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# Effect of corticosterone on growth and welfare of broiler chickens showing long or short tonic immobility

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ABSTRACT

Tonic immobility (TI) test is commonly used to assess fear. Animals showing different TI durations demonstrate distinct behavior and biochemical responses to stress. However, less is known about how TI phenotype affects growth and welfare of domestic fowl. In this study, broiler chickens (*Gallus gallus*) were classified into short and long TI duration (STI and LTI) phenotypes and treated chronically with vehicle (CON) or corticosterone (CORT). STI broilers demonstrated significantly higher growth rate with higher breast muscle yield ($P<0.05$) and liver weight relative to BW tended to be lower ($P=0.053$), which was accompanied by higher serum concentration of CORT ($P<0.05$) and uric acid ($P<0.01$), but lower serum level of T4 ($P=0.01$). CORT severely reduced body weight, as well as the relative weight of muscle, bursa of Fabricius and spleen ($P<0.001$), but relative liver weight was increased ($P<0.001$). CORT-treated chickens had reduced serum CORT, elevated heterophile/lymphocyte ratio, and increased serum levels of total and free T3. STI broilers displayed more preening behavior ($P<0.05$), yet CORT elicited more walking behavior ($P<0.05$). No difference was observed in the welfare assessment scores between STI and LTI phenotypes under basal situation, while LTI chickens showed significantly increased incidence of pad dermatitis compared to STI under CORT exposure. The results suggest that STI broilers demonstrate better growth performance and higher adaptability to stress compared to LTI chickens.



## 1. Introduction

Tonic immobility (TI) is an inborn response of animals when they are frightened, which is characterized by temporal freeze or paralysis. Tonic immobility is observed in a diverse variety of animals, including insects (Nishi et al., 2010), lizards (Hennig and Dunlap, 1977), rabbits (Woodruff and Lippincott, 1976), rats (Meyer et al., 1984) and chickens (Gallup et al., 1972; Rovee and Kleinman, 1974). Domestic fowl has been the most commonly used animal for TI induction due to its strong and clearly distinguishable reaction (Jones, 1986).

TI has been used as a measure for assessing fear (Hocking et al., 2005; Forkman et al., 2007; Zulkifli et al., 2009; Villagra et al., 2011). A long duration of TI is generally considered as an indication for high levels of fearfulness (Reese et al., 1984). In human and other mammals, high fearfulness or high anxiety traits are proposed to be a risk factor for the susceptibility of chronic stress-related disorders (Sandi and Richter-Levin, 2009). Stress response in vertebrates involves activation of the hypothalamus–pituitary–adrenal (HPA) axis which results in the release of glucocorticoids (corticosterone in birds) from the adrenal cortex. Glucocorticoids contribute to re-establish the homeostasis via inhibiting HPA reactivity (Tasker and Herman, 2011) and initiating adaptive immunological and metabolic responses (Sapolsky et al., 2000).

Japanese quails selected for long (LTI) or short (STI) duration of tonic immobility have been used as model to investigate the differences in behavior (Jones et al., 1991; Calandreau et al., 2011), reproductive performances (Minvielle et al., 2002), as well as the HPA responsiveness (Hazard et al., 2005, 2008a, 2008b; Calandreau et al., 2011). LTI quails are reported to show lower corticosterone (CORT) responses under restraint stress (Hazard et al., 2005, 2008b) and chronic stress induced by repeated exposure to negative stimulation (Calandreau et al., 2011). However, studies on chickens seem to contradict the findings on quails. Cobb broiler chickens with LTI phenotype demonstrated greater CORT response under crating and heat challenge (Zulkifli et al., 2009), which is consistent with an earlier report of greater adrenocortical activation in LTI than STI hens (Beuving

*Abbreviations:* BW, body weight; TI, tonic immobility; LTI, long tonic immobility; STI, short tonic immobility; CORT, corticosterone; GLU, glucose; TG, triglyceride; CHOL, cholesterol; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol; LA, lactic acid; T-AOC, total antioxidant capacity; UA, uric acid; LDH, lactate dehydrogenase; CK, creatine kinase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; H/L, heterophile/lymphocyte; T3, triiodothyronine; T4, tetraiodothyronine.

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et al., 1989). Therefore, the differences in the CORT response to chronic stress between long and short TI broiler chickens remain to be clarified.

Broiler chickens are known to confront a multitude of stressors, including human handling disturbance and adverse housing environment (e.g. heat, cold, noise, crowding, harmful gas and wet litter). The responses against these stressors are associated with several serious welfare problems that impair the health and meat quality of broiler chickens (Hocking et al., 2005; Meluzzi et al., 2008a). Different approaches have been proposed to alleviate the stress responses including improving the housing conditions (Hocking et al., 2005; Deep et al., 2010), decreasing the stock density (Meluzzi et al., 2008b; Buijs et al., 2009), implementing the environment enrichment (Leone and Estevez, 2008; Ventura et al., 2010), or compromising the productive efficiency through genetic selection for reduced growth rate (Decuypere et al., 2010). TI phenotype may serve as a selection target for a low stress response broiler line, yet the influence of TI on the growth performance, metabolic profiles, as well as the behavior and welfare status of broiler chickens has not been clearly elaborated.

Therefore, we tested, in the present study, whether broiler chickens with inborn long or short duration of tonic immobility differ in growth performance, health and welfare status under basal situation, and whether chronic stress may highlight or mask such innate differences. We used oral administration of corticosterone in drinking water for 2 weeks as an experimental model of chronic stress in broiler chickens which was established and employed in other studies (Post et al., 2003; Lin et al., 2006; Shini and Kaiser, 2009). Growth performance, serum biochemical and endocrine parameters, as well as the behavior and welfare assessment were determined. The results will provide essential basis for the selection of a stress-resistant line of broiler chickens.

## 2. Materials and methods

### 2.1. Animals and management

A total of 600 broiler breeder eggs (Ross 308) were purchased from a commercial breeding company and incubated under standard conditions. Newly hatched chickens were wing-banded and housed in an environmentally controlled room following the feeding standard set by the breeding company. The temperature was maintained at 34 ± 3 °C for the first 3 d, and then decreased gradually to 21 ± 3 °C until 28 d of age. Standard commercial broiler starter crumble (12.5 ME/kg; 21% CP) and finisher pellet (12.8 ME/kg; 19.5% CP) were provided from 1 d to 20 d and 21 d to 42 d, respectively. Continuous lighting was conducted during the first week, and the lighting time decreased by 2 h/week gradually to 18L:6D until 21 d and kept constant thereafter to 42 d. Water was available ad libitum. The experimental protocol was approved by the Animal Ethics Committee of Nanjing Agricultural University.

### 2.2. Tonic immobility test

To establish two segregated groups of STI and LTI phenotypes, all chickens were tested for TI twice on 10 d and 21 d, respectively. The 3rd TI test was performed on 41 d to all the chickens registered in the experiment. The TI test followed the protocol described previously (Mills and Faure, 1991). Briefly, chicken was carried individually to another room devoid of other birds. The chicken was placed on its back on the floor and restrained for 20 s (with one hand on the sternum and one lightly cupping the head of the bird). The experimenter remained silent and virtually motionless in the room, out of the bird's sight. If more than 10 s elapsed until the bird righted itself, the duration of TI was recorded. If TI was not attained after 3 attempts, a score of 0 s was given. Conversely, if the bird failed to right itself after 10 min, the test was terminated and a maximum score of 600 s was given for tonic immobility duration.

Chickens demonstrating two extremes (shortest and longest) of TI duration were registered in the experiment. Eighty chickens showing the shortest TI duration (29.6 ± 2.3 s) and 80 scoring the longest duration (246.2 ± 26.8 s) were classified into STI and LTI groups, respectively. The other chickens with intermediate TI duration were excluded from the study.

### 2.3. CORT administration

Chickens of STI and LTI groups were respectively allocated into control (CON) and CORT-treated subgroups to four 2 × 2.7 $m^2$ pens. From 27 d to 42 d, chickens in CORT groups of both STI and LTI phenotypes (40 per phenotype) were supplied water supplemented with 5 mg/L corticosterone, whereas those in control groups (40 from LTI and 40 from STI) were supplied water supplemented with equivalent volume of the solvent (absolute ethanol). Corticosterone (C2505, Sigma, USA) was first dissolved in absolute ethanol to a concentration of 5 mg/mL, and diluted into drinking water at 1:1000. Therefore, corticosterone content in the drinking water was 5 mg/L, while that of ethanol was 1 mL/L. Each broiler chicken consumed approximately 0.2–0.3 L water per day in average during the experimental period (27 d to 41 d). Therefore, the daily intake of corticosterone and ethanol was about 1.0–1.5 mg and 0.2–0.3 mL, respectively.

### 2.4. Video-recording of behavior

Six broilers from different groups were colored with various paints as candidates. Four cameras connected to computer were in one-to-one correspondence with four pens. The behavior of the colored broilers was video-recorded from 10:00 to 16:00 h for 2 consecutive days from 38 to 39 d. Behaviors were categorized as feeding, drinking, foraging, standing, sitting, walking, running, and preening, according to the method described previously (Ventura et al., 2012), and the definitions of the various behaviors are shown in Table 1. The percentage of the accumulative length of each behavior during the total sampling period per day (6 h) was calculated to represent collectively the frequency and duration of each specific behavior.

### 2.5. Welfare evaluation

Welfare status was assessed according to the Welfare Quality® Assessment protocol for poultry EU standards of animal welfare (Welfare Quality®, 2009). Scores were given to plumage cleanliness, hock burn, pad dermatitis, and gait as 3-point scales. The definition of pad dermatitis and hock burn is as follows: 0 = no evidence of lesions; 1 = moderate, superficial lesions; and 2 = large or deep lesions, ulcers,

**Table 1**
Experimental behavioral echograms.

| Behavior | Definition |
|---|---|
| Feeding | Bird is located next to feeder and has its beak inside the feeder. |
| Drinking | Bird's head is raised toward nipple drinkers and is either attempting to or is currently contacting its beak with the drinker. |
| Foraging | Bird is pecking or scratching at the ground. |
| Sitting | Bird has ceased locomotion and its breast is in contact with the ground. Eyes may or may not be closed. |
| Standing | Bird maintains upright position on motionless, extended legs. |
| Walking | Relatively low-speed displacement of bird on the ground in which the propulsive force is derived from the action of the legs. |
| Running | Higher speed displacement of bird on the ground in which the propulsive force is derived from the action of the legs. |
| Preening | Bird is using its beak to peck, stroke or comb plumage. |
| Dust bathing | Bird is lying on the ground and tossing dirt onto its back/wings by ruffling and shaking its feathers. |