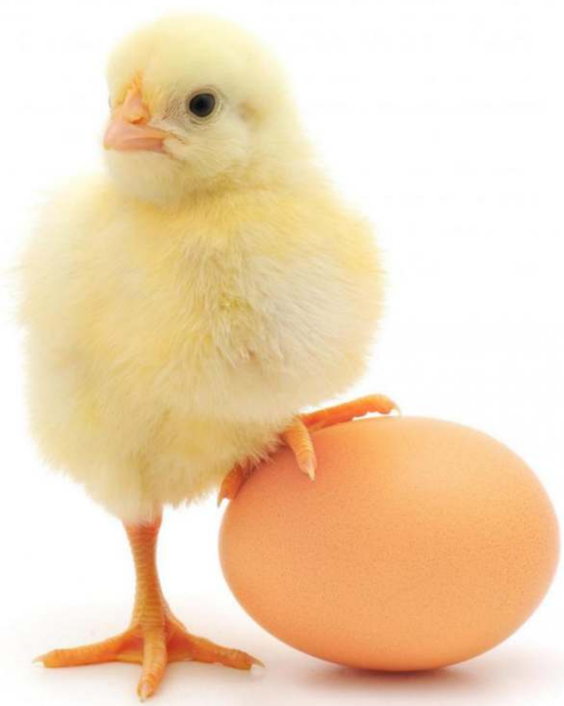
ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 14 (11): 602-605, 2015
ISSN 1682-8356

Evaluation of a Modified Manual Restraint Test for Estimating Fearfulness in Laying Hens

Natsuki Fukano, Natsuho Wada, Takao Oka and Takashi Bungo
Laboratory of Animal Behavior and Physiology, Graduate School of Biosphere Science,
Hiroshima University, Higashi-Hiroshima 739-8528, Japan

Abstract: The aim of the present study was to evaluate effect of a new manual restraint test for the detection of fearfulness on the blood parameters in hens. Each chicken was held in an underarm with mild pressure, whereas the other hand was used to restrain the legs. Thereafter, latencies to the first struggle and frequencies of struggling were recorded for 5 min. At the end of the test, the blood samples were collected. At one week after the test, their bloods were collected again as control. Median of latency (sec.) or frequency (number) of struggling in laying hens was 116.0 and 4.5, respectively. No significant differences were detected in the levels of glucose and FFA by manual restraint while the level of corticosterone just after the test was higher than that at one week after the test. These findings demonstrate that the newly developed restraint test is useful for detecting fearfulness in chickens.

Key words: Chicken, fearfulness, restraint, stress, corticosterone

INTRODUCTION

Behavioral or temperament traits in livestock have been the concern of animal scientists and producers because they not only induce conflict among animals but may also affect the efficiency of daily farm management. In addition, animal welfare should be considered to improve sustainability. For the reasons, it is important to quantify and estimate behavioral or temperament traits and to determine whether selection programs alter these traits, which in turn could impair the adaptation of animals to husbandry systems.

There are various tests for fearfulness in birds, such as the open field (Faure *et al*., 1983), novel object (Jones, 1985; Barnett and Hemsworth, 1989), isolation (Yanagita *et al*., 2011; Bungo *et al*., 2015) and tonic immobility tests (Jones *et al*., 1996; Mignon-Grasteau *et al*., 2003; Nakasai *et al*., 2013). In general, these tests have shown that fear responses, as measured by the specific reactions in a certain test, have a high heritability. However, the results among these tests did not clearly indicate high correlation of the estimations for fearfulness (Uitdehaag *et al*., 2008) because fear is related to many physiological and neural pathways.

Manual restraint is used to measure the fearful-related response (Uitdehaag *et al*., 2008). Freezing during manual restraint tests is generally interpreted as a fear reaction in chickens (Forkman *et al*., 2007), while high activity during restraint could reflect low fear (Uitdehaag *et al*., 2008). However, it is difficult for large fowls, such as Plymouth Rock, to be restrained manually. Recently, we developed a new technique for large fowls useful for detecting fearfulness in chickens (Oka and Bungo, 2014). However, the effect of stress response on blood parameters remains unknown.

The objective of this study, therefore, was to investigate the effectiveness of modified manual restraint method for estimating fearfulness on blood parameters in chickens.

MATERIALS AND METHODS

The experiment was conducted in accordance with the regulations of the Animal Experiment Committee of Hiroshima University.

Animals: The data were collected from commercial layer-type chicken (Julia Light). Hens (28-wk of age, n = 6) were housed in individual cages under ambient temperature (23-24°C). The room was maintained on a 14L:10D lighting schedule with light turned on at 0800 h and off at 2200 h. They were provided *ad libitum* with commercial layer feed (17% CP, 2,800 kcal/kg of AMEn) and had free access to water.

Manual restraint test: Hen was taken out of its home cage and restrained for 5 min according to the modified method as described by Bolhuis *et al*. (2009). Briefly, the experimenter held a hen in his left underarm with mild pressure. The left hand of the experimenter was placed on the abdomen of the tested bird. The right hand of the experimenter was used to stretch the legs of the chicken at the start of the test, after which the right thumb and index finger remained loosely around both legs and

Corresponding Author: T. Bungo, Laboratory of Animal Behavior and Physiology, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima 739-8528, Japan

were only used to prevent the bird from escaping when struggling. After each struggle, hens were gently brought back to the original test position (Oka and Bungo, 2014). Observations of bird responses during either restraint test included the latency to first struggle and frequency of struggles as an escape attempt. Hens were gently taken from a cage in random order. Each restraint manipulation was performed by one of two persons between 1300 and 1400 h.

Plasma levels of glucose, free fatty acid (FFA) and corticosterone (CORT): Immediately after the observation test, all hens were bled by wing vein puncture and blood was collected into heparinized tubes and centrifuged for 15 min. At one week after the test, same birds were gently taken from a cage and their bloods were collected as control (no stress manipulation). Harvested plasma was stored at -20°C until assayed.

The plasma concentrations of glucose and FFA were measured using a commercial kit (Glucose C II-Test Wako and NEFA C-Test Wako, Wako Pure Chemical Industries Ltd., Osaka, Japan).

Plasma CORT concentrations were measured by enzyme immunoassay, according to the method used in a previous study (Tanizawa *et al*., 2014). In brief, plasma was extracted with dichloromethane and the organic phase was decanted to a glass tube. After drying the tubes with the extracted organic phase, borate buffer was added and used for the assay. The reconstituted samples were applied into wells of microtitre plates coated with goat anti-rabbit IgG antibody followed by the addition of anti-corticosterone antibody (COSMO BIO Co., Tokyo, Japan) and HRP-conjugated corticosterone (COSMO BIO Co., Tokyo, Japan). After 2 h, incubation plates were washed and 3, 3-5, 5-tetramethylbenzidine solutions were applied to the substrate. Then, optical density was calculated at 450 nm wavelength using an Ultramark Microplate Reader (BIO-RAD Laboratories, Tokyo, Japan). All samples were run in the same assay to avoid inter-assay variations. Intra-assay coefficient of variation was 6.2%.

Statistical analysis: The data were analyzed using the commercially available package, StatView (SAS Institute, 1998) and the differences between treatments were determined by Student-t test. Differences were considered to be significant when P was less than 0.05. Results of blood parameters are presented as means±SEM.

RESULTS

The latency and frequency of struggling during 5 min restraint test in hens are shown in Table 1. Median and standard deviation of latency (sec) or frequency (number) of struggling in laying hens was 116.0±75.5 and 4.5±0.8, respectively.

Figure 1 shows the effect of manual restraint on blood parameters in laying hens. After 5 min of the treatment, the levels (ng/ml) of CORT in control (no treatment) and restrained chicks differed significantly. The restraint-treated chicks exhibited a higher level of plasma CORT than the control ($p<0.05$), while the levels of glucose and FFA did not change with manual restraint.

DISCUSSION

For the manual restraint test, it has been reported that high activity during restraint could reflect low fear (Uitdehaag *et al*., 2008), while immobility during restraint tests is interpreted as a reflection of fear in chickens (Forkman *et al*., 2007). In the previous study using two breeds of Japanese native chicken, Uzurao and Chabo (Oka and Bungo, 2014), it revealed that less fearful Uzurao chickens displayed more activity (latency: 63±25 sec, frequency: 4.0±1.6) under the new manual restraint test than the more fearful Chabo breed (latency: 170±111 sec, frequency: 1.5±1.3). Because layers in this study (latency: 116±76 sec, frequency: 4.5±0.8) showed more active response and started struggling sooner than Chabo in the manual restraint test, layers might be less fearful than the Chabo. Although it is difficult to directly compare the results with other manual restraint method, there was a positive correlation of frequency between the restraint methods (Oka and Bungo, 2014). Uitdehaag *et al*. (2011) showed that frequencies of White Leghorn and Rhode Island Red birds at 47 weeks of age were 0.7±0.3 and 1.9±0.3, respectively. Also, White Leghorn have shown low activity (frequency: 0.7±0.2) when subjected to the manual restraint test (de Haas *et al*., 2012). Therefore, layer hens (Julia Light) might be less fearful than the Rhode Island Red and the White Leghorn. Because more fearful hens have shown decreased egg production due to stress (noise, human contact and so on), it seems reasonable to consider that they have been selected unintentionally to be less fearful of human handling. In fact, there are some reports of such relationships between production and behavior traits, as a side effect of selection (Schütz *et al*., 2002).

Plasma CORT is the primary glucocorticoid used as a measure of endocrine response to stress in the hypothalamic-pituitary-adrenal (HPA) axis and it is well known that plasma CORT rapidly increases after exposure to stress stimuli (Charmandari *et al*., 2005). The treatment in this experiment appeared to be stressful to the chicks because manual restraint treatment could increase the concentration of CORT with HPA axis activation (Fig. 1). Korte *et al*. (1997) demonstrated that there was a time-dependent increase in the level of plasma CORT of chickens during the manual restraint test. Thus, it seems that the new method of manual restraint test may cause a similar physiological condition.

It is well known that increased CORT induces gluconeogenesis (Charmandari *et al*., 2005) and results

Table 1: Medians, standard deviations and ranges of struggling in the manual restraint test for laying hens

Struggling	Median	SD	Range
Latency (sec.)	116.0	75.5	75-253
Frequency	4.5	0.8	3-5

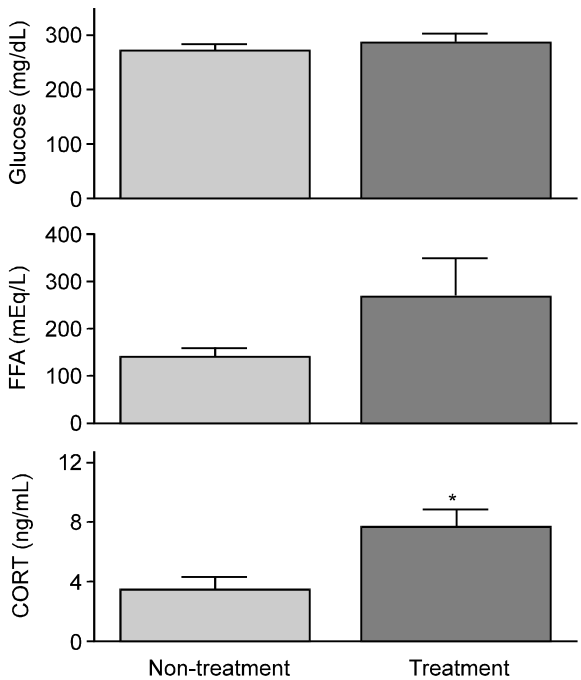


Fig. 1: Effect of manual restraint on plasma glucose, free fatty acid (FFA) and corticosterone (CORT) in laying hens. Data are presented as mean±SEM. Asterisks refer to the level of statistical significance (*, p<0.05).

in hyperglycemia in mammals (Ricart-Jané *et al*., 2002). The activation of the HPA axis under stressful conditions is also accompanied by the sympathetic-adrenal-medullary axis, as suggested by the increase in plasma catecholamines (Kregel *et al*., 1991). Increased plasma catecholamines, thereafter, induce beta-adrenoceptor-mediated lipolysis (Bowers *et al*., 2004). Increased plasma FFA by lipolysis is used as a measure of neural stress response in the sympathetic-adrenal-medullary axis. Our result, however, shows that the treatment did not affect the concentration of plasma glucose and FFA in hens (Fig. 1). Consistent with our findings, a study of isolation stress in chicks shows that exposure to novel environment for 10 min did not affect plasma glucose and FFA levels (Yanagita *et al*., 2011). There is a possibility that we could not find CORT-induced gluconeogenesis and beta-adrenoceptor-mediated lipolysis because the time of treatment (5 min) might be too short.

Conclusion: It is usual for estimation of fearfulness to apply manual restraint on chickens (Uitdehaag *et al*., 2008, 2009; Bolhuis *et al*., 2009) but the new manual restraint method is also applicable because both test results indicated a similar tendency. The new restraint method could be useful for large chickens, such as Plymouth Rock because the experimenter employing hand restraint must use one hand to restrain the upper part of the body of the tested bird.

ACKNOWLEDGEMENTS

The authors acknowledge the staff of the Laboratory of Animal Behavior and Physiology, Hiroshima University, for their technical support in maintaining the animals. This study was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science. Financial support was also received from the Graduate School of Biosphere Science, Hiroshima University (Grant-in-Aid for Fundamental Research). The authors are grateful to Dr. L.M. Liao for proofreading the manuscript.

REFERENCES

Barnett, J.L. and P.H. Hemsworth, 1989. Fear of humans by laying hens in different tiers of a battery: behavioural and physiological responses. Br. Poult. Sci., 30: 497-502.

Bolhuis, J.E., E.D. Ellen, C.G.V. Reenen, J. De Groot, J.T. Napel, R.E. Koopmanschap, G.D.V. Reilingh, K.A. Uitdehaag, B. Kemp and T.B. Rodenburg, 2009. Effects of genetic group selection against mortality on behaviour and peripheral serotonin in domestic laying hens with trimmed and intact beaks. Physiol. Behav., 97: 470-475.

Bowers, R.R., W.T.L. Festuccia, C.K. Song, H. Shi, R.H. Migliorini and T.J. Bartness, 2004. Sympathetic innervation of white adipose tissue and its regulation of fat cell number. Am. J. Physiol. Reg. Integr. Comp. Physiol., 286: R1167-R1175.

Bungo, T., E. Nakasai, M. Takawaki, H. Tanizawa and S. Kawakami, 2015. Brain monoamine asymmetry in chicks subjected to separation-stress procedure with litter substrate. J. Poult. Sci., 52: 37-41.

Charmandari, E., C. Tsigos and G. Chrousos, 2005. Endocrinology of the stress response. Ann. Rev. Physiol., 67: 259-284.

de Haas, E.N., M.S. Kops, J.E. Bolhuis, T.G.G. Groothuis, E.D. Ellen and T.B. Rodenburg, 2012. The relation between fearfulness in young and stress-response in adult laying hens, on individual and group level. Physiol. Behav., 107: 433-439.

Faure, J.M., R.B. Jones and W. Bessei, 1983. Fear and social motivation as factors in open-field behaviour of the domestic chick. Behav. Biol., 8: 103-116.

Forkman, B, A. Boissy, M.C. Meunier-Salaun, E. Calani and R.B. Jones, 2007. A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. Physiol. Behav., 91: 531-565.

Jones, R.B., 1985. Fear responses of individually caged laying hens as a function of cage level and aisle. Appl. Anim. Behav. Sci., 14: 63-74.

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

Korte, S.M., G. Beuving, W. Ruesink and H.J. Blokhuis, 1997. Plasma catecholamine and corticosterone levels during manual restraint in chicks from a high and low feather pecking line of laying hens. Physiol. Behav., 62: 437-441.

Kregel, K.C., J.M. Overton, D.G. Johnson, C.M. Tipton and D.R. Seals, 1991. Mechanism for pressor response to nonexertional heating in the conscious rat. J. Appl. Physiol., 71: 192-196.

Mignon-Grasteau, S., O. Roussot, C. Delaby, J.M. Faure, A. Mills, C. Laterrier, D. Guemene, P. Constantin, M. Mills, G. Lepape and C. Beaumont, 2003. Factorial correspondence analysis of fear-related behaviour traits in Japanese quail. Behav. Processes, 61: 69-75.

Nakasai, E., H. Tanizawa, M. Takawaki, K. Yanagita, S.I. Kawakami, T. Oka, M. Tsudzuki and T. Bungo, 2013. Age-dependent change of tonic immobility response in chicks of a native Japanese chicken breed, Tosa-Jidori. J. Poult. Sci., 50: 321-325.

Oka, T. and T. Bungo, 2014. Comparison of behavioral responses to manual restraint tests in two breeds of Japanese native chicken. Int. J. Poult. Sci., 13: 545-548.

Ricart-Jané, D., V. Rodríguez-Sureda, A. Benavides, J. Peinado-Onsurbe, M.D. López-Tejero and M. Liobera, 2002. Immobilization stress alters intermediate metabolism and circulating lipoproteins in rat. Metabolism, 51: 925-931.

SAS Institute, 1998. Statistical analysis Systems Institute, version 5. INC., Cary, North Carolina, USA.

Schütz, K., S. Kerje, Ö. Carlborg, L. Jacobsson, L. Andersson and P. Jensen, 2002. Analysis of a red junglefowl x White Leghorn intercross reveals trade-off in resource allocation between behavior and production traits. Behav. Genet., 32: 423-433.

Tanizawa, H., J.I. Shiraishi, S. Kawakami, M. Tsudzuki and T. Bungo, 2014. Effect of short-term thermal conditioning on physiological and behavioral responses to subsequent acute heat exposure in chicks. J. Poult. Sci., 51: 80-86.

Uitdehaag, K.A., T.B. Rodenburg, Y.M. van Hierden, J.E. Bolhuis, M.J. Toscano, C.J. Nicol and J. Komen, 2008. Effects on mixed housing of birds from two genetic lines of laying hens on open field and manual restraint responses. Behav. Processes, 79: 13-18.

Uitdehaag, K.A., T.B. Rodenburg, C.G. Van Reenen, R.E. Koopmanschap, G.D.V. Reilingh, B. Engel, W.G. Buist, H. Komen and J.E. Bolhuis, 2011. Effects of genetic origin and social environment on behavioral response to manual restraint and monoamine functioning in laying hens. Poult. Sci., 90: 1629-1636.

Yanagita, K., J.I. Shiraishi, S. Kawakami and T. Bungo, 2011. Time course changes in the blood parameters and the expression of diencephalic CRH and AVT mRNA due to acute isolation stress in chicks. J. Poult. Sci., 48: 130-134.