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# TONIC IMMOBILITY AND ADRENAL RESPONSE IN CHICKENS FED SUPPLEMENTAL TRYPTOPHAN

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## Abstract

GUDEV, D., P. MONEVA, S. POPOVA-RALCHEVA and V. SREDKOVA, 2011. Tonic immobility and adrenal response in chickens fed supplemental tryptophan. *Bulg. J. Agric. Sci.,* 17: 560-566

Tonic immobility duration was tested in 24 Label chickens at 34 and 49 d of age, whereas and adrenal response to catching and subsequent 30 min long crating were studied before (36 d of age) and following dietary tryptophan supplementation for 15 d (51 d of age).

Seventy nine percent of the studied chickens showed short duration of tonic immobility (less fearful) whereas the rest of the chickens had several times longer tonic immobility duration (extremely fearful). Adrenal response to catching was higher in the chickens which had longer duration of tonic immobility but the response to 30 min crating after catching was similar in all chickens irrespective of the tonic immobility duration. Dietary tryptophan supplementation for 15 d reduced ($P>0.05$) tonic immobility duration in the extremely fearful chickens. Supplemental tryptophan alleviated adrenal response to catching and crating ($P>0.05$), but plasma corticosterone did not correspond to the duration of tonic immobility in each individual. Our data suggest that tonic immobility duration as an indicator of fear does not correspond to the strength of adrenal response to catching and crating because of the complexity and transient nature of the fear emotion.

*Key words:* tonic immobility, fear, animal welfare, handling, crating, tryptophan, corticosterone, chickens

## Introduction

Fear is an adaptive behavior whose biological role is to protect the animal from psychochemical damage. It is defined as a reaction to the perception of actual danger (Forkman et al., 2007). The increased intensity of fear response can have deleterious consequences on poultry welfare and performance (Faure et al., 2003). Rough handling increases susceptibility to tonic immobility in broilers (Jones, 1992). Crating of broilers has been shown to be more potent stressor than handling (Kannan, 1998). Various behavioral and endocrine reactions to fearful events are widely used as indicators of fear. It is not possible to attribute a given behavior to any single emotion because of the complexity of the mechanisms underlying fear-related responses (Boissy, 1998). One of the most commonly used fear tests for poultry is tonic immobility elicited by manual restraint. A number of studies support the notion that under natural conditions tonic immobility serves in the capacity of predator defense (Gallup et al., 1971; Ratner et al., 1967). The enhanced tonic immobil-

ity is believed to be reflection of heightened fear (Gallup, 1979; Jones et al., 2005; Jones et al, 1991; Maser and Gallup, 1974). Hitherto there has been no standardized method for fear measurement, because of the complexity of the concept of fear (R. B. Jones). A consistent trend towards greater adrenocortical activation was reported in high-fear hens, which showed long tonic immobility reactions when compared to low-fear counterparts (Beuving et al., 1989). Similarly, plasma corticosterone correlated positively with the duration of tonic immobility in chickens during transport (Duncan, 1989) and shackling (Bedanova et al., 2007). Also, continuous corticosterone administration via an osmotic minipump, predisposed birds to react more fearfully to alarming stimulations (Jones et al., 1988). However, there still exists controversy over the concept of fear and its reliable measurement (Jones, 1996). Relatively few studies have been focused on the relation between the hypothalamic-pituitary adrenal axis and the state of anxiety and fear despite the demonstrated stress-induced anxiety (Selye, 1974). It is argued that at least part of the corticosteroid response to restrain stress is mediated by an increase in serotonergic activity that is dependent on increased supply of the precursor, tryptophan (Joseph and Kennet, 1983). Hoes (1980) speculates that hypercorticism causes disorders in the L-tryptophan metabolism and thus reduces cerebral serotonin synthesis. Serotonin is important for the regulation of the adaptional processes. It appears that serotonin plays essential role in the control of respiration, muscular tension and level of anxiety (Hoes, 1980).

This study evaluated consistency between the rate of plasma corticosterone elevation and corresponding duration of tonic immobility as a behavioral marker of fear in chickens exposed to handling and crating. In addition we tested the effect of supplemental dietary tryptophan on both tonic immobility duration and plasma corticosterone response to handling.

## Materials and Methods

Twenty four female Label chickens were randomly allocated into 2 groups and reared in battery cages with wire floors. The cages were housed in solid broiler building. Temperature was maintained within the required range. Feed and water were provided *at libitum*. Twelve of the chickens were fed commercial mash throughout the experiment (control group), whereas the rest the chickens (n=12) were given the same feed supplemented with 1% tryptophan from 36 d to 51d of age (experimental group).

The birds were subjected to tonic immobility test at 34 d (1$^{st}$ test) and 49 d (2$^{nd}$ test). Tonic immobility was induced by inverting the chicken on its side and applying lateral manual restraint until the chicken stops struggling. Tonic immobility duration was recorded from the moment the chicken became immobile until the bird righted itself. Tonic immobility test was conducted in separate room within the same building.

Adrenal response to handling and crating was conducted at 36 d and 51d of age. It was assessed immediately following capture (0 min). Then the chickens were placed in crates for 30 min. Adrenal response was tested again immediately following the crating. Blood samples were collected by the wing vein within 1-3 min after catching.

Plasma corticosterone level was measured by immunoassay kit (IBL, Gesellschaft fur immunchemie und immunbiologie, MBH, D 22335 Hamburg, Germany).

The results are expressed as means ±S.E.M. and were analyzed by ANOVA.

## Results and Discussion

Tonic immobility duration in 79.16% of the chickens tested at the age of 34 d (1$^{st}$ test), varied between 40 and 220 seconds. The rest of the chickens showed higher duration of tonic immo-

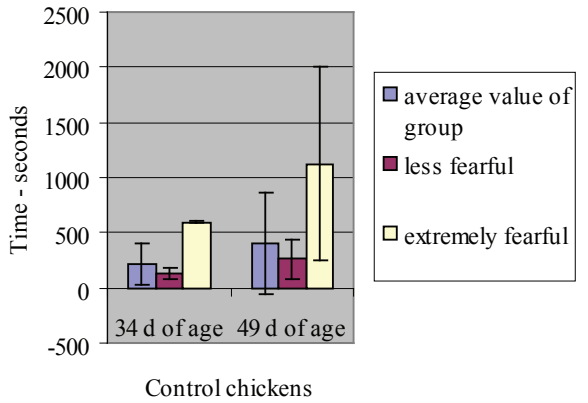


**Fig. 1. Tonic immobility duration in control chickens tested at 34 d and 49 d of age**

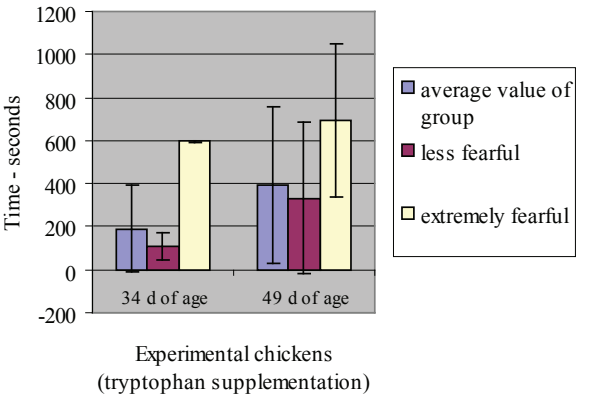


**Fig. 2. Tonic immobility duration in experimental chickens before (at 34 d of age) and following 14 d of tryptophan supplementation (at 49 d of age)**

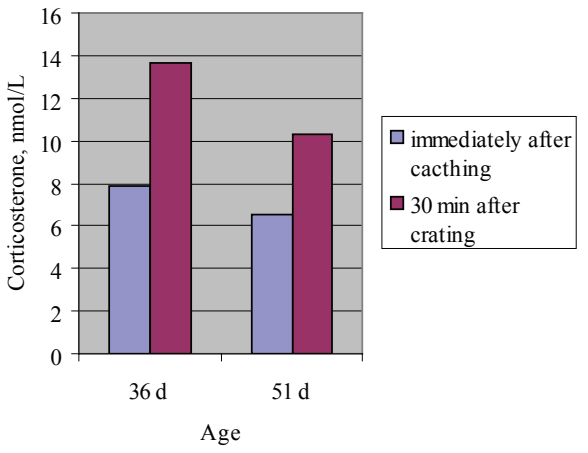


**Fig. 3. Adrenal response of less fearful chickens to handling and crating at 36 d of age and following 15 d of tryptophan supplementation (51 d of age)**

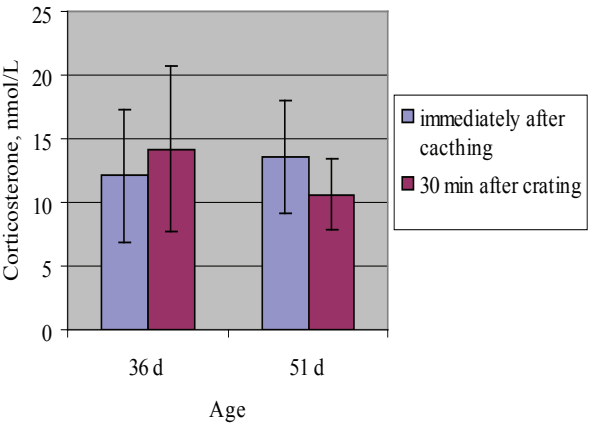


**Fig. 4. Adrenal response of extremely fearful chickens to handling and crating at 36 d of age and following 15 d of tryptophan supplementation (51 d of age)**

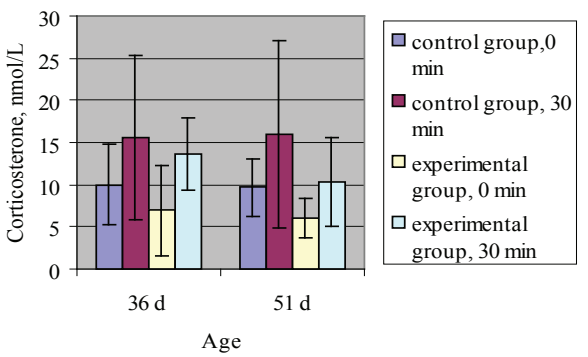


**Fig. 5. Adrenal response to catching and crating in control and tryptophan supplemented chickens at 36 and 51 d of age (mean values)**

bility that was within 590-600 seconds. Numerous studies that have examined the direct and indirect contribution of fear in relation to tonic immobility (TI) provided strong support for the notion that fear underlies tonic immobility in domestic fowls (Gallup et al., 1971; Gallup et al., 1972; Gallup et al., 1970). Therefore, we can assume that around 20% of the tested chickens were extremely fearful. Adrenal response of the extremely fearful chickens immediately after catching did not differ significantly (Figure 4) from that of the less fearful chickens (Figure 3) at 36 d and 51 d of age

although it tended to be higher in the extremely fearful chickens.

Supplemental dietary tryptophan given for 15 d had no effect on the average value of tonic immobility duration tested at 49 d (Figure 2) as compared to control group (Figure 1). However, tonic immobility duration in the extremely fearful chickens (from the experimental group was almost twofold lower ($P>0.05$) at 49 d of age relative to their control counterparts. Our results are not consistent with the reported dose-dependent increase in tonic immobility following systemic injections of tryptophan (Gallup et al., 1977). These controversial results could be due to the different way and duration of tryptophan administration in both cases. Our results are in agreement with the reported shorter duration of tonic immobility in broiler chickens supplemented with tryptophan (Newberry and Blair, 1993). It is noteworthy that tonic immobility duration during the second test (at 49 d) increased in both, control (Figure 1) and tryptophan supplemented chickens (Figure 2) as compared to the I$^{st}$ test (at 34 d of age), but the rate of enhancement was higher in control birds. However, when we scrutinized individual data, we found unchanged or slightly lower duration of tonic immobility in 20% of the chickens from both groups (control and tryptophan supplemented). This finding could be related to individual behavior responses of each bird (Cockrem, 2007) or to specificity of the repeated TI induction which could lead either to habituation and the eventual loss of the response or to sensitization of the response over time (Marx et al., 2008). Also, it is widely accepted that brain serotonin is implicated in the control of tonic immobility duration (Henning et al., 1986; Farrabollini et al., 1984; Walnak et al., 1981) and the level of individual tryptophan uptake could change brain serotonin concentration.

Individual plasma corticosterone level did not correspond strictly to the duration of tonic immobility in both extremely fearful and less fearful chickens. These results are consistent with the view that corticosterone responses and fear behavior responses vary in individual birds (Cockrem, 2007). Plasma corticosterone levels were similar in the high-fear and low-fear chickens following 30 min of crating (Figures 3 and 4). These results are in contrast with the reported higher corticosterone response in quill selected for short duration of tonic immobility (Hazard et al., 2008). This discrepancy could be due to the fact that plasma corticosterone level in their experiment was measured at the end of each tonic immobility episode, whereas plasma corticosterone and tonic immobility in our experiment were measured separately at different days. Chickens under tonic immobility were reported to have decreased heart rate, respiration rate, body temperature (Nash et al., 1976), analgesia and decreased autonomic arousal (Marx et al., 2008), whereas chickens under stress are known to have increased arousal, cardiovascular tone and respiration rate (Dettmer, 2009).Therefore, the only similarity between stress and tonic immobility episodes is the heightened analgesia. These facts bring tonic immobility as a fear marker into question.

Plasma corticosterone level in tryptophan treated chickens tended to be lower immediately after catching and following 30 min long crating, relative to control chickens (Figure 5). This is consistent with the reported decreased peak of heart rate in pigs during transport simulation (Peeters et al., 2004). Also, the decreased adrenal response to crating in tryptophan supplemented chickens occurred against the background of unchanged mean values of tonic immobility duration, relative to their control counterparts. These data and the reported inverse relationship between corticosterone response to restraint and tonic immobility duration in quail (Hazard et al., 2008) give further support of the view that adrenal response to stress does not correlate to tonic immobility duration. In our earlier study supplemental tryptophan had no effect on corticosterone level in chickens that were deprived of feed and water for seventeen hours each day in four consecutive days (Moneva et al., 2008)

These controversial results are consistent

with the widespread acceptance of serotonin as a modulator of hypothalamic-pituitary- adrenal axis. There is considerable evidence that any change in serotonin activity alters plasma corticosterone level (Harbuz et al., 1993; Chung et al., 1999; Hemrick-Luecke, 2002). Dietary manipulation of brain serotonin was reported to be influenced by stress (Markus, 2008). Furthermore, it has been demonstrated that circulating serotonin stimulates cortisol secretion (Dinan, 1996). Similarly, serotonin transporter knockout mice are reported to have reduced corticotrophin-released factor expression in the hypothalamus and reduced glucocorticoid receptor expression in hypothalamus, pituitary and adrenal cortex (Jiang et al., 2008). Lately a new concept concerning tryptophan metabolism under stress was proposed (Miura et al., 2008). According to this concept proinflammatory cytokines promote kynurenine pathway, which is the main tryptophan metabolic pathway, thus depriving 5-HT pathway of tryptophan.

Our results concerning adrenal response to handling and crating in tryptophan supplemented chickens could be reconciled with serotonin concept by the finding that acute and chronic treatment with serotonergic drugs may have opposite effect on hypothalamic-pituitary-adrenal axis as a result of adaptations with serotonin system (Nadeem et al., 2004; Ahrens et al., 2007).

## Conclusion

Taken together our results considered within the context of the available literature, suggest that tonic immobility duration does not seem to be reliable marker of fear because of its complex nature and phenomenology. Also, the rate of adrenal response to handling and crating did not strictly correlate with the duration of tonic immobility in individual birds.

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