulating levels of adrenaline (Breggin, 1964) and through a series of biochemical changes the hormone can indirectly catalyzes the breakdown of glycogen ante mortem (McVeigh *et al*., 1982). Hence, the present findings suggest that underlying fearfulness and meat quality are interrelated in chickens. Enzyme creatine kinase is released into the blood stream after muscular fatigue or damage (Knowles and Warriss, 2000) and have been used as indices of physiological stress response in mammalian (Kannan *et al*., 2000) and avian (Mitchell *et al*., 1992) species. However, the effect of environmental stress on blood CK activity has been inconsistent. Elevation in CK activity even with mild stress or exercise has been noted in rabbits and turkeys (Bacou and Ressot, 1976; Tripp and Schmitz, 1982). On the contrary, Hicks *et al*. (1998) reported that heat, cold and shipping stress had no influence on CK activity in pigs. In the present study, despite the high ambient temperature and long transit time, serum CK levels were not affected.

Closed-house system is recommended to improve management of environment and biosecurity in poultry production. In countries like Malaysia, closed-house system can alleviate poor growth performance and high mortality attributed to heat distress in broiler chickens. However, the present findings and those of Al-Aqil and Zulkifli (2009) suggested that rearing chickens in closed-house system was detrimental to their coping ability and meat quality following road transportation under high temperature. Irrespective of housing system, transit time and feeding regimen, all the birds survived the transportation procedure. Under the conditions of the experiment, broiler chickens that were gently caught and provided optimum crating density can be transported for 6 h under high ambient temperature without causing any mortality.

CONCLUSION

In this study, there is evidence that early age feed restriction can further improve the ability of birds to cope with transportation.

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