Adrenocortical Responses of Japanese Quail to a Routine Weighing Procedure and to Tonic Immobility Induction

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ABSTRACT Tonic immobility (TI) is induced by brief manual restraint, and it is a commonly used test of fearfulness, particularly in poultry. However, in view of increasing ethical concerns about experimentation on living animals, there is a need to ensure that the tests used do not elicit unacceptable distress. In the United Kingdom, there is some debate as to whether TI should be classified as a regulated experimental procedure that requires a Home Office license to experiment on living animals under the Animals (Scientific Procedures) Act of 1986. The present study compared plasma corticosterone (PC) concentrations in undisturbed Japanese quail (controls) with those exposed to the induction of TI or those exposed to a routine weighing procedure. Circulating PC concentrations were higher following TI induction or weighing than in the controls, but the 2 treatments elicited similar adrenocortical responses. Further, the PC levels found here were lower than those reported elsewhere when quail were exposed to crating, transport, or mechanical immobilization stressors. We therefore concluded that the induction of TI might be best regarded as a mild stressor. Furthermore, studies using this technique can identify ways of alleviating fear and thereby improving well-being. Collectively, we believe that the use of TI is justified, and it does not require classification as a regulated procedure in the United Kingdom or elsewhere.

(*Key words*: tonic immobility, corticosterone, weighing, regulated procedure)

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INTRODUCTION

Fear can seriously damage poultry welfare and performance, and the reduction is of major importance (Jones, 1996; Faure et al., 2003). Of course, it is necessary to induce and to then measure this harmful state before ways of alleviating it can be developed. Tonic immobility (**TI**) has been one of the most widely used tests, and it is regarded as a relatively robust measure of underlying fearfulness, particularly in poultry (Gallup, 1979; Jones, 1986, 1996; Faure and Mills, 1998; Schutz et al., 2001; Riedstra and Groothuis, 2002). Briefly, TI is a state of reduced responsiveness to external stimulation that can be easily induced by brief manual restraint in a wide variety of avian, mammalian, and invertebrate species. The duration of TI is considered positively related to the antecedent fear state because it correlates in the predicted direction with other scores of fear, and it is sensitive to fear-inducing stimuli and fear-reducing stimuli (Jones et al., 1991; Faure and Mills, 1998).

Ethical concerns about experimentation on living animals are becoming more prevalent worldwide, and in some cases legislative requirements can constrain the use of TI tests. For example, because TI is often classified as a technique of particular interest that involves induction of psychological stress integral to the procedure, a researcher cannot conduct TI tests in the United Kingdom without first securing a license to experiment on living animals under the Animals (Scientific Procedures) Act of 1986. **[AUTH QUERY: Please add publication information to the references section]** On the other hand, other tests of fear such as approach and avoidance responses to a novel and, hence, frightening object, open field, social separation, and emergence from shelter do not require to be licensed. We also know that TI is a natural adaptive response to capture and restraint, that the reaction is transient, and that repeated induction results in relatively rapid habituation (Gallup, 1979; Jones, 1986, 1996). These findings suggest that its elicitation may not be a particularly stressful event. In other words, the level of fear associated with TI may be mild to moderate rather than severe. Not surprisingly, therefore, there is some uncertainty about whether TI should actually be considered a regulated procedure. Therefore, in an attempt to contribute to the debate about the implications on well-being of this technique, the present study was designed to deter-

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mine whether the induction of TI is more or less stressful (in terms of the adrenocortical response) than a routine management procedure such as weighing. We used Japanese quail because they are inexpensive and useful models for chickens as well as an important agricultural species in several countries (Baumgartner, 1994; Faure et al., 2003).

MATERIALS AND METHODS

The Japanese quail used in this study were from a larger population of 240 birds of a nonselected line maintained at Louisiana State University for 30 generations. Egg incubation, chick brooding, feeding, and lighting procedures were similar to those described elsewhere (Jones and Satterlee, 1996) with the exception that chicks were brooded from 1 d of age in mixed-sex groups of approximately 60 within each of 4 compartments of a Petersime battery brooder (model 2SD-12, Petersime Incubator Co., Gettysburg, OH) modified for quail.

At 30 d of age, the quail were sexed by plumage coloration, and 72 randomly selected males were rehoused in individual cages measuring 51 × 15 × 26.5 cm (length × width × height) in 4 tiers of 2 battery cage units (36 birds per unit, Alternative Design Manufacturing and Supply, inc., Siloam Springs, AR). Feed (quail breeder diet: 21% CP, 2,750 kcal of ME/kg) and water were available ad libitum, and a photoperiod of 14L:10D was used.

The birds were randomly allocated to 1 of 3 treatment groups, each comprising 24 birds, when they were 38 d old. Each bird was used only once. In treatment 1, quail in the control group remained undisturbed in their home cage before bleeding (see below). In treatment 2, quail were carried individually by hand to a separate room where they were induced into TI. Each bird was placed on its back in a v-shaped polystyrene cradle covered with cloth so that its head and neck hung over the end of the cradle. It was restrained in this position for 15 s with 1 hand on the sternum and 1 lightly cupping the head. The experimenter then retreated about 2 m but remained in sight. After the quail had remained immobile for 1 min it was returned to its home cage where it remained for 8 min 30 s before 0.5 mL of blood was withdrawn by cardiac puncture for subsequent assay of plasma corticosterone (**PC**) concentration (see below). In treatment 3, each bird was taken from its cage and held in a crate for 1 min before being restrained in a cylindrical weighing tube for 30 s. It was then returned to the home cage where it remained undisturbed for 8 min 30 s until blood (0.5 mL) was withdrawn. Bleeds were always completed within a maximum of 30 s to minimize the likelihood of an adrenocortical response to handling and bleeding per se. Anesthesia was not used because it can significantly elevate PC concentrations (Scanes et al., 1980). Treatments and bleeding were randomized to minimize any effects of time of day or order of testing.

The whole blood samples were dispensed into chilled heparinized containers and stored on ice for approximately 1 h before centrifugation at 555 × *g* for 20 min. The plasma samples were then withdrawn and stored deep frozen (–20°C) until their PC concentrations were determined by radioimmunoassay (ICN Biomedicals, Costa Mesa, CA).

The results were transformed to log10 before they were subjected to 1-way ANOVA. This was followed by least significant difference posthoc comparisons of treatments.

Table 1. Plasma corticosterone (PC) concentrations in 38-d-old male quail that had remained undisturbed (controls) or that were exposed to tonic immobility induction or a routine weighing procedure (means ± SEM)

Treatment (n)	PC concentration (ng/mL)
Control (23)	1.428 ± 0.147[a]
Induction of tonic immobility (23)	2.400 ± 0.417[b]
Weighing (24)	2.317 ± 0.300[b]

[a,b]Means without a common letter differ significantly ($P < 0.05$).

RESULTS AND DISCUSSION

The results are shown in Table 1. One-way ANOVA showed that there was a significant effect of treatment ($F(2,67) = 3.18$; $P = 0.048$). Posthoc tests revealed that PC concentrations were significantly higher in quail exposed to TI induction ($P = 0.043$) or to the weighing procedure ($P = 0.025$) than in the undisturbed controls. There were no detectable differences between the circulating PC levels of birds from the weighing or the TI groups.

The elevated PC concentrations found here in quail that had been weighed or induced into TI confirm that both of these procedures were perceived as stressful. However, these circulating PC levels are substantially lower than those of 10.47 and 4.60 ng/mL previously reported (using similar assay techniques) when Japanese quail were exposed to brief periods of transport in a crate containing strangers or of mechanical immobilization, respectively (Satterlee et al., 1993; Jones et al., 2000). Such a comparison suggests that the induction of TI might be best regarded as a mild rather than a moderate or severe stressor. Collectively, the present findings, the transient nature of the TI response and its rapid habituation indicate that the beneficial information that may be obtained from the studies involving this technique, in terms of identifying ways of alleviating fear and thereby improving well-being, justify its use. We further conclude that classification of TI as a regulated procedure, [see the United Kingdom's Animals (Scientific Procedures) Act 1986], may not be strictly necessary.

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REFERENCES

Baumgartner, J. 1994. Japanese quail production, breeding and genetics. World's Poult. Sci. J. 50:227–235.

Faure, J. M., W. Bessei, and R. B. Jones. 2003. Direct selection for improvement of animal well-being. Pages 221–245 in Poultry Genetics, Breeding and Biotechnology. W. M. Muir and S. Aggrey, ed.CABI Publ., Wallingford, UK.

Faure, J. M., and A. D. Mills. 1998. Improving the adaptability of animals by selection. Pages 235–264 in Genetics and the Behaviour of Domestic Animals. T. Grandin, ed. Acad. Press, San Diego.

Gallup, G. G., Jr. 1979. Tonic immobility as a measure of fear in domestic fowl. Anim. Behav. 20:166–169.

Jones, R. B. 1986. The tonic immobility reaction of the domestic fowl: A review. World's Poult. Sci. J. 42:82–96.

Jones, R. B. 1996. Fear and adaptability in poultry: Insights, implications and imperatives. World's Poult. Sci. J. 52:131–174.

Jones, R. B., A. D. Mills, and J. M. Faure. 1991. Genetic and experiential manipulation of fear-related behavior in Japanese quail chicks (*Coturnix coturnix japonica*). J. Comp. Psychol. 105:15–24.

Jones, R. B., and D. G. Satterlee. 1996. Threat-induced behavioural inhibition in Japanese quail genetically selected for contrasting adrenocortical response to mechanical restraint. Br. Poult. Sci. 37:465–470.

Jones, R. B., D. G. Satterlee, D. Waddington, and G. G. Cadd. 2000. Effects of repeated restraint in Japanese quail genetically selected for contrasting adrenocortical respones. Physiol. Behav. 69:317–324.

Riedstra, B., and T. G. G. Groothuis. 2002. Early feather pecking as a form of social exploration: The effect of group stability on feather pecking and tonic immobility in domestic chicks. Appl. Anim. Behav. Sci. 77:127–138.

Satterlee, D. G., R. B. Jones, and F. H. Ryder. 1993. Effects of Vitamin C supplementation on the adrenocortical and tonic immobility fear reactions of Japanese quail genetically selected for high corticosterone response to stress. Appl. Anim. Behav. Sci. 35:347–357.

Scanes, C. G., G. F. Merrill, R. Ford, P. Mauser, and C. Horowitz. 1980. Effects of stress (hypoglycaemia, endotoxin and ether) on the peripheral circulating concentration of corticosterone in the domestic fowl (*Gallus domesticus*). Comp. Biochem. Physiol. 66:183–186.

Schutz, K. E., B. Forkman, and P. Jensen. 2001. Domestication effects on foraging strategy, social behaviour and different fear responses: A comparison between the red junglefowl (*Gallus gallus*) and a modern layer strain. Appl. Anim. Behav. Sci. 74:1–14.