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«Reseacrh Note»

Effects of the Calls and Presence of Roosters on Egg Incubation Behavior of Nagoya Laying Hens

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The incubation behavior of the Japanese Nagoya chicken breed is a commercial issue because it often causes a sudden and sharp drop in egg production. In this study, whether the incidence of incubation behavior in Nagoya laying hens was associated with calls and the presence of roosters in the same laying house was investigated. Four experiments were conducted using commercial layer-type Nagoya hens where the hatching time of the experimental birds and the treatment order in the presence of males were changed . In Experiment 1, the proportion of incubation behavior in the presence of roosters kept in another pen located between pen-rearing hens (51.3%) was higher than that in their absence (15.9%) or with only rooster calls (23.8%). In Experiments 2, 3, and 4, the proportion of incubation behavior in the presence of roosters (47.3%, 33.3%, and 37.9%, respectively) was higher than that in their absence (33.3%, 17.4%, and 25.6%, respectively). In all experiments, approximately 70% of the incubating hens observed in the absence of roosters exhibited incubation behavior, even in the presence of roosters. Therefore, the presence of roosters may enhance egg incubation behavior in Nagoya laying hens.

Key words: incubation behavior, laying hen, Nagoya, rooster

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Introduction

The Japanese Nagoya chicken is a dual-purpose breed that produces meat and eggs. This breed originated from a cross between a local chicken from Nagoya and a Chinese Buff Cochin in the early 1880s. In 1905, it was recognized as the first practical breed used for poultry farming in Japan. The Nagoya breed was formally established in 1919 after removing shank feathers and fixing gray-colored legs. The pure breed (Nagoya × Nagoya), which produces meat and pink eggs, has been commercialized without crossing it with other breeds. In Japan, this breed has been established as the most common chicken brand, and the market prices of its meat and eggs are much higher than those of the usual broiler meat and layer eggs.

Broodiness, a precise incubation behavior, is a behavioral trait observed in most common breeds of domestic chickens, except for White Leghorn[1,2]. Red junglefowl (*Gallus gallus*) is considered a single ancestor of domestic chickens, and native breeds, such as Silkie and Chabo (Japanese Bantam), show strong incubation behaviors[1,3,4]. In contrast, commercial chickens subjected to intensive artificial selection rarely exhibit this behavior[1,2]. Because incubation behavior may be reduced or eliminated by selective breeding, these data suggest that this instinct is a heritable trait in chickens.

Incubation is the natural behavior of incubating a clutch of eggs to obtain offspring[5]. After laying a clutch of eggs, hens that incubate them usually become broody and sit on the eggs. Once hens become completely broody, they do not lay eggs for a considerable period. Consequently, incubation behavior results in ovarian regression and a loss of egg production. Therefore, incubation behavior is an undesirable trait in modern poultry farming in Japan.

Nagoya hens lay approximately 200–250 eggs annually. Although this breed has been selected to remove the incubation behavior trait over the years, a few individuals still exhibit strong incubation behavior. Incubation behavior is a commercial issue in Nagoya farming because it often causes a sharp drop in egg production. In our previous study[6], a flock of Nagoya hens

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hatched in autumn began exhibiting incubation behavior at 28 weeks of age and showed the highest incidence of incubation behavior (approximately 11%) at 33 weeks of age. The incidence changed by approximately 2%–4% at and after 38 weeks of age. The flock of chickens hatched in the spring started exhibiting incubation behavior at 28 weeks of age and showed the highest incidence of incubation behavior (approximately 8%) at 35 weeks of age. The incidence changed by approximately 2%–4% at and after 41 weeks of age. Furthermore, the incidence of incubation behavior in Nagoya hens hatched in autumn was higher than that in hens hatched in spring.

As described above, certain factors (age and hatching season) observed in Nagoya chickens may affect the intensity of the incubation behavior[6]. Additionally, several environmental conditions (high temperature, dark nests, and photoperiod) in a laying house may be conducive to the incubation behavior of chickens[1,7]. Another factor that causes incubation behavior is keeping eggs or dummy eggs in the nest for long periods, which may encourage hens to exhibit incubation behavior[1,5]. Thus, understanding the factors that cause the incubation behavior may be useful for reducing the incidence of this behavior in poultry breeding.

The development of a molecular technique to select against incubation behavior has been desirable for many years. The genetics of incubation behavior in chickens have been previously investigated[1–3]; however, they have not yet been clarified. To develop a molecular selection technique against incubation behavior, it is crucial to more accurately distinguish incubating hens from non-incubating hens. Additionally, it is difficult to distinguish incubating hens from egg-laying hens, which promptly leave the nest after laying eggs because the patterns of these behaviors are similar. If this issue is resolved, the genetic differences between incubating and non-incubating hens may be more accurately analyzed and the genetics of incubation behavior in chickens may be clarified. Moreover, if a flock of Nagoya hens is kept in an environment that easily induces incubation behavior, only non-incubating hens may be selected for breeding; consequently, the incidence of incubation behavior in the breed may be reduced in subsequent generations.

In female gray-headed juncos (*Junco hyemalis caniceps*) and red-faced warblers (*Cardellina rubrifrons*), the absence of males resulted in less time spent incubating eggs[8]. However, whether the presence of roosters in the laying house affects incubation behavior in chickens has not been investigated. Thus, in this study, whether the incidence of incubation behavior in Nagoya laying hens was associated with rooster calls and the presence of roosters in the same laying house was investigated.

Materials and Methods

Experimental birds

Four experiments were conducted. In Experiments 1 to 4, commercial layer-type Nagoya hens hatched on December 18, 2019, December 23, 2020, June 23, 2021, and June 21, 2022, were used.

Experiment 1

The layout drawings and photographs of the pens used in this study are shown in Fig. 1. Forty-three and 39 hens, respectively, were reared in two free-range slatted floor pens (9.4 m^2/pen: 3.6 m × 2.6 m) of an open laying house. Each pen contained 15 nesting boxes (23 × 31 × 31 cm) with sloped floors that allowed the eggs to roll outside. To induce hen incubation behavior, 12 dummy eggs (wooden pink-colored eggs) were laid on the slatted floor of the pen and not collected after initiating Experiment 1. Freshly laid eggs were removed daily. The hens were 180 days old at the start of the experiment and were already laying eggs. Hens were fed normal feed (CP 17.0%, ME 2,830 kcal/kg; JA Higashi Nihon Kumiai Feed Co., Ltd., Aichi, Japan) and fresh water *ad libitum*. The lighting program began at 6:00 and ended at 20:00, with light and dark periods of 14 and 10 h, respectively.

Observations of the incubating hens were divided into three periods. The first period began on June 15, 2020, when the hens were 180 days old. Hen incubation behavior was observed for 25 days out of 6 weeks. The behavior of the hens was observed from 16:00 to 17:00 h, when most had already laid eggs. Hens that exhibited typical signs of incubation behavior, such as sitting on fresh egg(s) and/or dummy egg(s), gathering egg(s) under the chest using their beak, or sitting still in the nest or in one place for long periods were identified as incubating hens. The incubated hens were fitted with a metal leg band, with an individual identification number for each leg. The second period began on July 27, 2020, when the hens were 222 days old. In pen-rearing hens, rooster crowing sounds recorded by a voice recorder (ICD-UX523, Sony Corporation, Tokyo, Japan) were continuously played by speakers from 6:00 to 18:00 h. The length of the investigation period and method for identifying incubated hens were the same as those used in the first period. The third period began on September 7, 2020, when the hens were 264 days old. During this period, five Nagoya roosters were kept in a pen located between the rearing hens (Fig. 1). The pens were separated by wire netting to observe the roosters and hear their calls. The length of the investigation period and methods were the same as those used in the previous periods.

Experiment 2

Fifty-one and 40 hens were reared in two pens in the same laying house used in Experiment 1. To induce hen incubation behavior, one dummy egg was continuously placed outside the collection space of each nesting box, unlike in Experiment 1. Before starting the experiment, 10 Nagoya roosters were kept in another pen, as shown in Fig. 1. The conditions for rearing laying hens were the same as those used in Experiment 1.

Observations were divided into two periods. The method used to identify incubating hens was the same as that used in Experiment 1. The first period began on June 21, 2021, when the hens were 180 days old. In the presence of roosters, hen incubation behavior was observed for 25 days out of 6 weeks. After 6 weeks, the roosters were moved to a different house. Before starting the second period, hens were allowed a 3-week resting interval. The second period, in the absence of roosters, began on August 23,

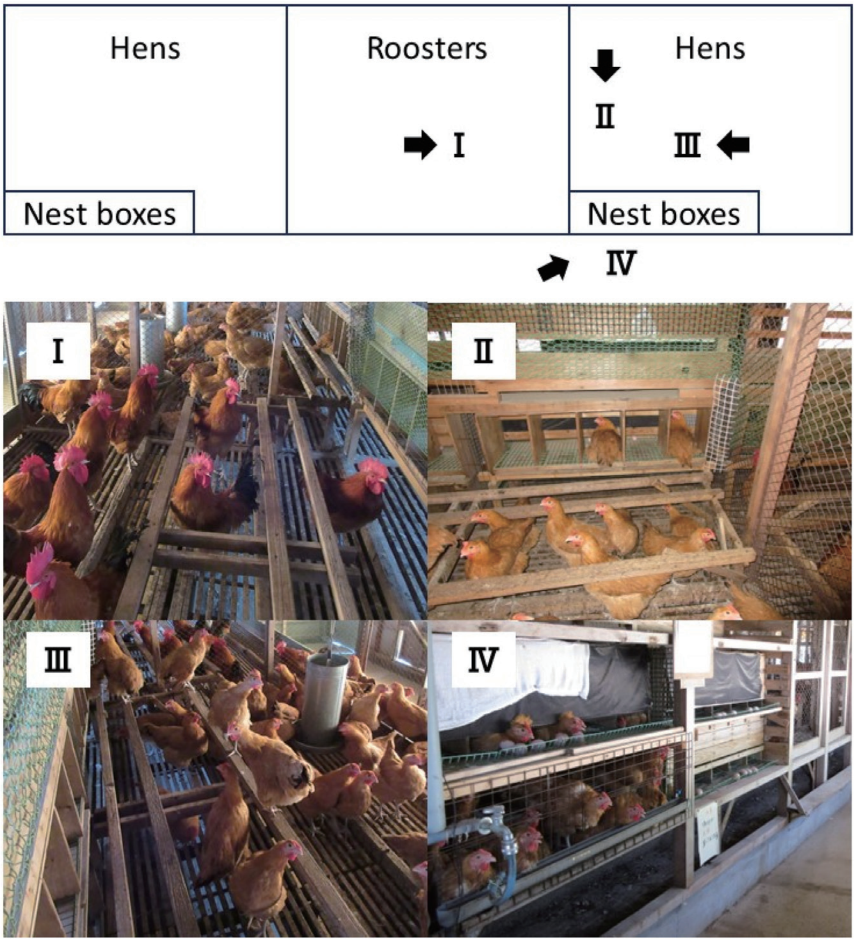

Fig. 1. **Schematic layout and photographs of pens used in all experiments.** Roman numbers indicate the location of the photographs. Arrows indicate the direction of the photographs.

2021, when the hens were 243 days old. The length of the second investigation period was the same as that of the first period.

Experiment 3

Forty-six hens were reared in one pen of the same laying house as in the above experiments. One dummy egg was continuously placed outside each nesting box, as in Experiment 2. The conditions for rearing laying hens were the same as those used in the above experiments.

Observations were divided into two periods. The method used for identifying the incubating hens was the same as that used in the above experiments. The first period began on January 10, 2022, when the hens were 201 days old. In the absence of roosters, hen incubation was observed for 25 days out of 6 weeks. The second period began on February 21, 2022, when the hens were 243 days old. During this period, 10 Nagoya roosters were kept in another pen, as shown in Fig. 1. The length of the investigation period was the same as that of the first period.

Experiment 4

Forty-four and 43 hens were reared in two pens in the same laying house used in the above experiments. One dummy egg was placed continuously as in Experiments 2 and 3. Before starting the experiment, 16 Nagoya roosters were kept in another pen as in Experiment 2 (Fig. 1). The conditions for rearing laying hens were the same as those used in the above experiments.

Observations were divided into two periods. The method used to identify the incubating hens was the same as that used in the above experiments. The first period began on December 26, 2022, when the hens were 188 days old. In the presence of roosters, hen incubation behavior was observed for 25 days out of 6 weeks. After 6 weeks, the roosters were moved to a different house. Before starting the second period, the hens were allowed a 3-week resting interval, as in Experiment 2. The second period, in the absence of roosters, began on February 27, 2023, when the hens were 251 days old. The length of the investigation period was the same as that of the first period.

Ethical approval

All animal experiments were approved by the Animal Care and Use Committee of the Aichi Agricultural Research Center (20–23, 21–24 and 22–25).

Table 1. **Number and percentage of incubating hens observed (Experiment 1).**

Period	Examined hens	Incubating hens	Percentage (%)
1 (Absence of roosters)	82	13	15.9
2 (Calls of roosters)	80	19	23.8
3 (Presence of roosters)	78	40	51.3

Combined data of hens in two pens.

Table 2. **Number and percentage of incubating hens observed (Experiment 2).**

Period	Examined hens	Incubating hens	Percentage (%)
1 (Presence of roosters)	91	43	47.3
2 (Absence of roosters)	78	26	33.3

Combined data of hens in two pens.

Table 3. **Number and percentage of incubating hens observed (Experiment 3).**

Period	Examined hens	Incubating hens	Percentage (%)
1 (Absence of roosters)	46	8	17.4
2 (Presence of roosters)	45	15	33.3

Table 4. **Number and percentage of incubating hens observed (Experiment 4).**

Period	Examined hens	Incubating hens	Percentage (%)
1 (Presence of roosters)	87	33	37.9
2 (Absence of roosters)	86	22	25.6

Combined data of hens in two pens.

Table 5. **Numbers of incubating hens observed in both the presence and absence of roosters in all experiments.**

Experiment	Absence of roosters	Both presence and absence of roosters	Percentage (%)[a]
1	13	10	76.9
2	26	17	65.4
3	8	6	75.0
4	22	16	72.7

[a]The value obtained by dividing the number of incubating hens observed in both the presence and absence of roosters by the number of incubating hens observed in the absence of roosters.

Results

The number and percentage of incubating hens observed in Experiment 1 are listed in Table 1. The proportion of incubating hens among the total number of hens examined in the absence of roosters was 15.9%, the proportion of incubating hens exposed to only rooster calls was 23.8%, and the proportion of incubating hens observed with roosters present was 51.3%. Thus, the proportion of incubating hens in the presence of roosters was higher than that in the absence of roosters or the presence of only rooster calls.

The numbers and percentages of incubating hens observed in Experiments 2, 3, and 4 are shown in Tables 2, 3, and 4, respectively. The proportions of incubating hens observed in the presence of roosters (47.3%, 33.3%, and 37.9%, respectively) were higher than those observed in the absence of roosters (33.3%, 17.4%, and 25.6%, respectively).

The numbers of incubating hens observed in both the presence and absence of roosters in all experiments are shown in Table 5. The numbers of incubating hens observed in both the presence and absence of roosters were counted by checking their individual identification numbers. Approximately 70% of the incubating hens observed in the absence of roosters exhibited incubation behavior even in the presence of roosters.

Discussion

This study provided particularly striking results in that the proportion of incubation behavior of Nagoya laying hens in the presence of roosters was consistently higher than that without roosters (Tables 1–4). In turkeys, the presence of males in the laying house has no significant effect on broodiness traits (number of broody periods, total broody days, and average broody period length)[9]. However, in two songbird species, gray-headed juncos (*Junco hyemalis caniceps*) and red-faced warblers (*Cardellina rubrifrons*), the absence of males causes increased vigilance in foraging females, resulting in less time spent incubating eggs[8]. Therefore, the presence of roosters may lower the vigilance of Nagoya laying hens, similar to songbirds, and enhance their incubation behavior. However, such an effect was not obtained using only rooster calls, as the proportion of incubating hens obtained using only rooster calls was not equivalent to that obtained in the presence of roosters (Table 1). Therefore, further research is required to determine the effects of other factors, such as vision and smell, that may enhance incubation behavior.

Hatching time and age may affect the intensity of incubation behavior in Nagoya laying hens[6]. In this study, the experiments were repeated four times, so that each experiment changed the hatching time of the experimental birds and the treatment order in the presence of males to more accurately confirm the effect of the presence of roosters. The proportion of incubating hens obtained in the presence of roosters in all experiments increased, regardless of the hatching time and treatment order.

As shown in Table 5, approximately 70% of the incubating hens observed in the absence of roosters in all experiments exhibited incubation behavior even in their presence. These reproducible results support the hypothesis that the presence of roosters strongly influences incubation behavior. Meanwhile, the remaining approximately 30% might not express incubation behavior in the presence of roosters by the effect of age or due to certain other factors.

Three methods (genetic improvement, manipulation of the rearing environment, and administration of veterinary products to alter hormone function) have been proposed to reduce incubation behavior[10]. Developing a novel approach to distinguish incubating hens from non-incubating hens is crucial to advance research on these methods. In the current study, the presence of roosters in the same house may enhance egg incubation behavior in Nagoya laying hens. Therefore, the results of this study contribute to the development of novel approaches to identify incubating hens. For example, when a flock of hens is kept in the presence of roosters and divided into incubating and non-incubating hens, the genetic differences between them may be more accurately analyzed. Thus, there is a high possibility of clarifying the molecular mechanisms underlying incubation behavior in chickens. Furthermore, when non-incubating hens are artificially selected in the presence of roosters for practical poultry breeding, the incidence of incubation behavior may be reduced in subsequent generations. Therefore, the findings of this study provide insights into the advancement of research on broodiness in poultry.

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Author Contributions

Akihiro Nakamura and Norio Kansaku designed the experiments and analyzed data. Akihiro Nakamura, Keizou Kobayashi, and Hiromitsu Miyakawa conducted the experiments. Akihiro Nakamura wrote the manuscript, and Hiromitsu Miyakawa and Norio Kansaku reviewed and revised it.

Conflicts of Interest

The authors declare no conflict of interest.

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