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Comparison of Behavioral Responses to Manual Restraint Tests in Two Breeds of Japanese Native Chicken

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Abstract: The aim of the present study was to evaluate the characteristic of two native Japanese chicken breeds, Uzurao (UZ) and Chabo (CB) using two types of manual restraint tests for the detection of fearfulness. Each hen was grabbed by one hand or 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. The latency in UZ was shorter than that in CB ($p<0.01$), but there was no significant difference between restraint types. The frequency of struggling in UZ during arm restraint test was significantly higher than others ($p<0.05$) but no significant differences were found among others. Between the restraint types, there was a positive correlation ($p<0.05$) in frequency, but not in latency to struggling ($p>0.1$). These findings demonstrate that UZ birds may be confirmed to respond less fearfully in fear-eliciting situations than CB birds and further suggest that the temperamental differences may correspond to genetic characteristics of breeds. Additionally, a newly developed technique, arm restraint, is useful to detect fearfulness in chickens, similar to hand restraint.

Key words: Restraint, fearfulness, latency, struggling, Japanese native chicken

INTRODUCTION

Selection with excessive emphasis on productivity has caused a number of negative side effects on animal well-being. For example, broilers have become too heavy for normal locomotion resulting to leg disorders. It is clear that their incidence may cause a major welfare problem because leg disorders are disabling and often associated with inflammation of joints, hocks and bone (Bradshaw *et al*., 2002). To improve sustainability, poultry breeders would also have to consider animal welfare. Additionally, it is important to determine whether selection programs alter behavioral traits, which in turn could impair the adaptation of animals to husbandry systems.

At the end of the last century, scientists and livestock producers became concerned about the potential loss of indigenous breeds and a program was launched by the FAO for the genetic conservation of livestock resources (FAO, 2007). In Japan, there is a great variety of indigenous chicken bioresources (approximately 50 breeds), and most were established for special plumage, crowing and fighting traits (Tsudzuki, 2003), as opposed to European and American breeds that were often developed for products (egg and meat). The study of Japanese native breeds as genetic resource, however has been given little attention from the vantage point of scientific interest and current research efforts have been directed primarily towards enhancing not only commercial production systems, but also animal behavior related to animal welfare.

The Uzurao (UZ) is characterized with a single comb, white earlobes, yellow shanks and rumplessness [the origin of the name is tail (the Japanese word “wo”) such as the quail (the Japanese word “Uzura”)]. The adult body weight is around 675 g for males and 600 g for females. This breed is thought to have been established during the late Edo era (Tsudzuki, 2003). The Chabo (CB: a Japanese bantam) is representative of bantam chickens, and has short shanks with adult body weight of around 730 g for males and 610 g for females. This breed is generally believed to have come from China or Indochina and introduced into Japan during the early years of the Edo era (1603-1867). CB is reared for entertainment in many countries in North America, Europe and Asia. However, despite being popular as ornamental birds, there is poor documentation of both behavior and temperament.

The aim of this study was therefore to investigate fear-related behavior of miniature breeds of Japanese native chickens using two types of manual restraint tests (fearfulness tests), in order to use valuable indigenous bioresources for improving commercial chickens.

MATERIALS AND METHODS

Animals: The data were collected from two Japanese chicken breeds, the indigenous Chabo (CB) and Uzurao (UZ) breeds, which are kept at our institute. Newly hatched chicks were wing-banded for individual identification and housed in a temperature-controlled brooder (Zenkei-N type, Zenkeien Manufacturing Co.

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Ltd., Shizuoka, Japan) with 24-h lighting until 10 weeks posthatch. Then, birds were transferred into a colony room that was illuminated by overhead fluorescent lights from 0500 to 1900 h. In the colony room, they were grouped in steel wire mesh cages until 17 weeks of age, and reared together in each pen with sawdust litter (3.0 x 1.2 m). They were vaccinated similarly and were subjected to the same managerial, hygienic and climatic conditions. Standard commercial starter (0-6 wks: CP, 20%), grower (7-10 wks: CP, 17%) and developer (after 11 wks: CP, 15%) diets were provided *ad libitum* and the birds had free access to water under all housing conditions. The handling of birds was performed in accordance with the regulations of the Animal Experiment Committee of Hiroshima University.

Manual restraint test

Hand restraint: Each individual hen (1-3 year of age) was taken out of its home pens and restrained for 5 min according to the method described by Bolhuis *et al.* (2009). In brief, the bird was placed on a table that was located within visual and auditory range of the other birds. The experimenter used one hand to restrain the legs, whereas his other hand was used to restrain the upper part of the body of the tested bird. The hand restraining the legs mainly functioned to prevent the birds from escaping when struggling. The thumb and index finger were used to loosely restrain both legs. The other hand put mild pressure on the upper body part when a bird was not struggling. After each struggle, birds were gently brought back to their original position.

Arm restraint: Similar to the hand restraint test, the same bird was restrained for 5 min according to modified method as mentioned above. 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 were only used to prevent the bird from escaping when struggling. After each struggle, hens were gently brought back to the original test position.

Observations: 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 pen in random order. Each restraint test was performed by 1 of 2 persons between 1300 and 1600 h. The numbers of hens used were: UZ, 11 and CB, 10.

Statistical analysis: The data were analyzed using the commercially available package, StatView (Version 5, SAS Institute, Cary, USA, 1998). Logarithmic and square root transformations were applied for skewed distributions of latency and frequency, respectively. Data were analyzed by two-way ANOVA with respect to the effects of breed and restraint method. For comparisons between means of each struggling frequency, a post-hoc test was done using the Steel-Dwass test. Correlation between the restraint methods in latency or frequency of struggling was analyzed using Spearman's rank correlation coefficient analysis. The level of significance was set at $p<0.05$ and at $p<0.1$ for a trend.

RESULTS

Figure 1 shows the latency of each manual restraint test in chickens of native Japanese chicken breeds. Significant effect of breed ($p<0.01$) was observed, but no significant effect of restraint type ($p = 0.165$) and interaction ($p = 0.494$) was detected. On both restraint tests, latencies to first struggle in UZ were shorter than those in CB.

The struggling frequency during 5-min restraint test in hens are shown in Fig. 2. A significant interaction between breed and restraint type was detected ($p<0.05$). Significant breed ($p<0.001$) and restraint type ($p<0.01$) effects were also observed. The value of arm restraint test in UZ was more than that of hand restraint test in UZ and those of both tests in CB ($p<0.05$).

The relationship between hand and arm manual restraints in latency or frequency of struggling is shown in Table 1. Statistical analysis showed a positive correlation ($r = 0.533$, $p = 0.017$) between the type of restraint in frequency, but not latency to struggling ($r = 0.352$, $p = 0.117$) in hens.

DISCUSSION

The present study shows that genetic group selection with ornamental appearance affects fear-related behavior in Japanese native hens subjected to manual restraint tests. UZ showed more active response, and started struggling sooner than CB in both manual restraint tests. Immobility during restraint tests is generally interpreted as a reflection of fear in chickens (Forkman *et al.*, 2007), while high activity during restraint could reflect low fear (Uitdehaag *et al.*, 2008). This implies that UZ hens are more docile and less fearful than CB hens. Uitdehaag *et al.* (2011) revealed that less fearful Rhode Island Red birds at 47 weeks of age displayed more activity (latency: 136±20 sec, frequency: 1.9±0.3) in the manual (hand) restraint test than more fearful White Leghorn ones (latency: 244±20 sec, frequency: 0.7±0.3). Although it is difficult to directly compare their results with ours because rearing conditions and age were different, UZ and CB hens might be less fearful than the White Leghorn and UZ hens might be less fearful than the Rhode Island Red. Because these native chickens, which are ornamental birds, have many opportunities (daily management or competitive show) for human contact, it seems reasonable to consider that they have been selected unintentionally to be gentle, docile and not fearful of human handling.

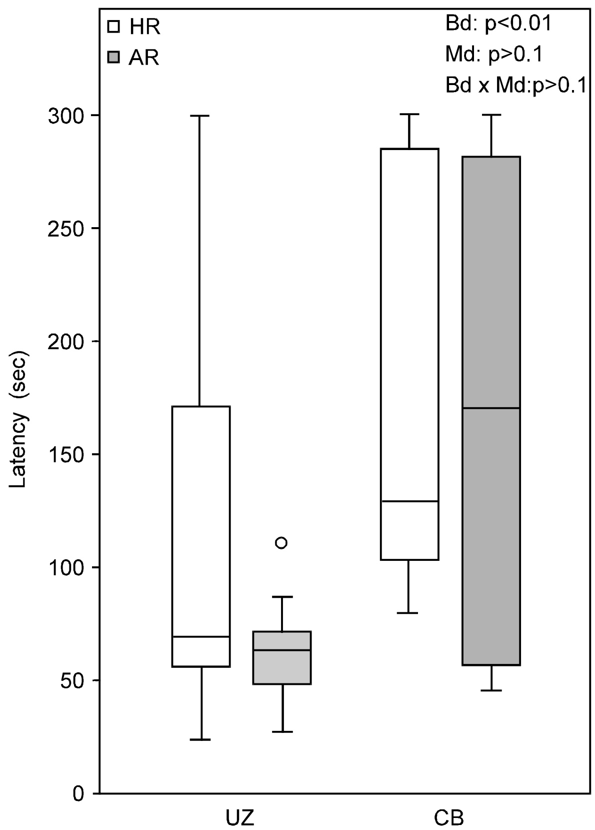

Fig. 1: Latencies of first struggle in Japanese native chicken breeds, Uzurao (UZ) and Chabo (CB). Box plots show the median (center line) and the interquartile range from the 25th to the 75th percentile. Whiskers above and below the box indicate the 10th and the 90th percentiles and circles indicate outliers. Bd, breed; Md, method; HR, hand restraint test; AR, arm restraint test

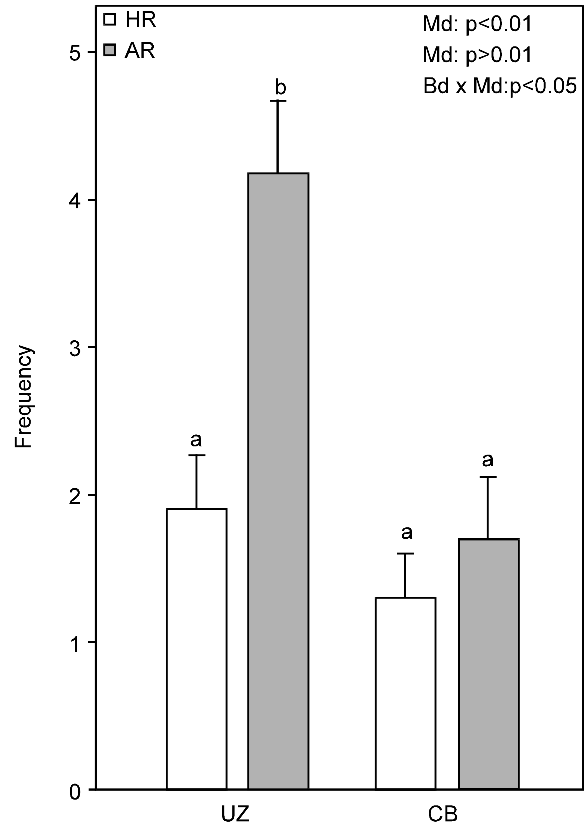

Fig. 2: Frequencies of struggle in Japanese native chicken breeds, Uzurao (UZ) and Chabo (CB). Md, method; HR, hand restraint test; AR, arm restraint test. Values are means±S.E.M. Means with different letters are significantly different at p<0.05

Table 1: Spearman correlation coefficient (r) between the restraint methods in latency or frequency of struggling in hens

	r	p
Latency (sec.)	0.352	0.117
Frequency (no.)	0.533	0.017

In birds, there are some tests for fearfulness, such as the novel object test (Jones, 1985; Barnett and Hemsworth, 1989), open field test (Faure *et al*., 1983; Yanagita *et al*., 2011), tonic immobility test (Jones *et al*., 1996; Mignon-Grasteau *et al*., 2003; Nakasai *et al*., 2013) and so on. Generally, these tests have shown that fear responses, as measured by the specific reactions in a certain test, have a high heritability. However, the results between the tests did not entirely indicate high correlation of the estimations for fearfulness (Uitdehaag *et al*., 2008) because fear is related to many physiological and neural pathways. In fact, Schutz *et al*. (2004) found some quantitative trait loci (QTL) in chickens related to fear responses in different tests (*e.g*., tonic immobility is affected by three different QTL on chromosome 1, behavior in a novel object test by a QTL on chromosome 4 and behavior in a restraint test by a QTL on chromosome 11). This complex genetic architecture may indicate that fear responses are unique under different types of stimuli, but it may also indicate a larger genetic network affecting one single emotional response. Although we could estimate fearfulness trend in birds using one of these tests, it is necessary to evaluate exact fearfulness from the result of various tests.

In the present study, we applied two similar restraint tests, hand and arm restraints. It is usual for estimation of fearfulness to apply the hand restraint in chickens (Uitdehaag *et al*., 2008, 2009; Bolhuis *et al*., 2009) but the arm restraint is also available because both test results indicated a similar tendency. In the case of big chickens, such as Plymouth Rock, the arm restraint method could be useful because the experimenter employing hand restraint must use one hand to restrain the upper part of the body of the tested bird. Furthermore, the arm restraint test might be more suitable in clarifying the difference between breeds. In the result of UZ breed, the statistical variability of the start of struggle in arm restraint was lower than that in hand restraint and then the difference in frequency between breeds also became clear. Because the reason of the difference between methods was not detected in CB and is not known to us, further investigation with various breeds is needed to aid future research of fearfulness in chickens.

Conclusion: In conclusion, UZ birds were confirmed to respond less fearfully in fear-eliciting situations than CB birds. This is the first report about the use of manual restraints in Japanese native chickens. These results suggest that responses to fear-eliciting situations are influenced by genetic factors, and it seems to be useful for the genetic selection of hens as valuable indigenous bioresources for improving commercial chickens. Moreover, it appears that the arm restraint method is effective depending on breed as a substitute for hand restraint. Further work on the behavioral characteristic tests is necessary for the selection of animals for both welfare and productivity purposes.

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