Doctoral Thesis

Behavioral studies on modified furnished cages from conventional cages for laying hens

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CONTENTS

INTRODUCTION

Recently, consumer interest in the welfare of laying hens has increased (Mench *et al*., 2011; Doyon *et al.,* 2015). A European Union (EU) directive banned the use of conventional cages in EU countries from 2012 (European Commission, 1999a). Furthermore, use of any cage system is banned in Switzerland and Austria. In California, the United States of America (USA), it was voted to ban the use of conventional cages from 2015 (California Proposition 2, Prevention of Farm Animal Cruelty Act, 2008). Other states in the USA such as Michigan, Ohio, and New Jersey are in a similar situation (Mench *et al*. 2011). Even in Japan, the guidelines for the welfare of laying hens were established in 2008 by the Japan Livestock Technology Association (JLTA) (Japan Livestock Technology Association, 2016).

According to the Japan Livestock Technology Association (JLTA, 2015), in 2015 more than 90% of laying hens were still housed in conventional cages in Japan. The change of housing systems for laying hens in the EU and USA may put pressure on the future egg industry of Japan. Some global companies have already set their own standards for supplying livestock products that reflect a concern with animal welfare (AW). Furthermore, the food strategy of the Tokyo Olympic and Paralympic in 2020 (https://tokyo2020.org/en/) may draw attention to the backgrounds of the food served, like previous Olympics such as London and Rio de Janeiro. Therefore, concern for animal welfare might be more prevalent in Japan in the near future. We must develop welfare-friendly housing systems that are suitable for Japan and scientifically verified. The system should be easy for farmers to introduce and manage. With concern for the future housing systems of laying hens that are suitable for Japan, this section looked at the current

situation and reviewed previous studies on the housing systems of laying hens inside and outside Japan.

High priority behavior of laying hens

Laying hens live in an environment that is provided by humans. It is completely different from nature. The expressions of behaviors differ according to the type of housing systems, resources, group sizes, stocking densities, and hen breeds (Abrahamsson and Tauson, 1995; Abrahamsson *et al*., 1995; Kim *et al*., 2017; Weeks and Nicol, 2006). The EU legislation requires a nest site, litter for scratching and foraging, and perches in furnished cages (European Commission, 1999b). This is for satisfying the hens' behavioral repertories. Weeks and Nicol (2006) reported that gaining access to the nest site is a high priority, foraging and dust bathing are behavioral needs, and spatial needs differ with stocking density and group sizes. However, Widowski and Duncan (2000) suggested that dust bathing is difficult to explain as a "need". "Needs" means that deprivation leads to a state of suffering; however, that was not shown in their study. Dust bathing also has the function of maintaining the plumage condition. Although hens use perches, it is yet unknown that how important perching is compared to the other behaviors. And, obviously, purposeless behaviors such as a high level of aggression, severe feather pecking, and cannibalism indicate that the housing system does not satisfy the hens' behavioral needs.

Current situation of laying hens

Production and consumption of eggs

In 2016, Japan was the fifth largest egg producer in the world after China, USA,

India, and Mexico (Statistics Bureau Ministry of Internal Affairs and Communication, 2016). It is still in the top five countries when the figures of the EU countries are combined. The egg consumption per person per year of Japan is the second in the world, after Mexico (International Egg Commision, 2017). Although the populations, economic situations, landscapes, and cultures differ among countries, the egg industry of Japan is among the largest in the world.

Housing systems of laying hens

By the beginning of the 20th century, laying hens were kept in small flocks in backyards for household consumption around the world (Fröhlich *et al.,* 2012). In the 1930s, the conventional cage system was developed along with a commercialization of egg production. This system was spread over the world and became large scale. However, the conventional cage system began to be criticized in northern Europe because of the restriction of movements and natural behavior of hens. This criticism was recognized by the consumer due to the publication of 'Animal Machines' by Ruth Harrison in 1964 (Mench *et al.,* 2011; Fröhlich *et al.,* 2012). This led to much research on the development of cages such as the Edinburgh modified cage (Appleby and Hughes, 1995; Abrahamsson *et al.,* 1996) and other alternative housing systems.

There are various housing systems for laying hens, and these are simply categorized as cages and non-cage systems. Cage systems are conventional cages and furnished cages. Non-cage systems include aviary and free-range (European Commission, 1999b; LayWel, 2006).

Conventional cage (CC)

A conventional cage is a small wire mesh cage with sloping floors (LayWel, 2006).

It is barren and equipped with only feed troughs and drinking facilities. One of the advantages of this system is good hygienic condition due to separation of hens and eggs from feces. Other advantages are that feeding, providing water, collecting eggs, and removing feces can be done automatically. This leads to the low labor and production costs. On the other hand, disadvantages of CCs are restriction of high priority behaviors such as nesting, perching, foraging, and dust bathing (Appleby *et al.,* 1993), and the negative effects on bone strength (AHAW, 2005).

Furnished cage (FC)

Furnished cages are cages equipped with resources such as perches, nests, and dust baths (European Commission, 1999a; Fröhlich *et al.*. 2012). The term 'enriched cage' is a synonym. FCs can be categorized by group sizes: small furnished cages (SFCs) for up to 15 hens per cage, medium furnished cages (MFCs) for 15 to 30 hens per cage, and large furnished cages (LFCs) for more than 30 hens per cage (Blokhuis *et al.,* 2007).

FCs have basic advantages like those of CCs. In addition to those, the resources in the FCs enable hens to express more behaviors than CCs. In addition, increasing activity could lead better bone quality than CCs (Tactacan *et al*., 2009). Barnett *et al.* (2009) suggested the effect of perches on strengthening bone. However, the advantages and disadvantages differ among group sizes. More diversity of behaviors was found in SFCs than in LFCs (Shimmura *et al.,* 2010a). Levels of cracked and dirty eggs in SFCs with well-designed nests were similar to those of CCs (Wall & Tauson, 2007). On the other hand, it was reported that the numbers of misplaced eggs were increased in larger group sizes (Wall, 2011). Nest use in LFCs needs further studies to improve egg quality. Although larger group sizes lead to more competition for resources, they have economic benefits and the larger total space enhanced exercise. Because of the higher cost

performance, recently LFCs are developed more than small ones in the EU (Mench *et al.,* 2011; Wall 2011; Widowski *et al.,* 2017). It was suggested that up to 40 non-beak-trimmed hens can live together in a LFC without adverse effects (Wall, 2011). The evaluation of six housing systems (small and large conventional cages, small and large furnished cages, aviary and free-range) conducted by Shimmura *et al.* (2010a, 2011) concluded that the welfare level of small furnished cages was similar to that of non-cage systems. Van Asselt *et al.* (2015) suggested a protocol for assessing the sustainability of housing systems (furnished cage, barn, free-range, and organic egg production). When equal weights were used for indicators such as the social (including food safety, animal welfare, and human welfare), environmental, and economic aspects in the Netherlands, the furnished cage was the most sustainable, although the weights and compensability were different between stakeholders.

Non-cage systems

Non-cage systems can be categorized as floor housing systems, which are single-tiered, or aviary systems, which are multi-tiered (Tauson, 2005). Other categories of non-cage systems, which are defined by possessing or not an outdoor run; barn systems (without outdoor access), free-range systems (with outdoor access), and organic systems (with outdoor access) (Rodenburg *et al.,* 2005, 2012).

Non-cage systems can provide more space per hen and allow hens to express various behaviors. It was reported that non-cage systems were better due to the freedom for expressing normal behaviors and freedom from fear and distress (Shimmura *et al.,* 2010a, 2011). On the other hand, they earn a poor score for freedom from pain, injury, and disease, and have some problems such as pale eggs and increased feed intake for production. Promoting the activity of hens increases bone strength (Fleming *et al.,* 1994;

Regmi *et al.,* 2016). In non-cage systems, the floor of hen house is usually covered with litter. This causes hygiene problems. David *et al.* (2015a, b) reviewed the air conditioning of housing systems of laying hens. The dust and ammonia levels were higher in litter systems than in cage systems. Furthermore, higher rates of bacterial and parasitic infections were found in hens kept in litter systems than in cages (Fossum *et al.,* 2009). The risk of cannibalism is higher in large flocks kept on the floor than in small groups in cages (Tauson, 2005; Fossum *et al.,* 2009). The hygiene problems and cannibalism cause a high mortality rate. Mortality rates in non-cage systems are usually higher than in cage systems (Tauson, 2005; Rodenburg *et al.,* 2012). It has been reported that genetic selection and whether the beaks of hens are trimmed or not are important for the reduction of cannibalism (Tauson, 2005; Janczak & Riber, 2015). Furthermore, the rearing environment affects the behaviors of laying hens and is important for improving the welfare of hens (Rodenburg *et al.,* 2012; Janczak & Riber, 2015).

Housing systems in commercial use

According to the report from the JLTA, in Japan, 91.6% of laying hens are kept in CCs, 0.4% are FCs, 6.2% are housed in a barn, 1.2% are free range, and 0.6% are other (Japan Livestock Technology Association, 2015). According to data from the European Commission, currently the housing systems of laying hens in the EU are FCs for 56%, barn for 26%, free range for 14%, and organic for 4% (European Egg Processors Association, 2016). From the annual review of the International Egg Commission 2017, currently most of the housing systems in the world outside the EU countries are cage systems, although cage systems are not classified as CC or FC (International Egg Commision, 2017).

A survey conducted in Flanders, Belgium, revealed that 33.9% of the egg farmers quit work after the ban on CCs in 2012 (Stadig *et al.,* 2016). Of the farmers who used CCs before 2012, 11.9% changed to FCs, 12.6% were changed to colony cages, 27.7% changed to floor housing, and 33.3% changed to aviary. The main reasons for their choice of these systems were whether the system suited their farm and consumer demand. Although the survey was conducted in just one part of the country, it could be a future reference for the countries in which poultry housing systems are moving to an alternative one. Egg farmers of Canada (2016) announced a transition from conventional egg production to other methods in the next 20 years. In 2016, 90% of egg production was in conventional cages in Canada. Their plan is about 50% transition in 8 years, about 85% in 15 years, and all production will be changed to alternative housing systems by 2036. Their plan concerned all aspects that could be connected to the transition of egg production systems, such as rearing environment for pullets, costs for new barns, stopping the production during replacement or rebuilding of hen houses, cost and time for importing housing systems, farmers, supply and demand of eggs.

Possibility of future housing systems of laying hens in Japan

Many studies to promote welfare of laying hens have been done, both developing housing systems and assessing methods for welfare levels. These studies were mainly conducted in EU countries. Lay *et al.* (2011) concluded that there was no single perfect housing system. Not only housing systems, but also rearing environments, breeds, and husbandry methods must be considered for improving the welfare of laying hens. Current situations such as laws and regulations, economics, culture, climate, and geography differ among the countries. In terms of sustainable egg production, the choice of the housing

systems should be based not only on the welfare of the birds but also environmental, economic, and human health aspects (De Boer & Cornelissen, 2002; Mench *et al*., 2011). As mentioned above, Japan has a large poultry industry and most laying hens are kept in CCs. Furthermore, Japan has a larger population, smaller farmland area, and more humid climate than the many of the EU member countries. Drastic changes to alternative systems may have a large impact on the poultry industry in Japan. Thus, housing systems that are suitable for Japan should be considered for a sustainable and stable egg supply.

With all these matters in mind, the aim of the present study was developing a housing system that is both practical and economical in the current Japanese situation. Results from many of the previous studies mentioned above suggest that furnished cages were well-balanced in various aspects. Shimmura *et al*. (2018) reported the usefulness of furnished cages re-using conventional cages, although the group size of the study was small with only four hens per cage. One advantage of the re-use of conventional cages is a lower introduction cost because the facilities around the cages can be used as they are. Most egg poultry farmers in Japan have conventional cages. Therefore, the furnished cage was modified from conventional cages, and behaviors and use of the resources were evaluated in this study. As mentioned above, nesting is a high priority behavior. When these housing systems were designed, enclosed nests lined with artificial turf were largely accepted (Abrahamson *et al*., 1996; Abrahamson & Tauson, 1997; Appleby, 1998a). Hunniford and Widowski (2018) concluded that the importance of enclosed nests for hens expressing nesting behaviors and provision of simple plastic curtains facilitate more settling nesting behavior. Artificial turf was widely used in alternative systems. However, results were not consistent. Hughes (1993) suggested that the enclosure and reduction of disturbance were more attractive to hens than the artificial turf. On the other hand, results

from studies by Struelens *et al*. (2005) showed that artificial turf was acceptable for both practical and welfare reasons. Wall and Tauson (2013) also reported that a nest with plastic netting and artificial turf was used more than the bare cage floor. Because the conventional cages were used for the modified cages in this present study, there were restrictions on layouts and sizes of cages. Therefore, a dust bath combined with nest areas that have no litter substrate was provided. As mentioned above, dust bathing is a highly motivated behavior. In addition, hens perform sham dust bathing without loose litter substrate. Hens performed sham dust bathing on the wire floor even when they could access a dust bath (Lindburg & Nicol, 1997; Olsson & Keeling, 2002). Lindburg and Nicol (1997) concluded that it is acceptable to provide furnished cages with adequate space and a loose pecking material in a location where sham dust bathing can occur undisturbed. It was reported that extra feed was an attractive stimulus for sham dust bathing (Moroki & Tanaka, 2016). Thus, evaluation of the use of a nest combined with a dust bath included observation of sham dust bathing and nesting behaviors.

In chapter 1, the furnished cage developed from 6 conventional cages is described. Nest areas were partitioned by a curtain and lined with artificial turf in the modified cages. Two experiments were conducted in this chapter. In experiment 1, hens' behavioral changes, establishment of social order, and use of resources after introduction to the cage were observed. In experiment 2, effects of artificial turf as nest linings were evaluated. In chapter 2, to promote the use of the nest areas, the effects of a commercially produced litter mat and a nest mat were evaluated. In chapter 3, a modified cage that was deeper than that in chapters 1 and 2 was developed and evaluated. In addition, the artificial turf used in chapter 1 and the commercially produced nest mat used in chapter 2 were compared by observing the behaviors and locations of hens. In chapter 4, comparison

between the two types of modified cages used in chapters 1–3 were carried out. In addition, behaviors for 30 min before oviposition were observed to evaluate use of the nest areas.

CHAPTER 1

Modification of furnished cages from conventional cages for laying hens: establishing social order, behavioral changes, and use of resources after introduction

1. 1. Introduction

In this chapter, a furnished cage was developed from conventional cages, and two experiments were carried out. One was the observation of behavioral changes, establishment of social order, and used of resources after introduction of hens to the cage. The other one was evaluation of the effect of artificial turf on the combined nest and dust bath.

Increasing public concern for the welfare of laying hens has resulted in a ban on the use of conventional cages in EU countries and some states in the U.S.A., and the decision by some global companies to avoid using hen eggs laid in conventional cages.

The main problem with conventional cages is restriction of high priority behaviors of hens such as nesting, perching, dust bathing, and foraging (Appleby *et al*., 1993). In addition, a negative effect on bone strength was reported (The Scientific Panel on Animal Health and Welfare, 2005). However, there are advantages to this system, such as hygienic conditions and low labor and production costs due to the automatic feeding, egg collecting, and feces removal. Although many alternative systems for laying hens have been developed, there are no single perfect housing systems for all hens (Lay *et al*., 2011). It is important to consider the housing design together with breed, rearing conditions, and

management. Six housing systems (small and large conventional cages, small and large furnished cages, aviaries and free-range) were previously evaluated by Shimmura *et al*. (2010a, 2011). They concluded that the welfare level of small furnished cages was similar to that of non-cage systems. Furthermore, in terms of sustainable egg production, the choice of a housing system should be based not only on the welfare of the birds but also environmental, economic, and human health aspects (De Boer & Cornelissen, 2002, Mench *et al*., 2011). Van Asselt *et al*. (2015) suggested a protocol for assessing the sustainability of housing systems (furnished cage, barn, free-range, and organic egg production). They set and assessed the indicators selected within the social (including food safety, animal welfare, and human welfare), environmental, and economic aspects in the Netherlands, and when equal weights were used for these indicators, the furnished cage was the most sustainable, although the weights and compensability were different between individuals.

In Japan, there are guidelines for the welfare of laying hens (Japan Livestock Technology Association, 2008), although there is no legal regulation. According to the report by the JLTA in 2015, more than 90% of the laying hens are kept in conventional cages in Japan (JLTA, 2015). Egg production in Japan was the fourth highest in the world in 2015 (Statistics Bureau Ministry of Internal Affairs and Communication, 2015), and annual per capita egg consumption is the third highest in the world (International Egg Commission, 2017). Thus, the egg industry in Japan is large. Because the main housing system of laying hens in Japan is conventional cages, a drastic change may disrupt the economy and sustainable egg supply.

With all these matters in mind, the aim of the present study was developing an easy-to-duplicate housing system that is both practical and economical in the current Japanese

environment. In many of the previous studies mentioned above, furnished cages were well-balanced in various aspects. The usefulness of furnished cages re-using conventional cages was reported by Shimmura *et al*. (2018), although the group size was small (4 hens per cage). One advantage of re-use of conventional cages is a lower introduction cost. Shimmura *et al*. suggested integration of dust bath and nesting areas with artificial turf. Recently, the group size of commercially used furnished cages is large (Widowski *et al*., 2017). The advantage of the large furnished cages is providing larger total cage area, enhancing hens' exercise. However, the increasing group size leads to aggressive pecking (Appleby *et al*., 2002; Shimmura *et al*., 2007a, 2010a). Shimmura *et al*. (2008b, 2009) reported that the separation of resources was effective in reducing competition for them in medium-sized furnished cages. In the present study, we modified the conventional cages that are commonly used in Japan to make furnished cages (7 hens per cage). To reduce the risk of competition for the resources, the nest areas were divided to both sides of the cage. We evaluated the modified cage by observing behaviors of hens and use of resources. In addition, artificial turf was placed on the nest areas as nest linings, and its effect on the nest use were evaluated.

1. 2. Materials and Methods

1. 2. 1. Animals and Housing

All experimental procedures were conducted according to the guidelines of the Animal Care and Use Committee of Azabu University (approval number: 170404_10). Experiment 1 was conducted from March to April 2017, and experiment 2 was conducted from May to June 2017.

1. 2. 2. Experiment 1

Fifty-six commercial White Leghorn hens (Julia) were used. All birds were beak trimmed and raised by a commercial poultry breeder. At the age of 123 days, they were transferred and introduced to the hen house at Azabu University.

Six conventional cages, 24 cm wide, 35 cm deep, and 41 cm high at the rear, were attached. Therefore, the total cage width was 144 cm (Figure 1). Cages were provided with 2 wooden perches (13.7 cm/hen), 2 nest areas, 2 claw sharpeners, 2 feed troughs (24.9 cm/hen), and 2 drinkers (24.9 cm/hen). Two nest areas were partitioned by curtains and placed at both ends of the cage to reduce the competition for them. The nest area was combined with a dust bath. Birds were allocated to 8 furnished cages with 7 birds per cage. The total cage area was 720 cm^2/hen. Because the cage was modified from conventional cages, there was a limitation to the layout and space of the cage. The cage density was as close as possible to meet the EU regulation (750 cm^2/hen).

The hens were fed and had access to water ad libitum. The illumination cycle was 11 h light (07.00 to 18.00) and 13 h dark for the first 4 weeks. After that, the lighting period was extended 30 min every other day until it was 14 h of light (06.00 to 20.00). Feeding and routine tasks were carried out from 08.00 to 09.30 and 16.00 to 17.00. The hen house was ventilated by 6 ventilators. The average temperature and humidity during the daytime of the experiment period were 14.9℃ (maximum 25.7℃, minimum 4.6℃) and 67.9% (maximum 96%, minimum 33%), respectively.

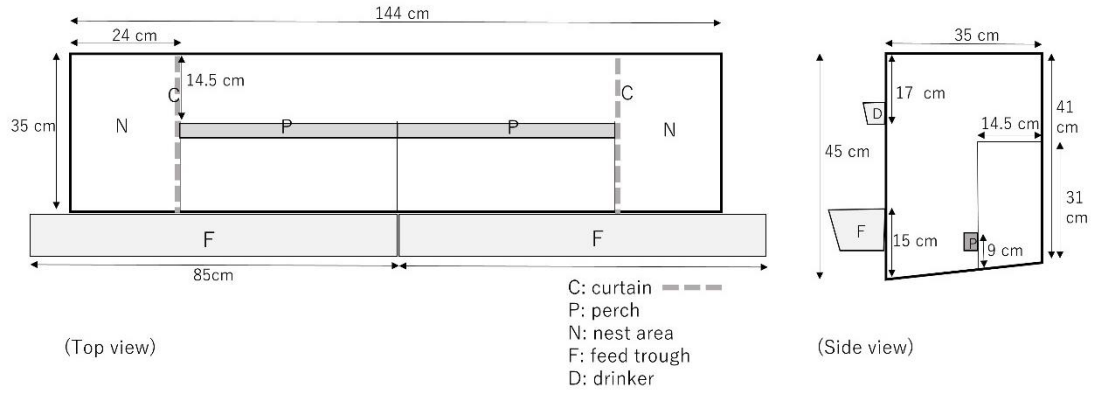

Figure 1. The modified furnished cages: top and side views

1. 2. 3. Experiment 2

The same 56 hens and cages as in experiment 1 were used when they were 26 weeks of age. Artificial turf (1236-524, Eco Kinzoku, Niigata, Japan) with 14 mm pile was placed on both sides of the nests of 4 cages. The artificial turf was cut into hexagonal shapes (Figure 2) to roll eggs out and minimize the droppings on it. The nest areas of the other 4 cages were without artificial turf as a control. The average temperature and humidity during daytime of the experiment period were 23.5℃ (maximum 29.3℃, minimum 17.4℃) and 63.5% (maximum 86%, minimum 36%), respectively.

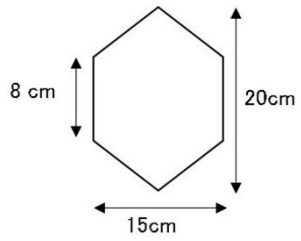

Figure 2. Size and shape of artificial turf

1. 2. 4. Observations and Measurements

Observation was conducted from March to May 2017. Behavioral observation was started immediately after introduction of the hens to the cages at 17 weeks of age. In addition to the direct observations, all cages were recorded additionally by a video camera (Sony Handycam HDR-CX675, Tokyo, Japan).

1. 2. 4. 1. Experiment 1

1. 2. 4. 1. 1. Dominance hierarchy

To identify hens, they were marked temporarily on the combs and numbered by leg bands. All aggressive behaviors toward other hens (aggressive pecking of head and body that causes the recipient to withdraw, and threatening) were directly observed for 10 min per cage in the morning and afternoon. Observation was conducted by one person from 10.00 to 12.00 and 13.00 to 15.00 for six days after introduction to the cages. Due to the low incidence of aggressive behavior, observation was continued 3 days a week for 2 weeks after the first observation of 6 consecutive days. Because the time of the introduction was late afternoon, observations of the first and second day were either in the morning or afternoon. Therefore, for a total 13 days, the duration of the observation time was 240 min per cage. The dominance ranking was calculated by David's score (Gammell, 2003).

1. 2. 4. 1. 2. Behavioral observation and use of resources

Direct visual scans at 10 min intervals was carried out from 10.00 to 12.00 and 13.00 to 15.00 for 14 days (3 times/week for the first 3 weeks, once a week from the 4^{th} to 6^{th} weeks). The numbers of the hens performing the following behaviors and each location were recorded: eating, drinking, resting (standing and lying), comfort behavior

(body shaking, wing-flapping, tail wagging, preening, head scratching, bill wiping, and stretching), sham dust bathing, exploring (looking, listening, and object pecking), aggressive pecking, and others. The positions were divided into left and right nest areas, perches, and floors.

1. 2. 4. 2. Experiment 2

A direct visual scan was conducted at 10 min intervals for 4 h per day (10.00 to 12.00, 13.00 to 15.00), 3 times per week for 4 weeks. In addition to the hens' behaviors and locations, the location of eggs, and behavior and location of the highest and lowest rank hens were recorded.

1. 2. 5. Statistical analysis

The proportions of hens performing each behavior and locations on each day were calculated in experiment 1. The numbers of behaviors on each day were analyzed by a Steel-Dwass test to evaluate the changes of behavior and use of resources after introduction to the cages. The proportions of hens performing each behavior and locations in each cage for 12 days were calculated in experiment 2. Mann-Whitney's U-test was used to compare the proportions of behavior and locations between cages with and without artificial turf. Because the ratio of the nest area to other areas was 1:2, use of the nest site was analyzed by a chi-square test with the expected value of 33.3%. The behaviors and positions of the highest and lowest rank hens were analyzed using a Wilcoxon signed rank test. Statistical significance was accepted at $P<0.05$.

1. 3. Results

1. 3. 1. Experiment 1

The proportion of each behavior of hens during the observation period is shown in Figure 3. There were significant differences in the proportions of behaviors of eating/drinking and resting between day 32 and each day before day 32 ($p<0.05$). The proportions of each behavior except eating/drinking and resting became stable in a few days after the introduction.

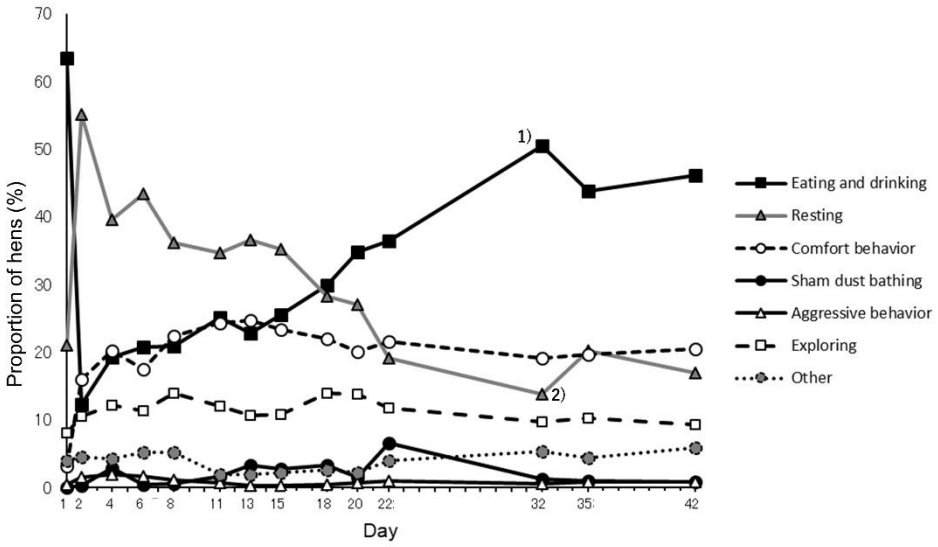

Figure 3. Change in proportion of behaviors of hens after introduction to the furnished cages. 1) and 2): there were significant differences between Day 32 and Day 1-22, $P<0.05$

The proportion of time in each location of hens during the observation period is shown in Figure 4. The proportion of hens' locations became stable 4 weeks after the introduction. The proportions of eating/drinking of hens gradually increased, and that of

resting simultaneously decreased for the first 3 weeks. Use of perches also decreased gradually for the first 3 weeks. The proportion of aggressive behavior increased to 1.9% for the first week, and after that, it was from 0.3% to 0.9%. The mean proportion of display aggressive behaviors was 0.9% throughout the observation period. Severe feather pecking was not observed throughout the observation period.

The first egg was laid at Day 15, when hens were 19 weeks of age, and 50% of hens were producing eggs at Day 22, when they were 20 weeks of age.

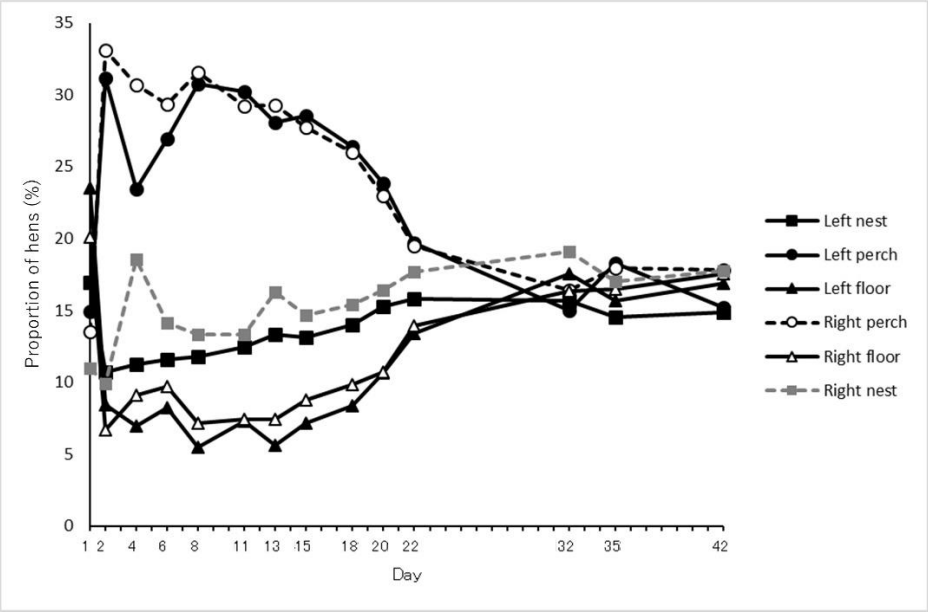

Figure 4. Change in proportion of hens located at each position during the observation period

1. 3. 2. Experiment 2

Tables 1 and 2 show the mean proportions of hens' behavior and locations respectively in the cages with and without artificial turf on the nest area. There was no significant difference in the mean proportions of behaviors and location between those with and without artificial turf on the nest area.

Table 1. Mean proportion of hens' behaviors

Behavior	No artificial turf (%)	With artificial turf (%)	Significance
Eating and drinking	41.2 ± 5.1	44.5 ± 1.8	NS
Resting	19.9 ± 3.7	14.7 ± 0.5	NS
Comfort behavior	21.3 ± 1.0	20.1 ± 2.5	NS
Sham dust bathing	2.2 ± 0.9	1.7 ± 0.8	NS
Aggressive behavior	0.3 ± 0.2	0.3 ± 0.1	NS
Explore	10.5 ± 2.3	13.2 ± 0.7	NS
Other	4.6 ± 0.8	5.5 ± 0.7	NS

Values are expressed as mean ± SD. NS, not significant.

Table 2. Mean proportions of birds' locations

Position	No artificial turf (%)	With artificial turf (%)	Significance
Nest area	32.1 ± 0.8	29.4 ± 1.4	NS
Perch	31.0 ± 5.6	28.7 ± 2.4	NS
Floor	36.9 ± 5.0	42.0 ± 1.3	NS

Values are expressed as mean ± SD. NS, not significant.

Locations where hens performed sham dust bathing are shown in Figure 5. There was no significant difference in the locations of hens performing sham dust bathing between the cages with and without artificial turf on the nest area. The hens significantly frequently performed sham dust bathing in the nest area ($p<0.01$). However, the artificial turf did not affect the place of performing sham dust bathing.

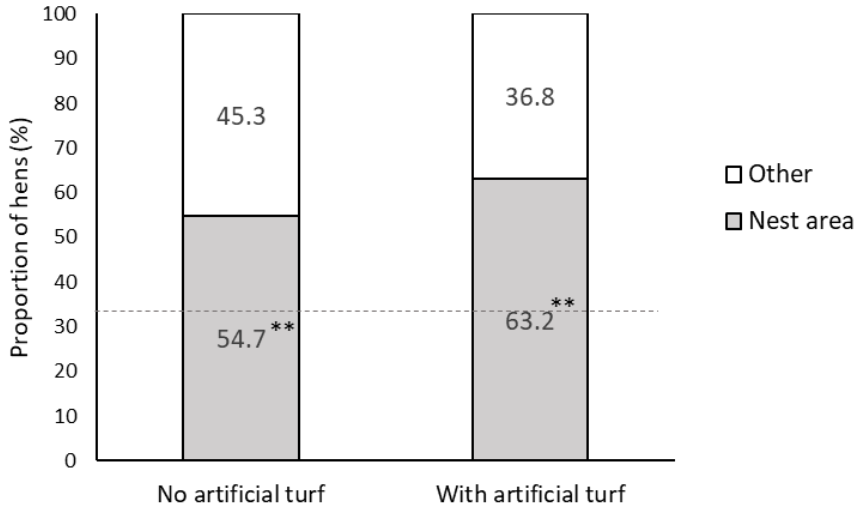

Figure 5. Proportion of locations of birds performing sham dust bathing. χ^2 test, expected value: 33.3%, **$p<0.01$

The proportions of locations where eggs laid are shown in Figure 6. More eggs were laid in the nest area with artificial turf than in the nest area without artificial turf ($p<0.01$). Hens significantly more frequently laid eggs in the nest area with artificial turf than the other areas ($p<0.05$). More eggs were laid in the other areas than in the nest areas without artificial turf ($p<0.01$).

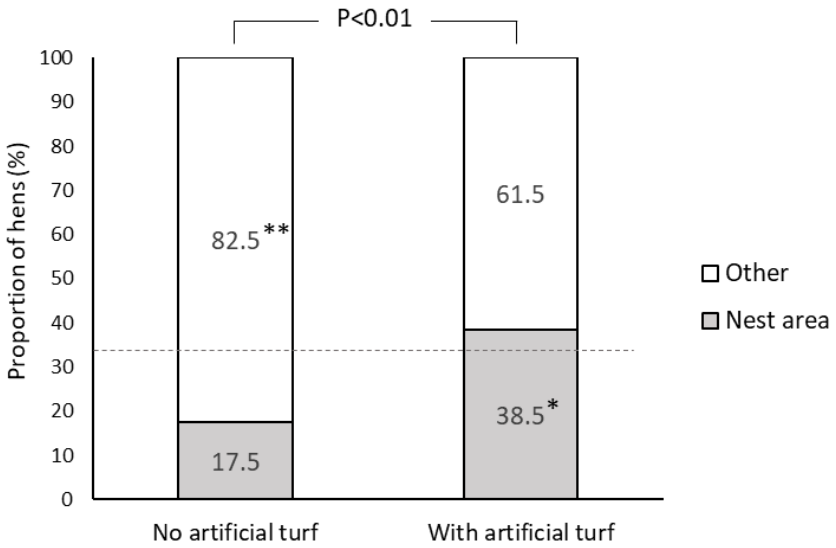

Figure 6. Proportion of areas where eggs were laid. χ^2 test, expected value: 33.3%, *p<0.05, **P<0.01

The behaviors of the highest and lowest rank hens are shown in Table 3. Also, the locations of the highest and lowest rank hens are shown in Table 4. The lowest rank hens moved significantly more frequently (p<0.05) and used the nest area more frequently (p<0.01) than the highest rank hens.

Table 3. Proportions of behaviors of the highest and lowest rank hens

Behavior	Highest rank hens (%)	Lowest rank hens (%)
Eating and drinking	47.12±7.77	46.14±6.45
Resting	15.48±6.05	11.58±5.22
Comfort behavior	20.38±2.72	19.96±4.74
Sham dust bathing	1.32±1.14	1.60±0.90
Aggressive behavior	0.32±0.36	0.31±0.43
Explore	11.62±3.32	12.38±2.11
Moving	2.27±1.25[a]	5.46±2.23[b]
Other	1.50±1.46	2.57±1.19

Values are expressed as mean ± SD.

Different letters in the same row indicate significant difference between social ranks of hens. [a, b]p<0.05.

Table 4. Proportions of locations of the highest and lowest ranking hens

Location	Highest rank hens (%)	Lowest rank hens (%)
Floor	46.07±7.48	38.14±5.11
Peach	30.79±7.97	23.40±5.92
Nest	23.15±2.82[a]	38.45±5.32[b]

Values are expressed as mean ± SD.

Different letters in the same row indicate significant difference between social ranks of hens. [a, b]$p<0.05$.

1. 4. Discussion

1. 4. 1. Experiment 1

The proportions of each behavior except eating/drinking and resting were stable within a few days after the introduction. This is partly in agreement with the study by Shimmura *et al*. (2006) which reported that hens' locations were stable from just after introduction into the furnished cages. Other studies also suggested that hens could be habituated to the new cages in a few days (Anderson *et al*., 1989; Tanaka & Hurnik, 1991). The proportion of time hens spent eating/drinking was gradually increased for about a month (day 32). This increase might be a result of increasing food consumption due to their growth as hens started to lay eggs; otherwise, it may be a reflection of adaptation to the new environment. Similarly, Tanaka & Hurnik (1991) reported that the number of hens eating/drinking in the last 3 days of 2 weeks' observation just after introduction to a new cage was higher than that in the first 7 days. Simultaneously with this increase of the proportion of eating/drinking, that of resting was decreased. And the proportion of hens'

location on the perch was decreased until day 32, and that of on the floor was increased until day 35. Hens stayed on the floor when they ate feed and drank water due to the placement of the feed trough and drinker. This might be reflected in these proportions of hens' locations. Furthermore, eating/drinking on the first day was observed more frequently than on other days. Hens might have been hungry when they were introduced to the cages because they were transported on that day and could not be fed until the introduction. Thus, the proportion of resting was low, and a simultaneous higher incidence of eating/drinking was observed on the first day.

The proportion of aggressive behaviors was from 1.0% to 1.9% in the first week, and after that it became less than 1%. Therefore, it is considered that the social hierarchy was established in the first week after introduction. Severe feather pecking was not observed during the present study. Feather pecking and aggressive pecking can be causes of cannibalism, and rates increased with the group size, especially in small groups of up to 12 hens (Hughes & Wood-Gush, 1977; Shimmura *et al*., 2010). To decrease the competition for resources, we provided 2 nest areas at each end of the cage. This might have affected the low incidence of aggressive behaviors in this study, since this result was in agreement with the previous study in medium-sized furnished cages (Shimmura *et al*., 2008b, 2009), although the present study did not have the control cages. Although further study for long-term observation for aggressive behaviors is needed, the low incidence of the aggressive behavior is one of the advantages of the modified cage in the present study.

1. 4. 2. Experiment 2

There was no significant difference in the proportions of behaviors and locations between cages with and without artificial turf on the nest area. There was no significant difference in the locations of sham dust bathing between the cages with and without

artificial turf in the nest area. Although the hens performed significantly more sham dust bathing in the nest area, artificial turf had no effect on the place where sham dust bathing was performed. Dust bathing is a highly motivated behavior for hens. This leads to an occurrence of sham dust bathing when adequate stimuli are absent. It is known that providing an adequate dust bath substrate entices hens to perform dust bathing (Lindberg & Nicol, 1997; Alvino *et al*., 2013; Guinebretiere *et al*., 2014). Lindberg and Nicol (1997) reported that most dust bathing behavior occurred by the feed trough even if there was a dust bath. They suggested that providing adequate space in which hens are not disturbed for sham dust bathing and access to a loose substrate in the place where dust bathing can occur was acceptable in a modified cage. In the present study, the nest area was combined with a dust bath, and loose substrate was not provided. Hens might be stimulated to perform sham dust bathing by the feed in the feed tough (Moroki & Tanaka, 2016) and prefer the nest area where fewer interruptions occurred.

More eggs were laid in the nest area with artificial turf in the present study. Although artificial turf itself had no effect on stimulating sham dust bathing, it had a significant effect on choosing a place to lay eggs. It was reported that more eggs were laid in a nest with a lining than on a bare wire floor (Reed and Nicol 1992; Struelens *et al*., 2005; Wall & Tauson, 2013). Although it was reported that loose material such as wood shavings attracted hens using nest boxes (Huber *et al*., 1985; Appleby & Smith, 1991), frequent use of artificial turf, as observed in this study, was also reported (Appleby *et al*., 1993; Abrahamsson *et al*., 1996).

Although more eggs were laid in the nest area than the other areas, a high proportion of eggs were still laid outside the nest area. In other studies of furnished cages, higher proportions of eggs laid in the nest boxes were reported (Appleby *et al*., 1993;

Wall & Tauson, 2013). There is a behavior called ‘gregarious nesting’ in which hens chose a nest already occupied even if other nests are unoccupied (Clausen & Riber, 2012). Furthermore, it was reported that subordinate birds left the nest because dominant birds pecked the birds when entering the nest (Shimmura *et al*., 2008b). The hens that could not enter the nest might lay eggs outside the nest areas. It was suggested that nest sites should be enclosed to entice hens to use them (Reed & Nicol, 1992). The nest area was partitioned by a curtain, but there was no curtain at both ends of the cages in the present study. This means hens could see the other flocks of hens in the neighboring cages. This might affect the high incidence of eggs laid outside the nests. Therefore, further development of cages which hens cannot see other hens while staying in the nests will be needed.

The lowest rank hens moved significantly more frequently and used the nest area more than the highest rank hens in this study. In agreement with the previous study (Shimmura *et al*., 2007, 2008a, b), this result demonstrated that the nest area was used not only for laying eggs but also by the low ranked hens to escape from other hens. This might reflect the low incidence of aggressive behaviors in the present study. It is important for welfare of hens to be able to escape from other hens because the severe feather pecking causes the removal of feather and injuries, and furthermore cannibalism (Savory, 1995; Rodenburg *et al*., 2013).

In conclusion, the hens became habituated to the environment in a few days after the introduction to the cage in the furnished cages modified from conventional cages in this study. The rate of aggressive behaviors was low, no severe feather pecking was observed, and a wide variety of behaviors were observed. The combined nest and dust bath was used not only for laying eggs and sham dust bathing but also for refuge by the

low ranked hens. Therefore, separation of resources and the combined nest and dust bath in this modified cage are functional in this cage and group size. However, further study is needed to improve the use of nest areas and reduce the number eggs laid outside the nest.

1. 5. Summary

The aim of the study was development of an alternative housing system that is easy to replicate. We constructed a furnished cage using 6 conventional cages. Two nest areas were provided both ends of the cage and were combined with a dust bath. Fifty-six White Leghorn (Julia) hens aged 123 days were allocated to 8 furnished cages. All aggressive behaviors were directly observed to calculate a dominance ranking Behaviors and use of the resources were recorded using a direct visual scan sampling technique at 10 min intervals. Locations where eggs were laid and the behaviors and locations of the highest and lowest rank hens were recorded. In the modified cages in this study, the hens were habituated the environment in a few days after the introduction to the cage. The rate of aggressive behavior was low, no severe feather pecking was observed, and a wide variety of behaviors were observed. The combined nest and dust bath was used not only for laying eggs and sham dust bathing but also for refuge by the low-ranked hens. Therefore, separation of resources and combined nest and dust bath are functional in this cage design and group size.

CHAPTER 2

Modification of furnished cages from conventional cages for laying hens: which do hens like a nest mat or a litter mat for the nest area?

2. 1. Introduction

In chapter 1, the usefulness of the modified furnished cages was suggested. Because there were still many eggs laid outside the nest, studies using nest linings to promote the use of nest areas are discussed in this chapter.

In EU member countries and some of states in the USA, use of conventional cages for laying hens has been banned. Hens in conventional cages cannot perform natural behaviors such as perching, nesting, dust bathing, and foraging. These are priority behaviors of hens (Tauson, 2005; Weeks and Nicol, 2006; Shimmura *et al*., 2010a), and this impairs the welfare of hens (Appleby *et al*., 1993). Therefore, many alternative housing systems, such as furnished cages and non-cage systems, have been developed. Although these housing systems have both pros and cons (Blokhuis *et al*., 2007; Lay *et al*., 2011), Shimmura *et al*. (2010) have pointed out that the benefits of the small furnished cage were similar to those of non-cage systems. Furthermore, it has been suggested that the furnished cage system is more sustainable than other housing systems (barn, free-range, and organic systems), although there are different weights and compensations between the stakeholders (van Asset *et al*., 2015). However, the introduction cost is one of the difficulties of altering housing systems (Tauson, 2005). It has been reported that

furnished cages re-using conventional cages could reduce the introduction costs (Shimmura *et al*., 2018), although the group size of the study was small with only 4 hens per cage. The group size of commercial furnished cages has been increased recently (Mench *et al*., 2011; Widowski *et al*., 2017). The larger group size allows hens more exercise by providing a larger area. On the other hand, more aggressive pecking is observed (Appleby *et al*., 2002; Shimmura *et al*., 2007b; 2010a). Separation of resources could reduce competition for the resources (Shimmura *et al*. 2008b; 2009). Thus, we developed furnished cages modified from conventional cages that were larger (7 hens per cage) than those of the previous study, and the resources were separated to reduce the competition for them (Kikuchi *et al*. 2018). In these cages, the nest areas are placed at both ends of the cage and combined with a dust bath. Due to the circadian rhythm, hens generally lay eggs in the morning and dust bathe in the afternoon (Vestergaard, 1982; Shimmura *et al*. 2008a). Therefore, the combination of a nest and a dust bath may work, and its effectiveness has been reported (Shimmura *et al*. 2018). However, in this previous study (Kikuchi *et al*. 2018), whereas the nest areas were used for laying eggs, sham dust bathing, and a refuge by the low-ranked hens, eggs were also laid outside the nest areas. Therefore, the aim of the present study was to evaluate nest linings suitable for the modified cages. To increase the nesting behavior and sham dust bathing in the nest areas, we used the mesh nest mat and the plastic litter mat commercially used in the Eurovent EU cage system (Big Dutchman, Vechta, Germany). Behaviors of hens were observed to evaluate the nest use.

2. 2. Materials and Methods

All experiments were carried out according to the guidelines of the Animal Care and Use Committee of Azabu University (approval number: 170404-10).

2. 2. 1. Animals and housing

Fifty-six White Leghorn (Julia) hens aged 32 weeks were used. All hens were raised by a commercial poultry breeder and had their beaks trimmed. They were introduced into the modified cages in a hen house at Azabu University at 17 weeks of age.

Eight modified cages used in the previous study were used (Kikuchi *et al*., 2018). Six conventional cages were connected to each other, making the size of the cage 144 cm (length) x 35 cm (width) x 41 cm (height) at the rear (Figure 1). Seven birds were housed in each cage (720 cm^2/hen). Two wooden perches (13.7 cm/hen), 2 claw sharpeners, 2 feed troughs, and 2 drinkers were provided. Both ends of the cage were nest areas (24 cm width, 35 cm depth) partitioned by polyethylene curtains. Nest areas were combined with a dust bath.

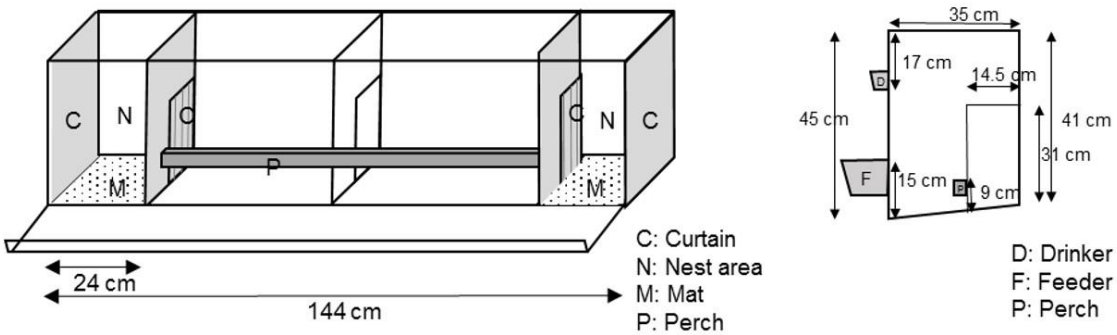

Figure 1. Layout and side view of the modified cage

Two types of commercially produced mats, the meshed nest mat and the plastic litter mat used in the Eurovent EU cage system (Big Dutchman, Vechta, Germany) (Figure 2), were used to line the nest areas. The entire nest areas of both ends of 4 cages were lined with litter mats, and those of the other 4 cages were lined with nest mats.

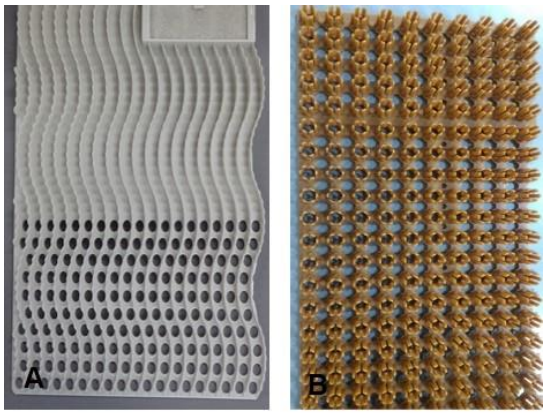

Figure 2. Images of the litter mat (A) and the nest mat (B) (Big Dutchman, Vechta, Germany)

Feed and water were provided ad libitum. Daily routines such as feedings and cleanings were carried out from 08.00 to 09.30 and from 16.00 to 17.00. The illumination cycle was 14 h of light (06.00 to 20.00). The hen house was equipped with 6 ventilators. The average temperature and humidity during the experiment were 28.0℃ (maximum 32.8℃, minimum 21.2℃) and 73.6% (maximum 92%, minimum 59%).

2. 2. 2. Observations

Observations were carried out from 32 to 36 weeks of age. Direct visual scans at 10 min intervals were conducted to count the behaviors and location of the hens for 4 hours per day (10.00 to 12.00 and 13.00 to 15.00), 3 days a week, for 4 weeks. All data were collected by the same observer, and all cages were additionally recorded by video

cameras (Sony Handycam HDR-CX675, Tokyo, Japan). Behaviors recorded were eating/drinking, resting, comfort behaviors (body shaking, wing flapping, tail wagging, preening, head scratching, bill wiping, and stretching), sham dust bathing, exploring (looking, listening, and object pecking), aggressive pecking, and others. The following locations of the hens were also recorded: nest areas, perches, and floors. In addition, the locations where eggs were laid were recorded.

2. 2. 3. *Statistical analysis*

The proportions of each behavior and location were calculated for each cage for 12 days. To analyze the effects of mats on the use of resources and behaviors, the proportions of each behavior and location were compared using Mann-Whitney's U tests. The effects of the mats on the locations of sham dust bathing and egg laying were analyzed using chi-square tests. Because the dimension of the nest areas was one third of the entire cage, the expected value was 33.3%. All analyses were carried out using the statistical software Statcel 4 (Yanai, 2015).

2. 3. Results

The proportions of each behavior and locations of hens are shown in Tables 1 and 2, respectively. There were no significant differences in mean proportions of each behavior between the cages with litter mats and nest mats. Hens were more frequently observed in the nest areas in cages with nest mats compared to the cages with litter mats (26.4% of hens, 20.9% of hens, respectively; $p<0.05$).

Table 1. Mean proportion of birds' behaviors in cages with litter mat and nest mat in the nest area

Behavior	With litter mat (%)	With nest mat (%)
Eating and drinking	45.4 ± 5.8	50.8 ± 2.4
Resting	17.7 ± 4.5	13.7 ± 1.2
Comfort behavior	18.9 ± 2.3	16.9 ± 1.2
Sham dust bathing	2.6 ± 0.8	3.9 ± 0.8
Aggressive behavior	0.1 ± 0.1	0.1 ± 0.04
Explore	9.3 ± 3.0	9.6 ± 0.7
Other	6.0 ± 1.6	5.1 ± 0.7

Values are expressed as mean ± SD.

Table 2. Mean proportion of birds located position in cages with litter mat and nest mat in the nest area

Position	With litter mat (%)	With nest mat (%)
Nest area	20.9 ± 2.1 [a]	26.4 ± 2.5 [b]
Perch	25.5 ± 4.7	19.9 ± 2.1
Floor	53.6 ± 5.7	53.7 ± 3.9

Values are expressed as mean±SD. Different letters in the same row indicate significant difference at $p<0.05$; Mann-Whitney's U test.

Sham dust bathing was observed more frequently in the nest areas than in other areas in the cages with nest mats (47.9% of hens, expected value: 33.3%; $p<0.01$) (Figure 3). On the other hand, in the cages with litter mats, sham dust bathing was observed less frequently in nest areas than other areas (11.3% of hens, expected value: 33.3%; $p<0.01$).

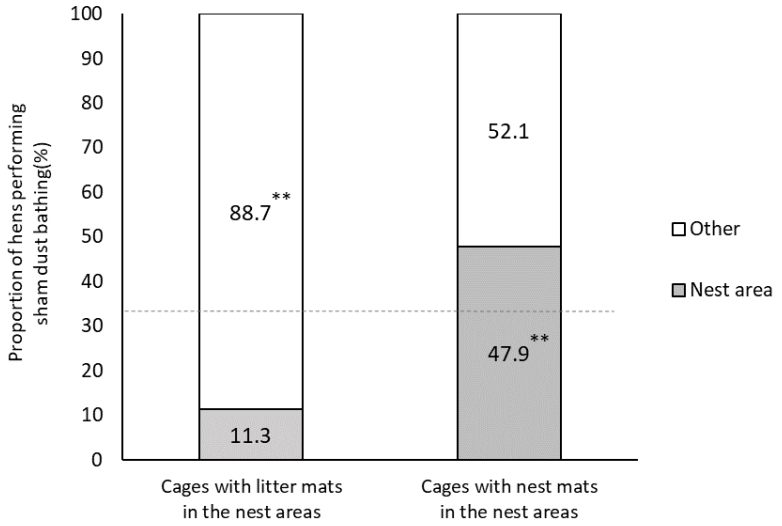

Figure 3. Proportion of hens performing sham dust bathing, χ^2 test, expected value: 33.3%. **p<0.01

Eggs were laid significantly more often in nest areas than other areas in both cages (Figure 4) (cages with litter mats: 50.9% of eggs, cages with nest mats: 53.6% of eggs, expected value: 33.3%; p<0.01).

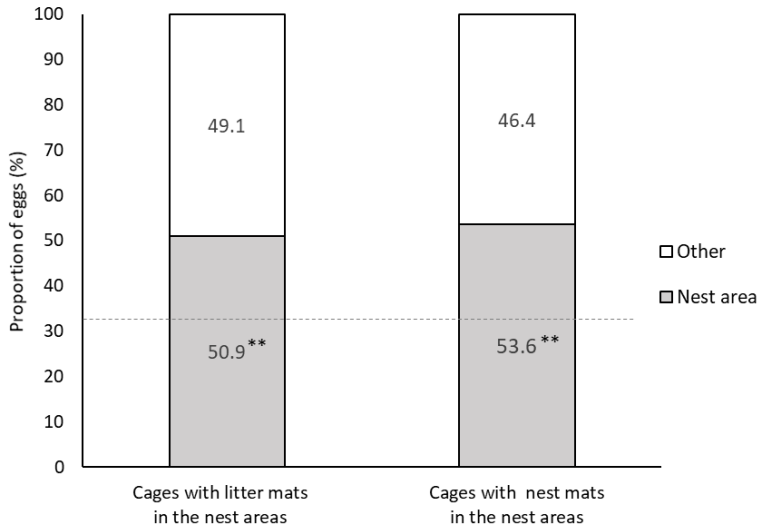

Figure 4 Proportion of eggs laid in nest areas and other areas, χ^2 test, expected value: 33.3%. **p<0.01

2. 4. Discussion

Although there were no significant differences in the mean proportions of behaviors of hens between the cages with different types of mats, hens in the cages with nest mats were more frequently observed in the nest areas than hens in the cages with litter mats. In the cages with nest mats, sham dust bathing was more frequently observed in the nest areas than in other areas. On the other hand, sham dust bathing was more frequently observed in areas other than nest areas in the cages with litter mats. It is reported that sham dust bathing is stimulated by access to the feed (Lindberg & Nicol, 1997; Moroki & Tanaka, 2016). In the present study, feed troughs extended the whole length of the cage. This means hens could access the feed troughs from any place in the cages. However, hens did not randomly perform sham dust bathing. Although the present study did not test a choice between the two mats, nest mats might be more attractive for hens than litter mats because they were observed more often and more frequently performed sham dust bathing in the nest areas with nest mats. In addition, the design of commercial litter mats may be based on the provision of litter substrates. In the present study, litter substrates were not provided on the litter mats, and this might have led to the lower use of the nest areas with litter mats. Although we did not measure the softness of the material of the mats, texture might also affect the use of the nest areas.

In both cage types, more eggs were laid in the nest areas than in other areas. However, eggs were still laid outside the nest areas. High use of nests with artificial turf in the small furnished cages was reported (Struelens *et al*., 2005; Wall *et al*. 2002; Wall &Tauson, 2013). Gregarious nesting on attractive nest sites was observed (Clausen &

Riber, 2012). In addition, it is reported that some hens are called “gregarious nesters,” preferring to nest with other birds rather than alone (Sherwin & Nicol, 1993). Solitary nesters might be disturbed by the gregarious nesters. This might lead to floor eggs. However, further study is needed on hens’ individual nesting behaviors.

In conclusion, the nest mat was used more frequently than the litter mat for sham dust bathing and nesting. The results suggest that nest mats are effective for the nest area combined with a dust bath in the modified cage.

3. 5. Summary

The aim of the study was to develop housing systems that are easy to introduce inexpensively. Six conventional cages were connected to each other. Nest areas combined with a dust bath were placed at both ends of the cage. We evaluated the effects of a commercially used litter mat and a nest mat to promote the use of the nest areas. Fifty-six White Leghorn (Julia) hens were allocated 7 birds per cage, and behaviors and locations of hens were recorded. Hens were more frequently observed in the nest areas in cages with nest mats than those with litter mats ($p<0.05$). Sham dust bathing was more frequently observed in the nest areas than the other areas in the cages with nest mats ($p<0.01$). On the other hand, it was less frequently observed in nest areas than in other areas in the cages with the litter mats ($p<0.01$). More eggs were laid in nest areas than in other areas in both cages ($p<0.01$). These results suggest that the nest mats are effective for the nest area combined with a dust bath in the modified cage.

CHAPTER 3

Modification of furnished cages from conventional cages for laying hens: square cage design and comparison of nest mats

3. 1. Introduction

Furnished cages have been developed as alternative housing systems to conventional cages. Conventional cages were criticized because hens cannot perform natural behaviors in a barren environment. Furnished cages provide opportunities for performing a variety of behaviors (Appleby & Hughes, 1995; Appleby, 1998a; 1998b; Appleby *et al.,* 2002). However, the cost of introducing furnished cages is high. This is one of the reasons for hesitation by farmers on changing the housing system of hens. Recently, most of the commercial furnished cages were enlarged (Mench *et al.,* 2011; Widowski *et al.,* 2017). Besides enhancing exercise and improving bone strength, one of the benefits of larger group size cages is lower capital cost per hen than small group size cages (Wall *et al.,* 2004).

In this series of study, conventional cages were modified to make furnished cages which were low in cost. The previous studies showed the effectiveness of modified cages from conventional cages with nest areas that included a dust bath (Shimmura *et al.,* 2018; Kikuchi *et al.,* 2018). To reduce the competition for resources that is observed in large flocks (Appleby *et al.,* 2002; Shimmura *et al.,* 2007b, 2010a), resources were separated. Furthermore, the previous study (Kikuchi *et al.,* 2018) of chapters 1 and 2 suggested the

effectiveness of artificial turf and commercially used nest mats in the nest areas combined with a dust bath. In this study, both linings were used and the effect on the nest areas in new layout of the modified cage was evaluated.

Because most commercial furnished cages are deeper than conventional cages, conventional cages were attached back to back to make deeper cages, making the cage square. The objective of this study was to evaluate the square cage design and effect of nest mats on a nest combined with a dust bath by observing the behaviors of hens and their use of resources.

3. 2. Materials and Methods

All experiments were carried out according to the guidelines of the Animal Care and Use Committee of Azabu University (approval number: 170907-2).

3. 2. 1. Animals and housing

Fifty-six White Leghorn (Julia) hens at 43 weeks of age were used. All hens had their beaks trimmed just after hatching and were raised by a commercial poultry breeder. They were introduced to a hen house at Azabu University when they were 17 weeks of age. They were randomly divided into 8 groups and housed in the modified cages used in the previous studies (Kikuchi *et al.* 2018).

Six conventional cages were used to make a square furnished cage (72 cm width, 70 cm depth, 41 cm height) (Figure 1). First, three conventional cages were linked together. Then, they were bound back to back. Two perches (13.7 cm/hen), 2 claw sharpeners, 2 nest areas, and 2 feed and water troughs were placed on both sides of the

cage. Polyethylene curtains partitioned 2 nest areas (24 cm width, 35 cm depth) per cage. The nest areas were combined with dust baths. Eight groups of hens were allocated to 8 modified furnished cages. Hens were housed 7 hens/cage. Due to the restriction of the cage design using conventional cages, the area per hen (720 cm^2/hen) was slightly less than that of EU regulations (750 cm^2/hen).

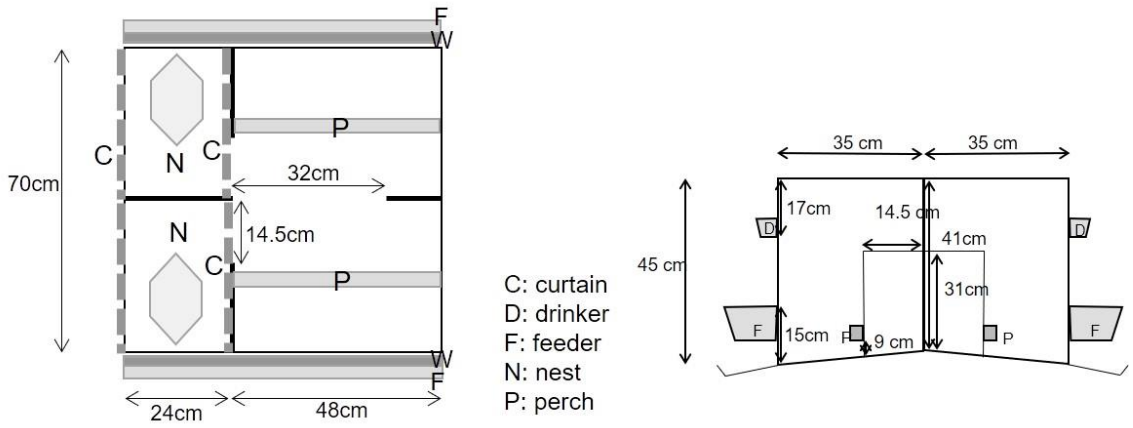

Figure 1. Top and side view of the modified cage

The nest areas of 4 cages were lined with the polyethylene artificial turf (1236-524, Echo Kinzoku, Niigata, Japan) described in chapter 1, and the nest areas of the other 4 cages were lined with commercially used nest mats for the Eurovent EU cage system (Big Dutchman, Vechta, Germany) described in chapter 2.

The light schedule was 14 h light (06.00 to 20.00). Hens were fed ad libitum. Daily routines such as feedings and cleanings were carried out from 08.00 to 09.30 and from 16.00 to 17.00. The average temperature and humidity during the experiment were 24.6°C (maximum 28.6°C, minimum 20.1°C) and 73.3% (maximum 85%, minimum 51%).

3. 2. 2. Observations

Behavioral observations were carried out at 10-min intervals using a direct visual scan technique for 4 h/d (10.00 to 12.00, 13.00 to 15.00), 3 d/wk for 4 weeks (total 12 days). The following behaviors were observed: eating and drinking, resting, comfort behaviors (body shaking, wing flapping, tail wagging, preening, head scratching, beak wiping, and stretching), sham dust bathing, exploring, aggressive pecking, and other behaviors. The locations of hens were also recorded as follows: nest area, perch, and floor. The places where eggs were laid were recorded. All data collections were carried out by the same observer, and all cages were additionally recorded by video cameras (Sony Handycam HDR-CX675, Tokyo, Japan).

3. 2. 3. Statistical analysis

Each behavior and hens' location in each cage are presented as percentages of the total observations. The effects of mats on each behavior and use of resources were analyzed using Mann-Whitney's U-tests. Use of the nest areas was analyzed using chi-square tests. The expected values were calculated from the ratio of 1 to 2 for the dimensions of the nest areas to other areas. All analyses were carried out using the statistical software Statcel 4 (Yanai, 2015).

3. 3. Results

Proportions of behaviors of hens are shown in Table 1, and proportions of hens' locations were shown in Table 2. There were no significant differences in proportions of each behavior and location between types of nest mats.

Table 1. Mean proportion of bird behaviors in cages with artificial turf and nest mat in the nest area

Behavior	With artificial turf (%)	With nest mat (%)
Eating and drinking	35.3 ± 2.4	40.0 ± 2.9
Resting	34.8 ± 2.7	30.5 ± 3.5
Comfort behavior	20.1 ± 3.5	20.2 ± 1.1
Sham dust bathing	1.9 ± 0.6	1.9 ± 0.3
Aggressive behavior	0.2 ± 0.1	0.2 ± 0.1
Explore	4.6 ± 1.8	3.7 ± 0.4
Other	3.1 ± 0.7	3.5 ± 0.6

Values are expressed as mean ± SD.

Table 2. Mean proportion of birds located position in cages with artificial turf and nest mat in the nest area

Position	With artificial turf	With nest mat (%)
Nest area	28.7 ± 1.3	26.4 ± 5.5
Perch	32.6 ± 6.1	31.1 ± 5.4
Floor	38.7 ± 7.1	42.5 ± 4.6

Values are expressed as mean±SD

Hens were significantly more frequently observed to perform sham dust bathing in nest areas with the artificial turf (50.7%, expected value: 33.3%, χ^2=20.3, df=1, p<0.01) (Figure 2). Hens in cages with the commercial nest mats tended to be observed to perform sham dust bathing less frequently in the nest areas (25.8%, expected value: 33.3%, χ^2=3.8, df=1, p=0.050).

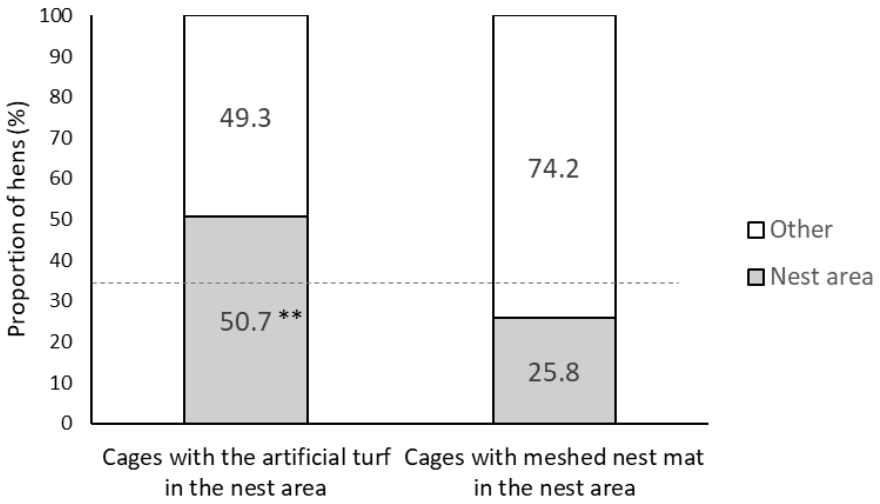

Figure 2. Proportion of hens performing sham dust bathing, χ^2 test, expected value: 33.3%. **p<0.01

Significantly more eggs were laid in the nest areas than other areas in both cages (artificial turf: 59.8%, expected value: 33.3% χ^2=99.8, df=1, commercial nest mat: 52.1%, expected value: 33.3%, χ^2=49.5, df=1, p<0.01) (Figure 3).

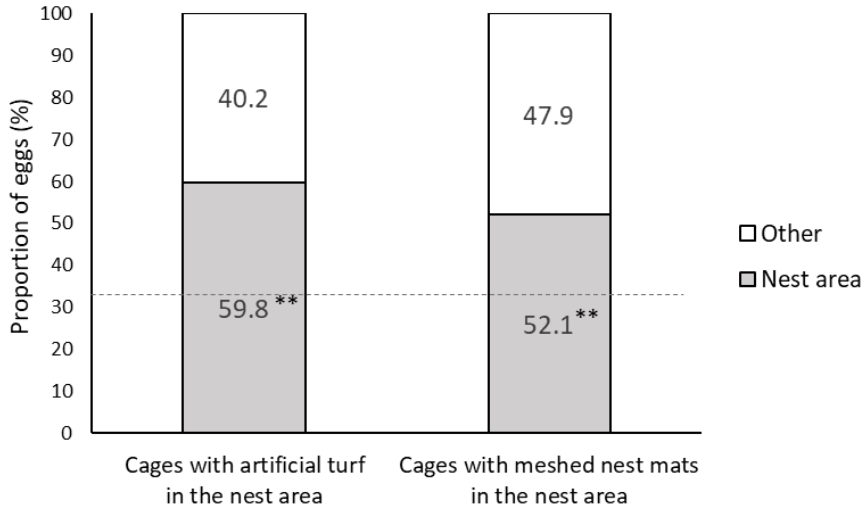

Figure 3. Proportion of eggs laid in nest areas and other areas, χ^2 test, expected value: 33.3%. **p<0.01

3. 4. Discussion

Furnished cages allow hens to perform more natural behaviors than conventional cages (Abrahamsson *et al.,* 1996; Appleby *et al.,* 2002; Shimmura *et al.,* 2007a; 2010a). The result in this study showed a variety of behaviors, and this is consistent with these previous studies. Not only the provision of the furniture, but also the layout of the cages are important for improving the welfare level of hens. The effectiveness of separation of resources on competition for them was previously reported (Shimmura *et al.,* 2008b, 2009; Xiang *et al.,* 2016). In this study, to reduce the competition for the resources, they were divided and placed on the other side of the cage. The proportion of the aggressive behaviors was low, and severe feather pecking was not observed through the observation period.

Proportions of sham dust bathing were same at about 1.9% in both cages. The percentages of hens' position in the nest areas with the artificial turfs were similar to those of the nest mats. However, sham dust bathing was observed more frequently in the nest areas with the artificial turfs than the other areas. In the cages with the nest mats, sham dust bathing tended to be observed less frequently in the nest areas than in the other areas. This result is unlike that of the experiment in chapter 2 that sham dust bathing was observed more frequently in the nest areas with the nest mats than the other areas. Furthermore, the results of experiment 1 showed that sham dust bathing was observed more frequently in the nest areas than the other areas regardless of absence of the linings. The cages used in the experiments 1 and 2 were the same design. The cage design might affect the use of the nest areas for performing sham dust bathing. Another possibility is

there might be preferences of hens to perform dust bathing, although the physical characteristics such as hardness of the artificial turf and nest mats were not examined. Hughes (2013) reported in their study that more hens dust bathed on artificial turf than on wire mesh. Previous experiences of hens may affect the place they perform dust bathing (Olsson *et al.,* 2002; Vestegaard *et al.,* 1990; Alvino *et al.,* 2013). Although the hens used in the present study were reared in cages without nest areas, dust bath, and linings, they had experiences of artificial turf and nest mats before the observation of this study, and this might affect the results.

More eggs were laid in the nest areas with both linings than the other areas. This result agrees with those of previous experiments in chapters 1 and 2. However, there were still many eggs laid outside the nest areas. Previous studies reported that high proportions of eggs were laid in the nests (Abrahamson *et al.,* 1996; Wall & Tauson, 2002; Struelens *et al.,* 2005; Wall & Tauson, 2007; Wall, 2011; Wall & Tauson, 2013). Gregarious nesting was one of the possible reasons for this. As mentioned in chapters 1 and 2, gregarious nesting is the phenomenon that hens choose to lay their eggs in a nest box occupied by one or more hens (Sherwin & Nicol, 1993; Riber, 2010). It was reported that there were "gregarious nesters", "solitary nesters", and "intermediate nesters" (Sherwin & Nicol, 1993; Tahamtani *et al.,* 2018). Some hens might be disturbed by the gregarious nesters. Higher ranking hens occupied more time in the nest boxes during the pre-laying period than lower ranking hens (Shimmura *et al.,* 2008a). Furthermore, Freire *et al.* (1997) reported that the hens used alternative routes to the nest box when there was a dominant or unfamiliar hen in it. All of these factors might affect the results.

In conclusion, artificial turf was used by hens in the square furnished cage for both dust bathing and nesting. The square design cage which had nest areas combined with a

dust bath with artificial turf was effective as a cage modified from a conventional cage.

3. 5. Summary

The aim of this study was to develop a furnished cage using the conventional cages that are mainly used in Japan. In this study, a cage design and effects of nest mats were evaluated by observing behaviors of hens and their use of resources. Six conventional cages were bound together to make a square furnished cage (72 cm width, 70 cm depth, 41 cm height) equipped with 2 perches, 2 claw sharpeners, and 2 feed and water troughs divided on both sides of the cage. Curtains partitioned 2 nest areas combined with dust baths. The 2 nest areas were all lined with polyethylene artificial turf (1236-524, Echo Kinzoku, Niigata, Japan), or commercial nest mats for the Eurovent EU cage system (Big Dutchman, Vechta, Germany). Then 4 replicates were made. Fifty-six White Leghorn (Julia) hens aged 43 weeks were used (7 hens/cage). Behaviors and location of hens were recorded at 10 min intervals by a direct visual scan technique. The places where eggs were laid were recorded. There were no significant differences in proportions of each behavior and location between the types of nest mats. Hens were significantly more frequently observed in nest areas with the artificial turf to perform sham dust bathing (50.7%, expected value: 33.3%; $p<0.01$). Hens in cages with the commercial nest mats were less frequently observed in the nest areas to perform sham dust bathing (25.8%, expected value: 33.3%; $p=0.050$). Significantly more eggs were laid in the nest areas than other areas in both cages (artificial turf: 59.8%, commercial nest mat: 52.1%, expected value: 33.3%; $p<0.01$). In conclusion, artificial turf was used by some hens in the square furnished cage for both dust bathing and nesting. The square design cage that had nest

areas combined dust bath with artificial turf was effective as a modified cage made from conventional cages.

CHAPTER 4

Modification of furnished cages from conventional cages for laying hens: comparison between rectangular and square cages, and observation of pre-laying behaviors

4. 1. Introduction

In the previous studies in chapters 1, 2 (Kikuchi *et al.,* 2018), and 3, the effectiveness of the two types of cages modified from conventional cages, which were rectangular and square was suggested. These cages had nest areas combined with a dust bath. The nest areas were used for nesting, sham dustbathing, and refuge by subordinate hens. These results suggested that effectiveness of the artificial turf as a nest liner for both sham dust bathing and nesting. Hens were observed more frequently to perform sham dust bathing in the nest areas with commercially used nest mats in the rectangular cage used in chapter 2. However, hens in the square cages in chapter 3 had a tendency to perform sham dust bathing in the nest areas with commercially used nest mats, but the difference was not significant. The reason might be that the commercially used nest mat was not attractive enough to hens. However, this is not consistent with the good result in chapter 2. Thus, the design of the cage might affect the places of sham dust bathing. Therefore, two types of modified cages were compared under the same provision in this chapter. Further, these previous studies reported that eggs were still laid outside the nest. One of the possible reasons for the floor laying was considered gregarious nesting. Gregarious nesting is a form of nest use in which hens chose nests occupied by other hens

(Sherwin & Nicol, 1993; Riber, 2010). Hens were categorized as gregarious nesters, solitary nesters, and intermediate nesters in previous studies (Sherwin & Nicol, 1993; Tahamtani *et al.,* 2018). However, the reasons for this behavior are still unknown (Tahamtani *et al.,* 2018). Pre-laying behaviors start approximately 1-2 hours before laying (Cronin *et al.* 2012; Nicol, 2015). First, the searching phase is observed as increasing activity and restlessness, and more vocalization (Wood-Gush & Gilbert, 1969; Meijsser & Hughes, 1989). Secondly, the sitting phase starts. It was reported that the sitting phase was observed from 100 min before laying and that all hens were sitting on the nest 5 min before the oviposition (Sherwin & Nicol, 1993). It was reported that individual hens had different nest site preferences and concomitantly different pre-laying behaviors (Zupan *et al*., 2008). The aim of the study was comparing two designs of cages by observing behaviors of hens and use of resources. In addition, to discover the use of the nest areas and reason for floor laying, video records of the behaviors were also observed for 30 min before laying.

4. 2. Materials and Methods

All experiments were carried out according to the guidelines of the Animal Care and Use Committee of Azabu University (approval number: 170907-2).

4. 2. 1. Animals

Fifty-six White Leghorn (Julia) hens were used when they were 54 weeks of age. All hens had their beaks trimmed at just after they hatched. At 17 weeks of cage, they were introduced to the modified furnished cages used in the previous study (Kikuchi *et al.,* 2018) at 7 birds per cage in a hen house at Azabu University.

4. 2. 2. Housing

4. 2. 2. 1. Rectangular shaped cage (RC)

The cage was used in the previous study (Kikuchi *et al.* 2018) and chapters 1 and 2. Six conventional cages were connected each other to make a rectangular shape (144 cm length, 35 cm depth, 41 cm height at the rear). Two perches (13.7 cm/bird), 2 claw sharpeners, 2 nest areas, 2 feed troughs, and 2 drinkers were provided. Nest areas combined with a dust bath were placed on both ends of the cages and partitioned by curtains.

4. 2. 2. 2. Square cage (SC)

The cage was used in the study in chapter 3. Six conventional cages were used to make a square cage. Three conventional cages were connected each other and they were bound back to back (72 cm width, 70 cm depth, 41 cm height). Two perches (13.7 cm/hen), 2 claw sharpeners, 2 nest areas, and 2 feed and water troughs were placed on both sides of the cage. Nest areas included a dust bath and were partitioned by curtains. Two nest areas were next each other.

The hexagonally shaped polyethylene artificial turfs (1236-524, Echo Kinzoku, Niigata, Japan) used in chapters 1 and 3 were put on all nest areas of both cages. Seven hens were housed in each cage (720 cm^2/hen). There were 4 replicates of each cage. Feed and water were provided ad libitum. The light schedule was 14 h light (06.00 to 20.00). The average temperature and humidity during the experiment were 14.5℃ (maximum 18.6℃, minimum 7.9℃) and 58.9% (maximum 76%, minimum 39%).

4. 2. 3. Observations

At ten min intervals, direct visual scans were carried out 4 hours per day (10.00 to

12.00 and 13.00 to 15.00), 3 days a week, for 4 weeks. The following behaviors of hens were recorded: eating/drinking, resting, comfort behaviors (body shaking, wing flapping, tail wagging, preening, head scratching, bill wiping, and stretching), sham dust bathing, exploring (looking, listening, and object pecking), aggressive pecking, and others. The following locations of the hens were recorded: nest areas, perches, and floors. All cages were video recorded (Sony Handycam HDR-CX675, Tokyo, Japan) and behaviors of hens that laid eggs during the observation time (from 10.00 to 15.00) were observed for 30 min. before the laying. Continuous sampling was used to count the number of nest visits, frequency of nest visits disturbed by the other hens, and measuring the time spent in the nest areas. The hens were regarded as entering the nest area when both legs were in the nest area. The numbers and locations of eggs laid were recorded. Feed intake for each cage was measured daily during the observation. All data collections were conducted by the same observer.

4. 2. 4. Statistical analysis

The proportion of each hen's behavior and location of total observations were calculated for each cage. The effects of cage designs on each behavior and use of resources were analyzed using Mann-Whitney's U-tests. Chi-square tests were used to evaluate use of the nest areas. Because the nest areas occupied 33.3% of that of total cage floor, the expected value was 33.3%. Student's T-tests or Welch's T-tests were used to compare the feed intake in both cages after an F-test was carried out. Numbers of visits to the nests and total duration spent in the nests in both cages were analyzed using Mann-Whitney's U-test. Time spent on the layer and floor layer of the nests were compared using Mann-Whitney's U-test. The statistical software Statcel 4 (Yanai, 2015) was used for all analyses.

4. 3. Results

There were no significant differences in the proportions of each behavior and location between the rectangular and square cages (Tables 1, 2).

Table 1. Mean proportion of bird behaviors in rectangular cage and square cage

Behavior	Rectangular cage (%)	Square cage (%)
Eating and drinking	33.8 ± 2.5	33.4 ± 1.4
Resting	39.4 ± 0.4	42.0 ± 1.7
Comfort behavior	16.8 ± 1.9	16.1 ± 1.5
Sham dust bathing	1.4 ± 0.6	0.9 ± 0.6
Aggressive behavior	0.5 ± 0.2	0.6 ± 0.2
Explore	2.3 ± 0.2	1.8 ± 0.5
Other	5.8 ± 0.7	5.2 ± 1.3

Values are expressed as mean ± SD.

Table 2. Mean proportion of birds located position in rectangular cage and square cage

Position	Rectangular cage	Square cage (%)
Nest area	24.1 ± 1.5	22.7 ± 4.0
Perch	48.1 ± 4.7	55.2 ± 2.9
Floor	27.9 ± 3.3	22.1 ± 2.6

Values are expressed as mean ± SD

Hens in rectangular cages were significantly more frequently observed to perform sham dust bathing in the nest areas than in the other areas (62.8%, expected value: 33.3%, $\chi^2 = 44.3$, df = 1, $p<0.01$) (Figure 1). There was no significant difference in places of performing sham dust bathing in the square cages (40.8%, expected value: 33.3%, $\chi^2 = 1.9$, df = 1, $p = 0.17$).

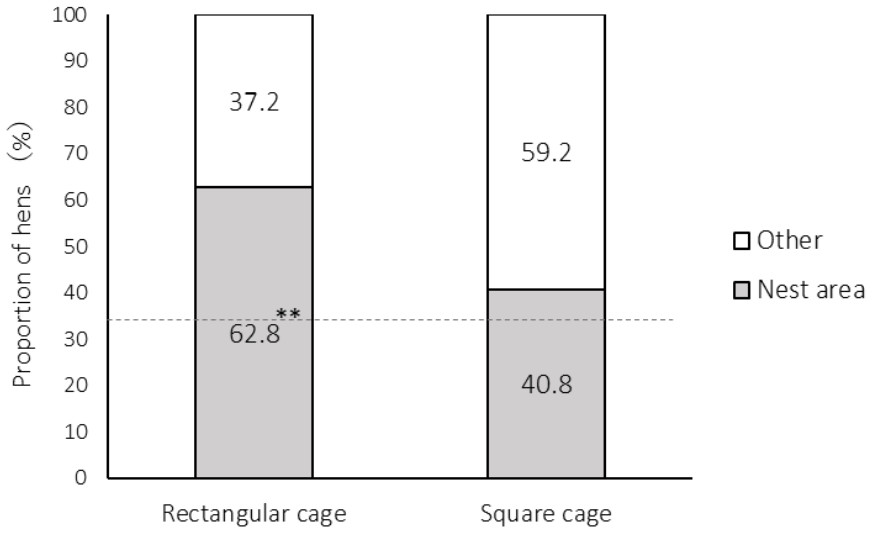

Figure 1. Proportion of hens performing sham dust bathing, χ^2-test, expected value: 33.3%, **p<0.01

Significantly more eggs were laid in the nest areas than the other areas in both cages (rectangular cage: 77.4%, $\chi^2 = 271.4$, df = 1, p<0.01, square cage: 57.2%, $\chi^2 = 81.8$, df = 1, p<0.01) (Figure 2).

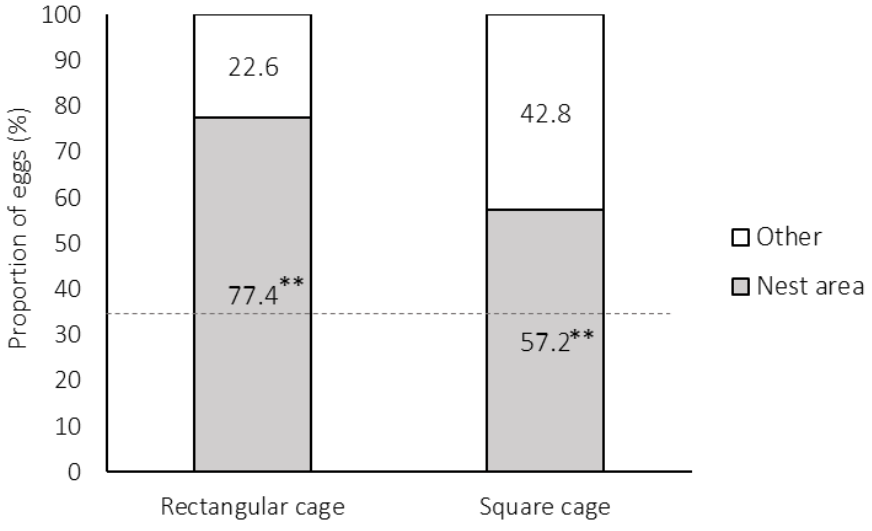

Figure 2. Proportion of eggs laid in the nest areas and the other areas, χ^2-test, expected value: 33.3%, **p<0.01

Table 3 shows nest visits for 30 min before laying. Visits to the nests were more frequent in the rectangular cage than the square cage ($p<0.05$). There was no significant difference between the cage types in numbers of nest visits where eggs were laid and total time spent in the nest.

Table 3. Nest visits of hens for 30 min. before laying

Variable	Rectangular cage	Square cage
Number of hens observed	17	26
Number of eggs laid outside of the nest	2	3
Mean number of nest visits (visit/hen)	8.9 ± 7.7^{a}	4.3 ± 4.6^{b}
Mean number of nest visits which the hens laid (visit/hen)	4.9 ± 4.3	3.4 ± 4.0
Mean total time spent in the nest (min/hen)	18.6 ± 9.5	20.0 ± 10.0
Mean time of the nest visits which the hens laid (min/hen)	10.0 ± 10.5	15.4 ± 12.8

Values are expressed as mean±SD. Different letters in the same row indicate significant difference at $p<0.05$; Mann-Whitney's U test.

Hens spent significantly longer in the nests than on the floor when they laid eggs (Table 4). Table 5 shows details of nest visits with other hens. Proportions of time spent visiting a nest occupied by more than one hen were similar in both cages at 48% in the rectangular cage and 45.9% in the square cage. The proportions of nest visits that were disturbed by other hens were 12.3% in the rectangular cage and 3.9% in the square cage, although frequencies were low, and statistical analyses could not carried out. Replacement of hens without any conflicts was observed. Some hens laid eggs whilst another hen was in the same nest.

Table 4. Details of nest layers and floor layers

Variable	Nest layer	Floor layer
Number of hens observed	38	7
Mean time spent in the place where they laid eggs (sec/hen)	744.4 ± 701.6**	72.1 ± 82.7**

** $p<0.01$, Mann-Whitney's U-test

Table 5. Nest visit

Variable	Rectangular cage	Square cage
Total number of nest visits	152	111
Proportion of nest visits that the nest was occupied by more than one hen (%)	48.0	45.9
Proportion of nest visits disturbed by other hens (visits disturbed/visits the nest occupied by more than one hen) (%)	12.3	3.9
Number of replacements without conflict	5	2
Number of hens laid eggs whilst the other hen was in the same nest	3	8

Table 6 shows the productivity in both cages. Egg productions per hen in 12 days were similar in both cage types. However, feed intake of hens in the square cage was significantly higher than that in the rectangular cage ($t = -3.35$, $df = 6$, Welch's T-test, $p<0.05$).

Table 6. Mean values of egg production

Variable	Rectangular cage	Square cage
Egg production (egg/hen/12days)	11.1 ± 1.4	11.1 ± 1.0
Feed intake (g/day/hen)	116.7 ± 7.7^{a}	121.3 ± 3.5^{b}

Values are expressed as mean ± SD. Different letters in the same row indicate significant difference. ($t = -3.35$, $df = 6$, $p<0.05$, Welch's t-test).

4. 4. Discussion

Behaviors and location of the hens were similar in both cage types. As mentioned in chapters 1–3, hens performed natural behaviors such as perching and nesting in furnished cages (Abrahamsson *et al.,* 1996; Appleby *et al.,* 2002; Shimmura *et al.,* 2007a; 2010; Lay *et al.,* 2011). Consistent with these authors, hens used perches and nest areas in the present study. Therefore, both cages allowed hens to perform natural behaviors.

For sham dust bathing, hens in rectangular cages were observed more frequently in the nest areas. This is consistent with results of the study that used the rectangular cages in chapters 1 and 2 (Kikuchi *et al.,* 2018). However, hens in square cages were observed to perform less sham dust bathing in the nest areas in the present study. In the study of the same square cage in chapter 3, sham dust bathing was observed more frequently in the nest areas with the artificial turf than the other areas. The reason is difficult to explain, but inconsistent use of the location of sham dust bathing in the square cage was found. One of the reasons of this might be the distance between the two nest areas. It was reported that low-ranked hens were often disturbed while sham dustbathing by higher ranked hens (Moroki & Tanaka, 2016; Moroki, 2018). It was suggested that hens prefer to perform dust bathing beside a familiar hen rather than in an empty cage (Shimmura *et al.*, 2010b). Moroki (2018) reported that it was important to see other hens to continue sham dust bathing. The nest areas were next to each other in the square cage, and hens in the nest area could see the other nest area. This might affect the choice of a place for performing sham dust bathing in the square cage.

More eggs were laid in the nest areas in both cages. However, there were still eggs

laid outside the nest, especially in the square cage. There were many factors reported that affect laying outside: genetic factors (Appleby, 1984; Wall, 2011), experiences (Cooper & Appleby, 1995), nest design (Wall & Tauson, 2002, 2007), presence of an attractive place (Wall, 2011), and social aspects (Cronin *et al*., 2009; Riber, 2010). However, Hunniford *et al*. (2014) suggested that not only the number of eggs laid in the nest may be an indicator of welfare of hens, but also that the nesting behavior itself is important.

In this study, behaviors for 30 min before laying were observed. The number of nest visits was more frequent in the rectangular cage then the square cage. The mean numbers of nest visits where eggs were laid in the present study seem not to be different from those in the previous studies (Appleby & Smith, 1991; Sherwin & Nicol, 1993), although these results could not be compared due to the difference between total observation times. There were no significant differences between the cages in the mean number of nest visits, time spent in the nest where eggs were laid, and total time spent in the nest. In a previous study (Appleby, 1990), an excessive number of nest visits observed were considered an abnormal behavior. Compared to that, the frequencies observed in the cages here seems to be acceptable. Floor layers that laid eggs outside the nest on the wire floor spent significantly shorter times in the places where they laid eggs than nest layers that laid eggs in the nest. This is consistent with the result of the previous study (Cronin *et al*. 2005). Also, litter layers that laid eggs in the litter tray spent more time exploring and less time in final nest visits than nest layers (Zupan *et al*., 2008). Floor layers in this present study laid eggs outside the nest even if they visited the nests. It is not clear if the floor layers were frustrated or not. It was suggested that hens had nest-site preferences (Zupan *et al.*, 2008). Gregarious nesting was observed in both cages. Proportions of visits to a nest occupied by more than one hen were 48.0% of total nest visits in the rectangular

cage and 45.9% of total nest visits in the square cage. Although frequencies were low., disturbance of nest visits was more frequently observed in the rectangular cage than the square cage. Contrary to expectations, gregarious nesting might not the reason for floor laying in this study, at least in the hens who laid eggs during the observation time. Some hens laid eggs whilst other hens were in the same nest even though the nest was packed with hens. As mentioned above, experiences of hens and individual preferences may affect the place of laying. In this present study, the behavior was recorded from 10.00 am to 15.00 pm, so not all the ovipositions were observed. Further, hens were not identified. Observation of individual hens in early morning will give more information about usage of the nest areas.

Feed intake of hens in the square cage was higher than that in the rectangular cage. The egg production per hen in 12 days was similar in both cages. Some studies showed that resting on perches led to energy savings, and reduced feed consumption (EFSA, 2005; Tauson & Abrahamsson, 1994; Hester *et al*., 2013). Although there were no significant differences found in proportions of behaviors and locations of hens between rectangular and square cages, mean proportions of resting were 39.4% vs. 42.0%, and mean proportions of hens on perches were 48.1% vs. 55.2%, respectively. These results and higher consumption of feed in the square cage were inconsistent with the previous studies. The reason is unknown, but physical observations such as feather loss and a more precise measure of energy use should be made to reveal the reason for this discrepancy.

To conclude, behaviors were not restricted, and no severe aggressive behaviors were observed in both cages. However, nest areas combined with a dust bath were used more in the rectangular cages for both sham dust bathing and laying eggs than in the square cages. Therefore, resources in the rectangular cage were more used by hens than

those in square cage.

4. 5. Summary

A series of studies was carried out to examine the modification of conventional cages to make furnished cages that were low in cost. The objective of the study was comparing two cage designs, which were rectangular and square shapes, by observing behaviors of hens and use of resources. Fifty-six White Leghorn (Julia) hens aged 54 weeks were housed 7 birds/cage, and 4 replicates were made for each cage. Behaviors and locations of hens were recorded using a direct visual sampling technique. Places where eggs were laid were recorded, and behaviors before laying were also observed for 30 min on video records. There were no significant differences in proportions of each behavior and location between the rectangular and square cages. Hens in rectangular cages were significantly more frequently observed to perform sham dust bathing in the nest areas than in the other areas (62.8%, expected value: 33.3%, $\chi^2 = 44.3$, df = 1, $p<0.01$). There was no significant difference on places of performing sham dust bathing in the square cages (40.8%, expected value: 33.3%, $\chi^2 = 1.9$, df = 1, $p = 0.17$). Significantly more eggs were laid in the nest areas than the other areas in both cages (rectangular cage: 77.4%, $\chi^2 = 271.4$, df = 1, $p<0.01$, square cage: 57.2%, $\chi^2 = 81.8$, df = 1, $p<0.01$). Nest visits were more frequent in the rectangular cage than the square cage ($p<0.05$). There was no significant difference in the number of nest visits where eggs were laid and total time spent in the nest between the cage types. Nest layers spent significantly more time than floor layers when they laid eggs. Proportions of nest visits occupied by more than one hen were similar in both cages (48% in the rectangular cage, 45.9% in the square cage). To conclude, behaviors were not restricted, and no severe aggressive behaviors

were observed in both cages. However, nest areas combined with a dust bath were used more in the rectangular cages for both sham dust bathing and laying eggs than in the square cage. Therefore, resources in the rectangular cage were used more often by hens than those in square cage.

GENERAL DISCUSSION

In this study, modified furnished cages were developed from conventional cages. They were equipped with 2 nest areas, 2 wooden perches, 2 claw sharpeners, 2 feed troughs and 2 drinkers. The nest areas were combined with dust baths. Evaluations were carried out based on the behaviors of hens, their locations, and use of nest areas. In chapter 1, establishment of social order, behavioral changes. and use of resources after introduction to the cage were reported. Besides that, effects of an artificial turf in the nest area were evaluated. In chapter 2, a nest mat and a litter mat used in the Eurovent EU cage system (Big Dutchman, Vechta, Germany) were used for nest linings and whether they increased nest use of hens for nesting and sham dust bathing was evaluated. Because most of the commercially available furnished cages were deeper than conventional cages, another design of cage that was deeper than that in chapter 1 and 2 was developed and reported in chapter 3. In addition, the artificial turf used in chapter 1 and the nest mat used in chapter 2 were used as nest linings and evaluated. In chapter 4, two types of modified cages were compared by observing the behaviors and used of resources. In addition, to find out the use of nest areas in the cage, the behaviors during the 30 minutes before laying were observed. In this chapter, the usefulness of the modified furnished cages was discussed with the previous studies. Finally, an effective combination of the resources and design of the cage was suggested.

As reported in the first experiment of chapter 1, hens were habituated the environment in a few days after introduction to the modified cage. This is consistent with the previous reports that hens could be habituated to the changes in the environments within few days (Anderson *et al*., 1989; Tanaka & Hurnik, 1991b; Shimmura *et al*., 2006).

It was reported that the adjustment from the battery cage to the new environment was delayed in an aviary compared with cages (Tanaka & Hurnik, 1992; Shimmura *et al*., 2006). Authors suggested that the reasons for the slower adjustment were differences in the environment between rearing and the experiments, and the complexity of the aviary design. It was generally agreed that the hens should be reared in an environment similar to that in which they will be housed as adults (Janczak & Riber, 2015). In the present study, hens were reared in cages without perches and nests before being introduced to the hen house at Azabu University. The simple design of the modified cage might affect the short adjustment period even if the hens had never used perches until introduced to them.

Severe feather pecking and aggressive pecking can lead to severe injury. This is one of the causes of cannibalism, which is a welfare problem that could lead to a high mortality rate (Savoy, 1995; Rodenburg *et al*., 2013). The proportion of aggressive behavior was low, and no severe feather pecking was observed in the study from chapters 1 to 4. In agreement with the study by Shimmura *et al*. (2008b, 2009), separation of resources could reduce the competition for them. In addition, the observation reported in chapter 1 found that the nest combined dust bath was used not only for laying eggs and sham dust bathing but also for refuge by the low-ranked hens. This is consistent with previous studies (Shimmura *et al*., 2007a, 2008a, b). Providing a place where hens can escape from other hens may play an important role in the low incidence of aggression in this study. Thus, low rates of aggressive behaviors and severe feather pecking was one of the advantages of this modified cage.

Sham dust bathing was more frequently observed in the nest area with the artificial turf, and commercially used nest mats and no linings in the other area in the rectangular cage as reported in chapters 1, 2, and 4. Although the proportions of hens that performed

sham dust bathing in the nest areas varied among the nest linings, the nest area in the rectangular cage was well used for sham dust bathing throughout the study. On the other hand, the inconsistency of the location of sham dust bathing in the square cage was found. Hens performed sham dust bathing in the nest area with the artificial turf in chapter 3; however, it was not observed in chapter 4, in which hens were less often observed performing sham dust bathing in the nest areas. Furthermore, the commercially used nest mats were well used for sham dust bathing in the rectangular cage in chapter 2, but they were not used in the square cage in chapter 3. As mentioned in chapter 4, one of the reasons for this inconsistency found in the square cages might be the distance and location between the two nests. Low ranked hens often disturbed the higher ranked hens by sham dust bathing (Moroki and Tanaka, 2016; Moroki, 2018). Hens prefer to perform dust bathing beside a familiar hen rather than in an empty cage (Shimmura *et al*., 2010b). Seeing other hens was important to continue sham dust bathing (Moroki, 2018). Lindberg and Nicol (1997) concluded that it is acceptable to provide adequate space and access to a loose pecking substrate in a place where sham dust bathing can occur without interruption, although providing a dust bath has additional welfare benefits such as improving foot health and performing foraging behavior. Furthermore, the place does not need to be a separate facility called a dust bath by humans. Hens used feed in feed troughs as a stimulus for performing sham dust bathing (Moroki & Tanaka, 2016). Hens in the present study did use the feed in the feed trough during their sham dust bathing behaviors. The nest areas in the rectangular cage in the present study may play a role as places where hens were not disturbed. This may be more important for hens performing sham dust bathing rather than the presence and kind of nest linings. In any event, nest areas combined a dust bath on both sides of the cage may be a better arrangement rather than

next to each other for performing sham dust bathing, although there was room for addition to the layout of the square cage in which the nest areas were placed diagonally. In addition, details of the sham dust bathing activities such as length of the bouts are needed to evaluate the dust bathing behavior.

Although more eggs were laid in the nest areas in the rectangular cage in the present study, proportions of eggs laid in the nest were lower in chapters 1 and 2 than chapter 4. The proportion increased in each study. Curtains were added on the end of cages to obstruct the view of neighbor cages after the study of chapter 2. In addition, the hen house was moved to the new place between the studies in chapters 2 and 3. The hen house used in chapters 1 and 2 had windows, while that of chapter 3 and 4 was windowless. Therefore, hen house used in chapters 1 and 2 might be brighter than those in chapter 3 and 4, even though the windows were shaded and the nest areas were enclosed by curtains. These differences might affect the increased number of eggs laid in the nest in each study. It was reported that nest choice is influenced by hens' experiences (Hughes, 1993; Struelens, 2005; Riber, 2010). It was suggested that hens preferred the nest to which they were habituated. Hens' experience might affect the choice of places where eggs laid in the present studies, especially in chapters 2 – 4. More eggs were laid in the nest areas with linings than on the bare cage floor in chapter 1. This is consistent with the previous study (Wall & Tauson, 2013). Although it was not compared with the artificial turf and commercially used nest mat in rectangular cage, the artificial turf used in the study was easier to purchase and costs far less than the commercially used nest mat when simply compared (artificial turf: about 100 yen/cage, commercially used nest mat (imported): 2,000 yen/cage). Therefore, artificial turf was adequate to meet the aim of this present study, which was developing a modified cage that allowed natural behaviors of hens and

was low in cost.

In conclusion, these results suggested the usefulness of the furnished cages modified from conventional cages. Recommendations could be made that the cage for 7 birds per cage would be rectangular shape and have two nest areas that combined dust bath partitioned by polyethylene curtains on both ends of the cage with hexagon-shaped artificial turf. Materials used in the modified furnished cage were purchased easily and inexpensively (total 200–300 yen/cage). In addition, because the cages were modified from conventional cages, the facilities of hen house can be used as before. Although field tests should be carried out and consideration should be given to how large a hen house may benefit from this modification including labor costs, compared to how much consumers were willing to pay for these eggs. The results of the present studies would contribute to the Japanese poultry industry which is recently requiring changes in housing systems for the welfare of laying hens, and farmers who have an interest in changing the housing systems for hens inexpensively.

Part of this dissertation has been published as follows:

1. Kikuchi A., Uetake K., Tanaka T.: Modification of furnished cages from conventional cages for laying hens: establishing social order, behavioral changes, and use of resources after introduction. *Animal Behavior and Management*, 54(3): 123-133, 2018.

SUMMARY

Increasing public concern for the welfare of laying hens has resulted in a ban on use of conventional cages in EU countries and some states in the U.S.A. and the decision by some global companies to avoid using hen eggs laid in conventional cages. The main problem of the conventional cages is the restriction of high-priority behaviors of hens such as nesting, perching, dust bathing, and foraging. More than 90% of the laying hens are kept in conventional cages in Japan. One of the barriers to conversion from conventional cages to alternative systems is the high introduction cost. The aim of the present study was development of an alternative housing system that is both practical and economical in the current Japanese environment.

Chapter 1 (Establishing social order, behavioral changes, and use of resources after introduction to the modified cage)

The furnished cage was modified from 6 conventional cages. Two nest areas were provided at both ends of the cage and were combined with a dust bath. Two experiments were reported in chapter 1. In experiment 1, behavioral changes and establishment of social order were observed. In experiment 2, artificial turf was put on the nest areas as nest linings and the effects on nest use were evaluated. Fifty-six White Leghorn (Julia) hens 123 days old were allocated to 8 furnished cages. In experiment 1, all aggressive behaviors were directly observed to calculate a dominance ranking. In experiments 1 and 2, behaviors and use of the resources were recorded using a direct visual scan sampling technique at 10 min intervals. Further, locations where eggs were laid and the behaviors and locations of the highest and lowest rank hens were recorded. The hens became habituated to the environment in a few days after introduction to the modified cage. The

rate of aggressive behavior was low, no severe feather pecking was observed, and a wide variety of behaviors were observed. More eggs were laid in the nest areas with artificial turf than on the bare cage floor. Artificial turf did not affect the place of sham dust bathing. The nest combined with a dust bath was used not only for laying eggs and sham dust bathing but also for refuge by low-ranked hens. In conclusion, separation of resources and combined nest and dust bath were functional in this cage design and group size. Artificial turf was effective for nest linings but not for sham dust bathing.

Chapter 2 (Comparison of two nest linings used in commercial furnished cages)

To promote use of the nest areas in the modified cage used in Chapter 1, the effects of a commercially used litter mat and a nest mat for the Eurovent EU cage system were evaluated. Fifty-six White Leghorn (Julia) hens were allocated 7 birds per cage, and behaviors and locations of hens were recorded. Hens were more frequently observed in the nest areas in cages with nest mats than those with litter mats ($p<0.05$). Sham dust bathing was more frequently observed in the nest areas than the other areas in the cages with nest mats ($p<0.01$). On the other hand, it was less frequently observed in nest areas than in other areas in the cages with the litter mats ($p<0.01$). More eggs were laid in nest areas than in other areas in both cages ($p<0.01$). These results suggest that the commercially used nest mats are effective in the nest area combined with a dust bath in the modified cage.

Chapter 3 (Development of another cage design and comparison of two nest linings)

Another cage design that was deeper than the cages in chapters 1 and 2 was developed. In addition, the artificial turf and commercially used nest mats that were found to be effective in chapters 1 and 2 were evaluated as nest linings. Six conventional cages were bound together to make a square furnished cage (72 cm width, 70 cm depth, 41 cm

height). Equipment was the same as in chapters 1 and 2. The two nest areas were partitioned by curtains, and all were lined with artificial turf or commercial nest mats. Fifty-six White Leghorn (Julia) hens aged 43 weeks were used (7 hens/cage). Behaviors and location of hens were recorded at 10 min. intervals by a direct visual scan technique. The places where eggs were laid were recorded. There were no significant differences in proportions of each behavior and locations between types of nest mats. Hens were significantly more often observed to perform sham dust bathing in the nest areas with the artificial turf ($p<0.01$). Hens in cages with the commercial nest mats tended to be less frequently observed in the nest areas to perform sham dust bathing ($p=0.050$). Significantly more eggs were laid in the nest areas than other areas in both cages ($p<0.01$). In conclusion, artificial turf was used by some hens in the square furnished cage for both dust bathing and nesting. The square design cage which had nest areas combined dust bath with artificial turf was also effective as a modified furnished cage.

Chapter 4 (Comparison between rectangular and square cages, and observation of pre-laying behaviors)

In chapter 4, the study compared two designs of cages which were rectangular and square shapes. Fifty-six White Leghorn (Julia) hens aged 54 weeks were housed 7 birds/cage, and 4 replicates of each cage were made. Behaviors and locations of hens were recorded using a direct visual sampling technique. Places where eggs were laid were recorded, and behaviors before laying were also observed for 30 min by video recordings. There were no significant differences on proportions of each behavior and location between the rectangular and square cages. Hens in rectangular cages were significantly more frequently observed to perform sham dust bathing in the nest areas than in the other areas ($p<0.01$). However, there was no significant difference in places of performing

sham dust bathing in the square cages ($p = 0.17$). Significantly more eggs were laid in the nest areas than the other areas in both cages ($p<0.01$). Nest visits were more frequent in the rectangular cages than the square cages ($p<0.05$). There was no significant difference in number of nest visits where eggs were laid and total time spent in the nest among the cage types. Time spent in the place by nest layers was significantly longer than that by floor layers when they laid eggs. Proportions of nest visits occupied by more than one hen were similar in both cages (48% in the rectangular cage, 45.9% in the square cage). Egg productions per hen in 12 days were similar in both cage types. Feed intake of hens in the square cage was significantly higher than that in the rectangular cage ($p<0.05$). To conclude, the resources of the rectangular cage were used more than those of the square cage.

In conclusion, these results suggest that the usefulness of producing the modified furnished cages from conventional cages. Recommendations could be made that the cage would be rectangular shape and have two nest areas combined with a dust bath and partitioned by polyethylene curtain on both end of the cage with hexagon shaped artificial turf. Materials used in this study were purchased inexpensively. These results would contribute to the Japanese poultry industry, which is concerned with housing systems and the welfare of laying hens.

和文要旨

アニマルウェルフェア(AW)に対する社会的関心の高まりから、EU 諸国や米国のいくつかの州では既に産卵鶏の従来型ケージでの飼育が禁止され、国際的に AW に配慮した飼育方法へ転換する動きがある。従来型ケージでは、主に鶏の行動の制限が問題とされるが、日本では 90%以上が従来型ケージを使用しているのが現状である。従来型ケージから代替飼育方法へ変換する際の問題のひとつとして、高額な導入コストが挙げられる。本研究は、導入が容易な AW に配慮した産卵鶏の飼育方法の提案を目的とし、従来型ケージを改良したエンリッチドケージを試作し、その効果を検討した。

【第 1 章：試作ケージへの鶏導入後の社会的順位確立と馴化過程および社会的順位と資源利用の関係】

従来型ケージ 6 個を横 1 列に結合し、止まり木、2 ヵ所の巣箱兼砂浴び場、爪とぎを設置したケージ 8 個を試作した。実験 1 として、鶏導入後の社会的順位確立と順化過程について観察を行い、実験 2 として、巣箱兼砂浴び場の敷き材として人工芝を設置し、その効果を検討した。

実験 1 では、試作したケージに 17 週齢の白色レグホーン 56 羽を 7 羽ずつ導入（720cm^2/羽）した。導入直後から連続 6 日間、午前 10～12 時、午後 13～15

時の間に、各ケージ 10 分間の直接観察により各個体間の敵対行動を記録し、社会的順位の観察を行った。また、導入後 3 週間にわたり週 3 回、その後は 5 週目まで週１回、午前 10～12 時、午後 13～15 時の間に 10 分間隔の瞬間サンプリング法により鶏の行動（摂取、休息、慰安、敵対、探査、その他)、資源の利用について記録し、順化過程を観察した。敵対行動は導入後１週間は 1～2%、その後は 1%以下となり、1 週間程度で順位が確立したものと考えられた。なお、導入直後から激しい敵対行動は認められなかった。各行動の発現割合は導入後数日でほぼ一定となり、馴化が完了したものと考えられた。各個体間の敵対行動の記録より David’s score を計算し、最上位、最下位個体を特定した。

実験２では、実験１と同じ簡易型エンリッチドケージの巣箱兼砂浴び場に、敷き材として人工芝を設置したものを 4 ケージ、対照として敷き材を設置しないものを 4 ケージ用意した。供試個体についても、実験１と同じ白色レグホーン 56 羽（26 週齢）を使用した。週 3 回 4 週間、午前 10～12 時、午後 13～15 時の間に 10 分間隔の瞬間サンプリング法により行動、資源の利用について観察を行った。さらに、産卵数・産卵場所についても記録した。また、実験１の結果より得た各群の社会的順位最上位の個体、最下位の個体の資源の利用状況についても観察を行った。人工芝設置の有無による各行動、資源の利用に有意な差は認められなかった。砂浴び様行動の発現場所について、面積（巣箱 33.3%、巣箱以

外 66.6%）を元に比較したところ、両群ともに巣箱兼砂浴び場で期待値よりも有意に多く砂浴び様行動が認められた(人工芝設置群 63.2%、人工芝無し群 54.7%、期待値 33.3%、$p<0.01$)。産卵場所について、人工芝無しの群に比べて人口芝設置の群において、巣箱兼砂浴び場で多く産卵が認められた（人工芝設置群 38.5%、人工芝無し群 17.5%、$p<0.01$)。また、人工芝設置群では巣箱兼砂浴び場における産卵が有意に多く（期待値 33.3%、$p<0.05$)、人工芝無しの群では巣箱兼砂浴び場における産卵が少なかった（期待値 33.3%、$p<0.01$)。社会的順位と資源利用との関係について、最下位個体は最上位個体と比べて頻繁に移動しており(最下位個体 5.5%、最上位個体 2.3%、$p<0.05$)、巣箱兼砂浴び場をより利用していた(最下位個体 38.5%、最上位個体 23.2%、$p<0.01$)。

試作ケージにおいて、激しい敵対行動はみられず、行動が大きく制限されることもなかったため、福祉ケージとしての機能を果たしていると考えられた。巣箱兼砂浴び場は産卵、砂浴び様行動に利用されるだけでなく、社会的順位の低い鶏の退避場所としても機能していた。また、人工芝の設置により巣箱兼砂浴び場での産卵が多く認められた。

【第 2 章：市販の敷き材 2 種類の効果の検討】

第 1 章で作製したケージの巣箱兼砂浴び場の利用促進を目的として、市販のエンリッチドケージに使用されている 2 種類の敷き材（巣箱用と砂浴び用：Big

Dutchman）を使用し、その効果を検討した。両端の巣箱兼砂浴び場に巣箱用敷き材を敷き詰めたケージ（N ケージ）と、砂浴び用敷き材を敷き詰めたケージ（D ケージ）を各 4 ケージ用意した。観察方法は第 1 章に準じた。また第 1 章と同じ白色レグホーン（32 週齢）56 羽を使用した。D ケージよりも N ケージにおいて、巣箱兼砂浴び場をより多く利用していた（N：26.4%、D：20.9%; $P<0.05$）。砂浴び様行動の発現場所について、面積（巣箱 33.3%、巣箱以外 66.6%）を元に比較したところ、N ケージでは巣箱以外の場所よりも巣箱兼砂浴び場において期待値よりも多く認められ（47.9%、期待値 33.3%、$P<0.01$）、D ケージでは、巣箱以外の場所において多く認められた（88.7%、期待値 33.3%、$P<0.01$）。産卵場所については、どちらのケージにおいても、巣箱以外よりも巣箱兼砂浴び場での産卵が多く認められた（D：50.9%、N：53.6%、期待値 33.3% 、$P<0.01$）。砂浴び様行動、産卵ともに、砂浴び用敷き材に比べて巣箱用敷き材の方がより多く使われていた。巣箱と砂浴び場を兼用とした本ケージにおいては、市販の巣箱用敷き材が効果的であることが示唆された。

【第 3 章：新規ケージレイアウトと敷き材 2 種の比較】

新規のレイアウトのケージを作製し、第 1、2 章において結果が良好であった人工芝と市販の巣箱用敷き材の比較を同時に行った。従来型ケージを 3 個ずつ横並びに連結したもの二つを背中合わせに連結し、正方形に近いケージを作製

した。資材はすべて第 2 章までと同じものを同じ数量使用したが、巣箱兼砂浴び場は隣り合い、止まり木、飼槽、水受けは 2 ヵ所に分散する形となった。二つの巣箱兼砂浴び場に人工芝を設置したケージ（AT ケージ）と、市販の巣箱用敷き材を設置したケージ（N ケージ）を各 4 ケージに、これまでと同じ白色レグホーン群を導入した（43 週齢）。観察方法、項目は第 1 章に準じた。AT ケージにおいて、巣箱以外の場所よりも巣箱兼砂浴び場においてより多く砂浴び様行動が認められた（50.7%、期待値 33.3%、P<0.01）。N ケージにおいて、巣箱以外の場所での砂浴び様行動が多い傾向が認められた（74.2%、期待値 66.6%、P=0.05）。また、どちらの敷き材のケージにおいても、巣箱兼砂浴び場での産卵が多く認められた（AT：59.8%、 N：52.1%、期待値 33.3%、ともに P<0.01）。今回作製した正方形のケージデザイン、巣箱と砂浴び場所の兼用はこのグループサイズの白色レグホーンにおいて、福祉ケージとしての機能を果たしていると考えられた。また、本ケージにおいて、人工芝を設置した巣箱兼砂浴び場が砂浴び様行動と産卵の場所としてより多く利用され、人工芝の有用性が示唆された。

【第 4 章：ケージレイアウト 2 種の比較】

第 3 章までに作製した長方形と正方形のケージにおける鶏の行動、資源利用の比較を行った。すべての巣箱兼砂浴び場には敷き材として人工芝を設置した。第 3 章までの実験と同じ鶏（54 週齢）を供試個体とし、同様の観察項目に加え、

飼料摂取量を測定した。また、巣箱兼砂浴び場における鶏の行動を観察するため、ビデオ録画より、産卵前 30 分間の鶏の行動について連続記録を行った。鶏の行動、利用場所ともにケージ間で有意差は認められなかった。長方形ケージにおいて巣箱兼砂浴び場での砂浴び様行動が多く認められ（62.8%、 期待値 33.3%、P<0.01)、正方形ケージにおける砂浴び様行動発現に場所による有意差は認められなかった（巣箱兼砂浴び場 40.8%、巣箱以外 59.2%)。産卵場所について、どちらのケージも巣箱兼砂浴び場における産卵が多く認められた（長方形 77.4%、正方形 57.2%、期待値 33.3%、ともに P<0.01)。産卵前行動について、巣箱兼砂浴び場への訪問は長方形のケージにおいて有意に多く認められた(P<0.05)。産卵を行った巣箱への訪問回数、総滞在時間は両ケージ間で差は認められなかった。産卵を行った際の産卵場所への滞在時間は、巣箱以外に産卵した鶏に比べ巣箱に産卵した鶏の方が長かった。巣箱に複数の鶏が滞在していた訪問の割合は両ケージ同等に認められた（長方形：48%、正方形 45.9%)。これらの結果より、長方形のケージにおいて巣箱兼砂浴び場がより活用されていたことが明らかとなった。

以上の結果より、従来型ケージを改良した簡易福祉ケージの有用性が明らかとなった。鶏の利用が多かった組み合わせは、長方形のデザイン、巣箱兼砂浴び場はふたつを両端に設置すること、巣箱兼砂浴び場はカーテンで仕切られるこ

と、六角形の人工芝を敷材として巣箱兼砂浴び場に設置することであった。資材はすべて容易に、そして安価に入手が可能である。農家などへの導入試験などさらなる検討が望まれるが、本研究の結果が AW に配慮した飼育方法導入の検討の一助となることを期待する。

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