Effect of a tranquilizer on stress control in birds under restraint.

Yojiro Yanagawa and Kaoru Suzuki*

Field Science Center, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

*Correspondence to Kaoru Suzuki, Field Science Center, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

Abstract

Birds are easily disabled by stress, which often has adverse effects on their health, and thus birds should be handled as little as possible to minimize stress. In the present study, the effects of the tranquilizer Diazepam against restraint stress were evaluated using the Japanese quail *Coturnix coturnix japonica*. When restraint stress was applied to the birds by squeezing the cage, the plasma corticosterone (hormone which increases under stress) levels increased markedly, while no difference was observed in the birds that had only undergone blood sampling. Thus, the squeezed cage is a suitable instrument for restraint stress. Intramuscular injection of diazepam before restraint stress resulted in no significant increase in the corticosterone level. In contrast, the corticosterone level increased markedly when saline was administrated as a placebo. These findings demonstrate that use of a tranquilizer is very effective in reducing stress, and that tranquilizers will be useful in the study of reductions in stress or investigation of the mechanisms of stress in birds.

Key Words: bird, corticosterone, diazepam, restraint, stress control, tranquilizer

INTRODUCTION

Birds easily become stressed, with many resultant adverse effects, including death. One common cause of stress in birds is being restrained, which is often required when birds are used for ecological or environmental studies or when being treated for injury. In addition, birds struggle under restraint that can be harmful to both birds and humans. Sick or injured birds exhibit “sick bird syndrome”, which manifests as puffing up, reduction in activity, and inappetence. The birds exhibiting “sick bird syndrome” should be handled carefully as little as possible to minimize stress and improve prognosis (Turner 1985). Even birds that are not severely disabled are likely to be very frightened and vulnerable to stressors (Cooper 1984). Thus, stress must be controlled when birds are handled.

Bird stress as evaluated by corticosterone (hormone secreted in response to stress) response to various kinds of stressors such as environmental stress (Smith et al. 1994), social stress (Satterlee et al. 1983) and immobilization stress (Jones et al. 1992, 2000) has been previously investigated. Restraint is one of the more common stressors of birds. One

particular requirement is that in the examination of immobilization stress, the stress applied needs to be identical in strength for each test. This point had been taken into consideration in order to make every treatment to be same in this study.

Although the control of stress is very important, few previous studies have attempted to reduce stress responses using drugs and to prove the effects by measuring stress hormone levels. Many studies on the effects of tranquilizers or anesthesia have focused on immobilization or ease of handling, not stress. The few studies that have examined the use of drugs against stress, evaluated the effects only by monitoring behavioral responses (Watanabe et al. 1966). However, whether birds still feel stressed when they are apparently calm or immobile is unclear. Thus, results obtained by observing behavior alone may be influence by the subjectivity of the observer. The effect of drugs against stress needs to be more objectively evaluated.

The aim of the present study was to monitor the stress response in birds under restraint precisely, and to investigate the effect of a tranquilizer

diazepam on the stress control. The study was conducted using the Japanese quail *Coturnix coturnix japonica*, which is widespread in Japan and has long been used in laboratory research.

MATERIALS & METHODS

Birds

Male Japanese quails from Saitama Experimental Animals Supply Co., Ltd. (,Japan), about eight-week-old and weighing about 100 g, were housed individually in laboratory cages for 1 to 3 weeks before the experiment. The birds had *ad libitum* access to water and food. The temperature in the room installing the cages was maintained at 20℃, and the photoperiod was kept at 9:00 to 17:00.

Experiment 1

Twelve Japanese quails were assigned to two groups of six: the Basal and Stressed groups. For the Basal group, a blood sample was collected from each bird and the bird returned to its cage. Blood was collected again 10 min later. For the Stressed group, each bird was captured in its cage and blood collected, after which the bird was placed into the “squeeze cage” and put under stress. The bird was kept in the narrow space of the squeeze cage for

10 min and then a blood sample again collected. All tests were performed between 12:30 to 14:00, and behavioral responses were also observed.

Experiment 2

Twelve Japanese quails were assigned to two groups of six; the Saline and Diazepam groups. For the Saline group, each bird was captured in its cage, a blood sample collected, and normal saline then immediately administered intramuscularly as a placebo. After the administration of saline, the bird was placed into the squeeze cage without squeezing. The birds was left alone for 20 min and restraint stress, as described above, then inflicted for 10 min, after which a blood sample was again collected. The Diazepam group received the same treatment as the Saline group, except that 5 mg/kg of diazepam (Cercine, Takeda, Japan) was administered to each bird instead of saline. The dosage of diazepam (5 mg/kg) and duration of sedation (20 min) were determined by assessment of the literature (Fukushi 1999) and confirmed by preliminary experiments. All treatments were performed from 12:30 to 14:00, and behavioral responses were also

observed.

Restraint and Blood sampling

The squeeze-cage was used for the infliction of a restraint stress. The squeeze-cage was 20×25×40 cm (W×H×D) and made of metal. To inflict stress, the space of the cage was narrowed to 20×25×3 cm (W×H×D).

Blood (0.5ml) was taken by venipuncture of the jugular vein and withdrawn through a 27-gauge needle into a 1 ml plastic plain syringe. The sample was then transferred to an EDTA coated tube. The interval between capture in the cage and collection of blood was always less than 2 min. The samples were centrifuged and the plasma were stored at －20℃ until the hormone assay.

Hormone Assays

Plasma corticosterone levels were measured using a modification of the radioimmunoassay reported by Nishida *et al.* (1976).

Statistics and ethics

Corticosterone levels in blood samples collected after each treatment were compared with the levels before treatment using a paired t-test for all groups. The differences were considered statistically significant at $P<0.05$. All data are presented as mean ± SE.

The protocols used in this experiment were approved by the Tokyo University of Agriculture and Technology Laboratory Animal Care and Use Committee.

RESULTS

In experiment 1(Fig. 1), in the Basal group the plasma corticosterone concentration did not significantly differ between the initial (0.49±0.12 ng/ml) and final blood samples (1.17±0.44 ng/ml). The plasma corticosterone level after the 10 min restraint (23.63±5.08 ng/ml) was significantly higher than before the treatment in the Stressed group (0.92±0.27 ng/ml, $P < 0.05$). The birds in the Stressed group struggled almost continuously whilst in the squeeze cage.

In experiment 2 (Fig. 2), in the Saline group, the plasma corticosterone concentration significantly increased after treatment (before: 0.70±0.27 ng/ml, after: 11.48±0.74 ng/ml, $P < 0.05$). In the Diazepam group, no significant difference in plasma corticosterone concentration was observed between before (1.51±0.57 ng/ml) and after (4.71±2.51 ng/ml) the diazepam treatment, although some birds did show an increase. Behavior in the squeeze cage also differed between the two groups, with birds struggling frequently in the Saline group, but rarely moving in the Diazepam group.

DISCUSSION

In response to a stressor, secretion of glucocorticoid from the adrenal cortex increases. The major glucocorticoid in birds is corticosterone (Carsia and Harvey 2000), and the concentration of corticosterone in bird blood is a known marker of stress and has been used in many studies of bird stress (Satterlee et al. 1983; Jones et al. 1992, 2000; Smith et al. 1994; Fowler 1999; Cockrem and Silverin 2002). Plasma corticosterone levels usually start to rise 2-3 minutes after capture (Cockrem and Silverin 2002). The adrenocortical stress response, when prolonged, can result in a number of physiological problems for birds. As the plasma corticosterone level increases, the growth and skeletal development of the young bird are depressed, while adults experience loss in weight and reduced reproductive capability, often despite increases in food consumption (Bartov et al. 1980). In addition, stress reduces the fitness of the individual, thus making the bird more susceptible to diseases in general (Siegel 1980).

The results of the present study suggest that restraint in a squeeze cage is sufficient for birds to feel stress. The corticosterone levels increased

markedly and the birds struggled constantly against the restraint. One of the advantages of the squeeze cage is that the space in which the bird is immobilized can be varied to some extent, thereby accommodating various sized birds. By reducing the internal space by the same extent for each treatment, the same amount of restraint, and thus stress, is applied to each bird.

The results of the present study suggest that the administration of diazepam can reduce the stress response in birds. Tranquilizers have been used against various stress disease in humans (Tsutsui 1990), and thus may be suitable for use against bird stress. Tranquilizers have been previously used for the sedation of birds (James and Peek 1966; Watanabe et al. 1966), and if the sedation is deep enough, the birds will close their eyes and sit quite still. Under sedation they also become indifferent to outside stimuli, and are thus very easy to handle. Although the level of sedation in the present study appeared sufficient, the corticosterone levels tended to increase a little in some birds, indicating individual differences in the response to the tranquilizer. Thus, for sufficient control of stress, clinical

symptoms and other factors, such as age or character, must be considered. The best approach is to comprehensively evaluate the conditions, and then adopt a combination of tranquilizer use and either another drug or covering of the eyes to keep the bird quiet, thereby reducing stress.

From the point of view of animal welfare, it is necessary to handle birds in the least stress-provoking way possible. This is also important when collecting clinical or biological data, as stress is not only a threat to the health of the bird, but also cause physiological changes as well, which may distort findings. Furthermore, the less the bird is handled the better, which can be facilitated by using a tranquilizer to calm the bird down and reduce struggling. The use of tranquilizers should result in more time effective research and better stress management for birds, enabling better research into the conservation of wildlife.

REFERENCES

Bartov, I., L.S. Jensen, and J. R. Veltmann Jr. (1980) Effect of corticosterone and prolactin on fattening in broiler chicks. Poultry Science 59:1328-1334.

Carsia, R. V. and S. Harvey (2000) Adrenals. In: Whittow, C.G. (ed.); Sturkie's Avian Physiolohy 5th ed. Academic Press, San Diego, pp.489-537.

Cockrem, J. F. and B. Silverin (2002) Sight of a predator can stimulate a corticosterone in the Great Tit (*Parus major*). General and Comparative Endocrinology 125:248-255.

Cooper, J. E. (1984) First aid for wild birds. In Practice 6:195-198.

Fukushi, H. (Translator, 1999) Diagnosis, basic examination and principals of treatment. In: Fukushi, H. and T. Yamamaguchi (Translators for the Japanese version); British Small Animal Veterinary Association Manuals of Psittacine Birds. Gakusosha Company Ltd., Tokyo, pp.23-33. (in Japanese, in English for original version).

Fowler, G. S. (1999) Behavioral and hormonal responses of Magellanic

penguins (*Spheniscus magellanicus*) to tourism and nest site visitation. Biological Conservation 90:143-149.

James, M. and M. S. Peek (1966) Chlordiazepoxide and pentobarbital as tranquilizers for Cowbirds and Coturnix quail. Journal of American Veterinary Medical Association 149:950-952.

Jones, R. B., D. G. Satterlee, and F. H. Ryder (1992) Fear and distress in Japanese quail chicks of two lines genetically selected for low or high adrenocortical response to immobilization stress. Hormones and Behavior 26:385-393.

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 responses. Physiology and Behavior 69:317-324.

Nishida, S., S. Matsumura, M. Horino, H. Oyama, and A. Tenku (1976) A radioimmunoassay for human plasma corticosterone. Endocrinologia Japonica 23:465-469.

Satterlee, D. G., L. A. Jacobs-Perry, and R. E. Truax (1983) Circulating

corticosterone response of wild-type Japanese quail to albinoquail intrusion. Poultry Science 62:310-313.

Siegel, H. S. (1980) Physiological stress in birds. Bio Science 30:529-534.

Smith, G. T., J. C. Wingfield, and R. R. Veit (1994) Adrenocortical response to stress in the common diving petrel *Pelecanoides urinatrix*. Physiological Zoology 67:526-537.

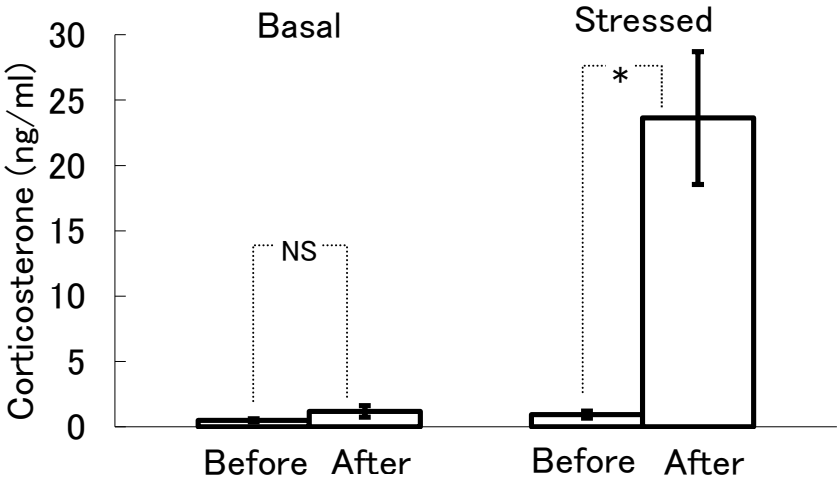
Tsutsui, S. (1990) Stress and medicine for modern people. Pharmacia 26: 138-142 (in Japanese).

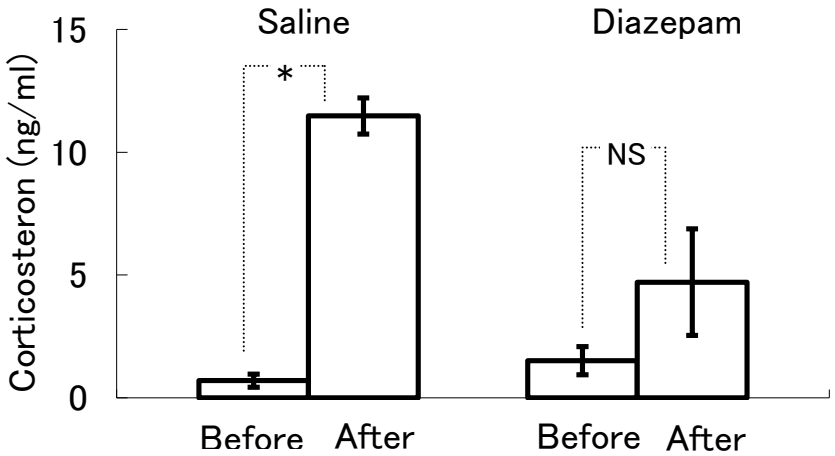
Turner, T. (1985) First aid for cage birds. In Practice 7:76-81.

Watanabe, T. , M. Saito, T. Tateiwa, and J. Kobara (1966) The sedative effect of diazepam to some animals. Journal of Japanese Association of Zoological Gardens and Aquarium 8:9-14 (in Japanese).

Fig 1: Changes in plasma corticosterone concentration before and after restraint in the Basal and Stressed groups. Data are presented as mean±SE. (*): Significant difference between before and after treatment ($P<0.05$), (NS): Not significant ($P>0.05$).

Fig 2: Changes in plasma corticosterone concentration before and after restraint in the Saline and Diazepam groups. Data are presented as mean±SE. (*): Significant difference between before and after treatment ($P<0.05$), (NS): Not significant ($P>0.05$).


Basal
Stressed
Corticosterone (ng/ml)
30
25
20
15
10
5
0
NS
*
Before
After
Before
After


Saline
Diazepam
15
10
5
0
Corticosteron (ng/ml)
*
NS
Before
After
Before
After