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Effect of Vitamin C, Shackling and Crating Stress on Tonic Immobility Reactions of Broiler Chickens in Preslaughter

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Abstract: The objective of this study was to assess fear responses of broiler chicks to vitamin C, different shackling and crating times in preslaughter. In this study, tonic immobility (TI) was used as an index of fear reactions. At 42 d of age, 80 male broiler chicks were randomly selected for 8 treatments in the experiment. The treatment included treated and untreated vitamin C for two shackling times (60 and 120 s) and subsequently two crating times (2 and 4 h). The equal numbers of birds were provided drinking water containing either 0 or 1000 ppm of L-ascorbic acid for 36 h. The TI was determined following the crating. Vitamin C and handling time resulted in a significant reduction in duration of TI in broilers drank treated water with vitamin C and for 60 s shackling times ($p < 0.05$). Time of the crate had no significant effect on the duration of TI ($p > 0.05$). Number of attempts to induce TI were not affected by vitamin C, shackling and crating time ($p > 0.05$). It was concluded that the use of vitamin C in drinking water during 36 h preslaughter may offer a practical method for alleviating underlying fearfulness and enhancing poultry welfare.

Key words: Broiler, vitamin C, shackling, crating, stress, tonic immobility

INTRODUCTION

There are some welfare problems in broiler chicken production related especially to pre-slaughter management and transport, such as meat quality, carcass bruising, physiological stress and elevated mortality (Nicol and Scott, 1990; Ali *et al.*, 2008; Vieira *et al.*, 2011). The slaughter of poultry differs from the slaughter of all other meat-producing animals because the live birds are hung upside down in shackles and crating prior to slaughter. Commercial broiler live haul involves a number of potential stressors, including shackling, crating and transportation that can cause increased production of adrenal hormones and affect the welfare of the birds. Kannan and Mench (1996) reported crating causes an increase in plasma corticosterone levels in broiler chickens. The method of crating (Duncan, 1989) and the duration of crating (Kannan and Mench, 1996; Yalcin and Guler, 2012) can also influence the stress response shown by the bird. Duncan (1989) found that birds that were crated and transported on a vehicle for 40 min had higher plasma corticosterone concentrations than birds that were crated and loaded onto the vehicle but not transported. Further, Cashman *et al.* (1989) reported that fear levels in birds were mainly determined by transportation and not just by catching and loading.

Knowles and Broom (1990) reported that the handling component was the most potent stressor for broiler chickens. The handling method adopted may have implications for bird welfare. According to Gentle and

Tilston (2000), the shackling of commercial poultry involves the insertion of each leg into parallel metal slots and holding the bird inverted for a period of time before stunning and slaughter. Shackling the birds in an inverted position on the shackles would probably also increase the plasma corticosterone response, because holding broilers by their legs in an inverted position has this effect (Kannan and Mench, 1996). Kannan *et al.* (1997) stated that the duration of shackling had a significant influence on plasma corticosterone concentrations in experiments with male broilers. Bedanova *et al.* (2007) reported the act of shackling is a considerable traumatic procedure for broilers and its stress effect is markedly dependent on duration of shackling period that the broiler chickens experience. Korte *et al.* (1997) studied the effect of manual restraint in chickens on plasma corticosterone concentrations and found plasma corticosterone level significantly higher during manual restraint (time-dependent elevation) compared with resting birds.

The preslaughter handling is a potentially traumatic process; the tonic immobility (TI) fear reactions of broilers to various preslaughter treatments are therefore measured. TI is thought to provide a useful measure of general fearfulness, because close relations have been found between TI reactions and its responsiveness to a variety of fear-eliciting situations in poultry (Jones, 1989). Scott *et al.* (1998) stated that frightened birds could be put into TI, an unlearned, catatonic state, the duration of which is positively related to the level of fear of the birds.

According to Bedanova *et al.* (2007) duration of shackling period was positively correlated with tonic immobility duration. Zulkifli *et al.* (2000) reported different handling methods (upright and inverted) had a significant effect on tonic immobility (TI) durations in broiler chicks.

However, the need for attenuating the adverse physiological and behavioral consequences associated with harvesting of broiler chickens is recognized within the poultry industry. One of the approaches that has yielded some promising results is the dietary supplementation of vitamin C. Vitamin C is perhaps the most studied vitamin in relation to perturbation of homeostasis in poultry. There is substantial evidence to suggest that under field and laboratory situations, treating poultry with vitamin C may enhance productivity, immune response, disease resistance and survivability under stressful conditions (Gross, 1988; Kutlu and Forbes, 1993; Zulkifli *et al.*, 1996). Avian species have been reported to be able to synthesize vitamin C (Roy and Guha, 1958). However, environmental insults cause a marked increase in vitamin C requirements and birds are not able to synthesize sufficient vitamin C to replace the severe losses of this vitamin during stress (Pardue and Thaxton, 1986). Zulkifli *et al.* (2000) reported that the addition of vitamin C to drinking water reduced TI duration in broiler chickens in preslaughter handling stress condition. Kannan and Mench (1997) attempted to habituate broiler chickens to preslaughter handling through repeated handling during the growing period but failed. Satterlee *et al.* (1989) reported that the addition of vitamin C to drinking water dampened adrenocortical response to the traumatic experience of harvesting and transport in broiler chickens. However, when subjecting broilers to a brief capture and cooping procedure, Satterlee *et al.* (1994) noted the opposite. Thus, additional studies are needed to further define the effect of vitamin C treatment on preslaughter stress responses.

This experiment was performed to study the effect of vitamin C, different periods of shackling and crating in preslaughter on fear responses by measuring tonic immobility in broiler chickens.

MATERIALS AND METHODS

Birds and treatments: Three hundred day-old broiler chicks (Ross 308) were housed in an experimental house. The birds were raised to 42 days of age on wood shavings litter with bird density of 10 chickens per m². The birds were under continuous lighting. Feed and water were provided *ad libitum*. The birds were fed a standard broiler starter mash (3000 kcal ME/kg; 21% crude protein) from 0 to 20 d of age, followed by a broiler grower mash (3050 kcal ME/kg; 19% crude protein).

At 42 d of age, 80 male broiler chicks were randomly selected for 8 treatments in the experiment. The treatment included treated and untreated vitamin C for two handling time (shackling treatments) 60 and 120 s and subsequently two crating time 2 and 4 h. The equal numbers of birds (2000 h) were provided drinking water containing either 0 or 1000 ppm of L-ascorbic acid for 36 h. Plastic bottle waterers with a capacity of 4 L were used (10 birds per drinker). To maintain the stability of crystalline vitamin C in solution, the waterers of vitamin C treated birds were replenished every 4 h with fresh vitamin C solution (Satterlee *et al.*, 1994; Zulkifli *et al.*, 2000). To avoid the confounding effect of periodic disturbance to the birds, the waterers of control birds were also replenished every 4 h. At 42 d of age, broiler chicks were tested for TI for two shackling times (60 and 120 s) and subsequently two crating times (2 and 4 h). For inducing shackling stress, one bird was held by both legs in one hand in an inverted position and allowed to flap freely and the experimenter walked and held the birds with both hands for 60 and 120 s and placed in the crates (86x56x23 cm) for 2 and 4 h, with 10 birds placed in each crate.

TI tests: A total of 80 birds (10 birds per each treatment) were tested individually for duration of TI. Immediately following the handling and crating treatment, the birds were carried to a separate room (no visual contact with other birds) and subjected to TI measurements. A modification of the procedure described by Benoff and Siegel (1976) was used. Tonic immobility was induced by laying the bird down on its right side and gently restraining it by hand for 15 s. Then the hand was removed and the experimentalist retreated approximately 1 m out of sight of the bird and remained silent. The time was measured from withdrawal of the hand until the bird straightened up. If the bird straightened up in less than 10 s, it was restrained repeatedly. If TI was not induced after 3 attempts, the duration of TI was considered 0 s. If the bird did not straighten up within 10 min, it was removed and given the maximum duration of 600 s. The number of inductions required to attain TI was also recorded for each bird.

Statistical analyses: The data for duration of TI were normalized using a logarithmic transformation prior to analysis. Duration of TI and number of attempts to induce TI data were analyzed using vitamin C, handling time and crating time as main effects along with their interactions. Statistical analyses were conducted using the ANOVA general linear models procedure of SAS software (SAS Institute, 1997). When ANOVA revealed significant effects, means were separated by Duncan's multiple range test. Treatment means differed significantly at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 shows that vitamin C and handling time resulted in a significant reduction in duration of TI in broilers drank treated water with vitamin C and for 60 s shackling time ($p < 0.05$). Time of crate had not significant effect on duration of TI ($p > 0.05$). Number of attempts to induce TI were not affected by vitamin C, shackling and crating times ($p > 0.05$).

In the present study the method that has been used to measure fear levels was to induce tonic immobility (Benoff and Siegel, 1976; Jones, 1992). Tonic immobility is an anti-predator behaviour shown in situations where the chicken has been caught by a predator. By pretending to be dead, there is a better chance to escape in an unguarded moment (Thompson and Liebreich, 1987). The duration of tonic immobility has been shown to correlate positively with fear and stress levels measured by the serum corticosterone (Lin *et al.*, 2006).

In agreement with our results, Zulkifli *et al.* (2000), reported birds were provided drinking water containing 1200 ppm of L-ascorbic acid during the 24 h period had the lower duration of TI in comparison with untreated water. In their experiment there was no difference in number of attempts to induce TI. Satterlee *et al.* (1994) indicated that the supplemental vitamin C shortened TI duration in 24-d-old broiler chicks stressed with capture and 10 min of cooping. Several studies have demonstrated the possibility of alleviating fear in Japanese quail by supplementation of vitamin C (Satterlee *et al.*, 1993; Jones *et al.*, 1996).

In the present study, the lower shackling time (60 s) shortened duration of TI. Bedanova *et al.* (2007) stated shackling duration had a significant influence on TI duration and decreased it. They reported a highly significant positive correlation between duration of shackling period and TI duration was found in their experiment, which indicated an increased level of fear in shackled broilers that grew with the extension of the shackling period. Similarly, Zulkifli *et al.* (2000) observed a prolonged TI duration in response of broiler chicks to hanging in an inverted position and claimed augmented fearfulness. Furthermore, Jones (1989, 1992), Scott *et al.* (1998) and many others also report increased TI duration in associated with rough handling in domestic poultry and related these changes to the fear level of birds. This statement is in agreement with Knowles and Broom (1990) who compared various preslaughter processes in broilers and concluded that the handling component was the most potent stressor for broiler chickens.

The results of our experiment indicated that crating time for 2 and 4 h had not effect on TI. In agreement with our findings, Kannan *et al.* (1997) stated crating time did not influence plasma corticosterone, epinephrine, or norepinephrine concentrations and meat quality in

Table 1: Mean (\pm SEM) duration of tonic immobility (s) and number of attempts to induce tonic immobility by vitamin C, Shackling and crating time

Variable	----- Tonic Immobility -----	
	Duration	Induction
Vitamin C		
+	154 \pm 18 ^b	0.75 \pm 0.13
-	204 \pm 20 ^a	0.66 \pm 0.12
Handling time		
60 s	160 \pm 15 ^b	0.78 \pm 0.13
120 s	203 \pm 17 ^a	0.62 \pm 0.11
Crating time		
2 h	181 \pm 25 ^a	0.78 \pm 0.12
4 h	180 \pm 22 ^a	0.62 \pm 0.11

^{a,b}Means within a column-subgroup with no common superscripts differ significantly ($p < 0.05$)

broilers, prior to processing. In their experiment male broilers were held in crates (10 birds per crate) for either 0, 1, 2, 3, or 4 h prior to processing. However, Chloupek *et al.* (2008) reported duration of crating for 8 and 12 h significantly influenced the stress response in broiler chickens. It may be related to the lower time of crate in our experiment than study of Chloupek *et al.* (2008).

Supplemental vitamin C may offer a feasible method for alleviating underlying fearfulness and enhancing poultry welfare, concomitantly. The potential fear-ameliorating effect of vitamin C in other breeds of chickens, particularly those of high susceptibility to fear, merits further investigations. As indicated in earlier studies (Satterlee *et al.*, 1993, 1994), there is no clear explanation for the fear-ameliorating effect of vitamin C, although it could be associated with neurobiological alterations such as synthesis of catecholamine and the conversion of dopamine to norepinephrine. Given the putative link between fearfulness and adrenocortical activity (Jones, 1996), the blocking effect of vitamin C on adrenal steroidogenesis is another likely route of action. According to the results of our study use of vitamin C in drinking water during 36 h preslaughter may offer a practical method for alleviating underlying fearfulness and enhancing poultry welfare.

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