Study on welfare of laying hens in various housing systems

-developments of overall welfare assessment and new modified cage system-

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Contents

Published papers

This thesis is based on the following papers:

CHAPTER 1: Shimmura, T, T. Suzuki, T. Azuma, S. Hirahara, Y. Eguchi, K. Uetake and T. Tanaka, Form not but frequency of beak use by hens is changed by housing system. Applied Animal Behaviour Science, 115: 44-54. 2008.

CHAPTER 2: Shimmura, T., S. Hirahara, T. Azuma, T. Suzuki, Y. Eguchi, K. Uetake and T. Tanaka, Multi-factorial investigation of various housing systems for laying hens. British Poultry Science. (revision required)

CHAPTER 3: Shimmura, T., S. Hirahara, Y. Eguchi, K. Uetake and T. Tanaka, Overall welfare assessment for laying hen – comparing science-based, environment-based and animal-based assessment. Animal Science Journal. (under review)

CHAPTER 4: Shimmura, T., Y. Eguchi, K. Uetake and T. Tanaka, Effects of separation of resources on behavior of high-, medium- and low-ranked hens in furnished cages. Applied Animal Behaviour Science, 113: 74-86. 2008.

CHAPTER 5: Shimmura, T., T. Azuma, Y. Eguchi, K. Uetake and T. Tanaka, Effects of separation of resources on behaviour, physical condition and production of laying hens in furnished cages. British Poultry Science. (in press)

INTRODUCTION

Although animal welfare was only a concept in the past, it has now progressed rapidly from a concept to laws or guidelines around the world. The beginning was the signing of the Treaty of Amsterdam in 1997, which amended the Treaty of the European Union (EU), in which EU countries agreed to consider animal welfare and to proceed from concept to law. The Treaty of Amsterdam also presented a comprehensive view of animal welfare from the viewpoint of five freedoms: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury and disease; freedom to express normal behaviour; and freedom from fear and distress (European Union, 1997). Promotion of animal welfare can lead to trade problems because farm products produced by animals with high welfare quality are differentiated by labels on the food package, which results in problems between nations at exportation and importation. Therefore, the EU presented a proposal for standardization of "animal welfare and agricultural trade" at the Agricultural Negotiation of the World Trade Organization (WTO) in 2000. Following the proposal, the World Organisation for Animal Health (OIE) decided two new missions, animal welfare and food safety as strategic plan from 2005-2010, and proposed these two missions at the 70th General Session of the OIE in May 2002, where permanent working groups for these missions were inaugurated (OIE, 2002). The Animal Welfare working group presented a "world animal welfare guideline" at the Global Conference on Animal Welfare in February 2004. The OIE is an intergovernmental organisation, in which 167 countries including Japan, 87% of the countries in the world, participate (value for 2004). This situation indicates that each country all over the world has to manage animal welfare. Actually, in the EU countries, regulation of animal welfare has been enforced by law, and conventional cages will be banned from 2012 (European Union, 1999). In the United States of America, an animal husbandry guideline has been prepared by the United Egg Producers (UEP), and the cage space per hen was increased, although conventional cages will not be banned (United Egg Producers, 2006). A guideline for farm animal welfare is also being developed in Japan. In this manner, animal welfare has progressed rapidly from a concept to laws or guidelines around the world.

In the EU countries, in which animal welfare is well developed, conventional cages will be banned from 2012, and a variety of housing systems that consider animal welfare have been developed, e.g., furnished cages, aviaries, and free-range systems (Tauson, 2005). Therefore, the development and modification of housing systems has become one of the central subjects in studies of farm animal welfare. In fact, the OIE declared its intention to establish world standards for housing systems and housing management by 2010.

The farm products produced in these housing systems are sold in many EU supermarkets. However, consumers are not able to fully understand the welfare level of the producing flock without any label on the food package. According to a consumer survey by the European Commission, the inability of EU consumers to actually find this information has reduced the interest of consumers in farm animal welfare (Eurobarometer, 2005), and so a system of grading eggs according to an integrated welfare assessment is needed. Since then, welfare assessment has been also one of the central subjects in studies of farm animal welfare. Such attempts have also been made in Japan, and it was decided to evaluate welfare from the viewpoint of the five freedoms, a concept of welfare widely accepted all over the world (Farm Animal Welfare Council (FAWC), 1992).

In these circumstances, basic information about the advantages and disadvantages of various housing systems is needed, and modifications of housing systems that resolve the disadvantages also must be developed. For example, it is important for producers to know what effect these housing systems have on productivity and immunity. In addition, to differentiate and sell stock farm products produced in the systems, welfare assessment evaluating various housing systems at the farm level need to be developed, which inform consumers of what added values are attached to the systems.

To clarify these advantages and disadvantages, comparisons between the housing systems are effective and have high scientific validity. However, almost all studies compare two housing systems: conventional and furnished cages (Appleby and Hughes, 1995; Abrahamsson and Tauson, 1997; Appleby et al., 2002; Wall and Tauson, 2002; Shimmura et al., 2007a, 2007b), different types of furnished cages (Abrahamsson et al., 1995, 1996; Vits et al., 2005; Weitzenbürger et al., 2005; Shimmura et al., 2008a, 2008b, 2009a), conventional cage and non-cage systems (Tanaka and Hurnik, 1991, 1992; Taylor and Hurnik, 1994, 1996; Abrahamsson and Tauson, 1995; Brand et al., 2004), and different types of non-cage systems (Odén et al., 2002; Shimmura et al., 2008c). Because there are many differences, such as hybrids and rearing conditions, between these studies, simple comparison of these studies is difficult, and therefore, the advantages and disadvantages of various housing systems have not been clarified completely. Moreover, parameters for welfare evaluation are limited, and so clarifying the advantages and disadvantages is difficult.

Therefore, six housing systems were selected: two types of conventional cages (small and large), furnished cages (small and large), and non-cage systems (single-tiered aviary and free-range). These housing systems were built and used at the same location for one year and a half, and the advantages and disadvantages were then investigated. In Chapter 1, comparison of beak-related behaviours for selection of welfare parameter is reported, and in Chapter 2, the overall evaluation of welfare levels, egg production, and immune response is reported. The welfare level was evaluated by many-sided investigation examining ethology, physiology, anatomy, production, and physical condition. In addition, clarification of the advantages and disadvantages for welfare of the housing systems from the viewpoint of the five freedoms by weighting each measurement was attempted.

In Chapter 3, a newly developed overall assessment system developed by using the welfare evaluation of Chapter 2 is reported. Many welfare assessment systems now exist, and the methods are varied (Botreau et al., 2007a). Among the assessments used in practice at the farm animal level, the Animal Needs Index (ANI) designed by Bartussek (1999) and the decision support system developed by Bracke et al. (2002a) are the best known. ANI is based mainly on environment-based measurements (e.g., group size, litter area). Although comparison with animal-based measurements is essential to evaluate an assessment, ANI scores have no correlations with animal-based assessments (Zaludik et al., 2007), and it was also pointed out that the weighting method of the measurements in ANI is not based on scientific studies (Bracke et al., 2002a; Kohari et al., 2006; Seo et al., 2007). On the other hand, the decision support system has the advantage of the weighting method in that it is based on scientific statements from studies of farm animal welfare. Being based on scientific studies would imply validity, which is important in animal welfare studies because they are continuously changing. However, an evaluation of the decision support system by comparing animal-based assessment has not been reported and therefore its usefulness is uncertain. In Chapter 3, a science-based assessment for laying hens that applies Bracke's modelling principles of the decision support system was proposed. The protocol of this study comprised the construction and evaluation of our model. To increase the validity of the evaluation and to facilitate expansion and maintenance of the assessment system, a basic strategy that used many accumulated studies on animal welfare to create a database of studies on the welfare of laying hens around the world was planned. On the basis of it, a science-based overall assessment for laying hens was devised, which can be evaluated from the viewpoint of the five freedoms that are essential in making the Japanese welfare assessment. The usefulness of the assessment was also evaluated by comparing it with the environment-based Animal Needs Index (ANI) and animal-based assessment of Chapter 2.

In Chapters 4 and 5, a new modified cage developed on the basis of the evaluation of Chapters 2 and 3 is reported. The results of both Chapters 2 and 3 indicated the high potential value of furnished cages, as suggested by a large number of previous studies (Appleby and Hughes, 1995; Abrahamsson et al., 1995, 1996; Abrahamsson and Tauson, 1997; Appleby et al., 2002; Wall and Tauson, 2002; Vits et al., 2005; Weitzenbürger et al., 2005; Shimmura et al., 2007a, 2007b, 2008a, 2008b, 2009a). However, in large furnished cages, competition for a restricted number of resources was frequently observed due to increased group size, while mobility and comfort behaviour are enhanced by providing a larger total cage area. The competition for a concentrated resource is one of the disadvantages of the large furnished cages. Actually, some researchers have demonstrated that competition for a dust bath occurs in furnished cages and even in non-caged systems (Van Rooijen, 1999; Shinmura et al., 2006a, 2006b). In my later research, it was found that dominant hens had priority using the dust bath (Shimmura et al., 2007c). In small furnished cages, higher-ranked hens used the dust bath and performed dust-bathing more frequently than lower-ranked hens. It was also confirmed in my studies that the priority using the dust bath by higher-ranked hens occurred remarkably in large furnished cages even with a large dust bath area (Shimmura et al., 2008a, 2008b, 2008c). These results indicated that the priority for use by higher-ranked hens leads to competition for a small dust bath, and that in large furnished cages, only a small number of hens (those that are high-ranking) may have priority using resources such as the dust bath, even if those resources seem to be used fully by many hens. It would be difficult to conclude, in these conditions, that furnished cages have an unequivocal advantage in removing behavioural restrictions. From these previous studies, it seemed that the problem was that a resource was placed on one side of the cage ('localised' resources). Therefore, a medium-sized furnished cage with resources on both sides of the cage ('separated' resources) was designed. One of the aims of this design was to reduce competition for the dust bath and to increase the use of the dust bath by lower-ranked hens. In Chapter 4, the behaviour of high-, medium- and low-ranked hens in this new type of furnished cage with 'separated' resources was compared with that of hens in the furnished cages with a 'localised' resource. In Chapter 5, the new cage with 'separated' resources was also evaluated thoroughly by many-sided investigations measuring behaviour, physical condition, and production.

CHAPTER 1

Comparison of pecking behaviour in six housing systems

1.1. Introduction

Feather pecking, which can develop into cannibalism, is a serious welfare problem in laying hens that can cause high mortality (Savory, 1995). Therefore, studies on ways to reduce this abnormal behaviour have been conducted. For example, there is a report that feather pecking was reduced by supplying a pecking device (Jones et al., 2002). Huber-Eicher and Wechsler (1998) showed that feather pecking decreases when birds are provided with litter substrates. These studies indicated that feather pecking can be reduced by redirecting pecking toward other materials. For other pecking behaviours, much of the evidence also indicated that pecking behaviour is decreased when other behaviours using the beak are increased. For example, Sandilands and Savory (2002) compared the behaviours of intact and beak-trimmed hens, and reported that the beak-trimmed birds spent more time in preening while less time in aggression. It was also reported that object pecking increased dramatically when feed was withdrawn for the purpose of induced moulting (Shimmura et al., 2008d).

In the European Union (EU), where conventional cages for laying hens will be banned in 2012, much development of alternative housing systems has occurred. These alternatives comprise furnished cages and non-cage systems such as aviaries and free-range, which must provide a litter area and, for free-range housing, an outdoor area (Blokhuis, 2004). Following the theory that feather pecking can be reduced by redirecting hens' pecking toward other materials, the provision of litter material and outdoor pasture could reduce feather pecking. In fact, in my study investigated pecking of laying hens in litter-floor and free-range housing, the proportion of hens grazing was very high in the free-range system while the other behaviours using the beak, such as eating and litter pecking, as well as feather pecking, were observed less frequently compared with the litter-floor system (Shimmura et al., 2008c). A more striking result was that total pecking behaviour was almost the same between the two housing systems, although the breakdown of types was different. Considering both this result and the evidence mentioned above, hypotheses that hens in different housing systems have the same motivation for using the beak and that the amount of feather pecking would be reduced by pecking other material was framed. This hypothesis is partly supported by our previous study (Shimmura et al., 2008c), but it compared only two non-cage systems. Therefore, in order to verify the hypothesis, comparison of the pecking behaviour of laying hens in following six housing systems was conducted: two types of conventional cages, two types of furnished cages, a single-tiered aviary, and free-range. By using a larger number of housing systems, it was possible to test the extent to which hens maintain their time budget for beak-related behaviours.

1.2. Materials and Methods

1.2.1. Animals and housing

In total, 300 chicks of a medium hybrid laying strain (a White Leghorn/Rhode Island Red cross-breed) were prepared and 284 were used for the trial. All chicks had their beaks trimmed at 1-day-old, and were raised in pens (100 chicks in each) with wood shavings on the floor and where three circular feeders and 16 water nipples were placed (1347.5 cm2 floor area per bird). Lighting was adjusted to give an intensity of 10 lx at the food troughs.

At the age of 16 weeks, 284 birds were randomly selected and introduced into one of the six housing systems at the same location as the rearing facility. Forty-eight birds were housed in 12 small and 12 large conventional cages with two birds per cage. To obtain four replicates, three cages were grouped, resulting in a total of four groups of each type of conventional cage; 20 birds in four small furnished cages with five birds per cage; and 216 birds in four large furnished cages, four single-tiered aviaries, and four free-range pens with 18 birds per cage or pen. There were four replicates of each system.

The building was ventilated with three ceiling fans. The average daytime temperature (±S.D.) during the observation period was 18.3 ± 6.7 °C at the centre of the house. Lighting was provided by miniature ceiling bulbs, adjusted to give an intensity of 10 lx at the food troughs. The illumination cycle was 14 h of light and 10 h of darkness, with the light period from 05:00 to 19:00 h. The birds had ad libitum access to water and feed. The feed contained more than 18.5% crude protein and 2840 kcal metabolic energy per kg. Feeding and other routine work, such as supplying wood shavings, was done between 09:00 and 09:30 h, and eggs were collected between 16:00 and 16:30 h.

1.2.2. Housing system

The design and equipment of all systems, except the small conventional cages, fulfilled the regulations in the EU (Blokhuis, 2004). Non-cage systems were not on a large commercial scale but small to facilitate behavioural observation. The same V-shaped feeders were used for all housing systems. The same type of wooden perch (4 cm deep and 3 cm high with a chamfered top edge) was used in the furnished cages and non-cage systems, and the same dry wood shavings were supplied to these housing systems. The other details were as follows.

Small conventional cage (SC). Laying cages, 23 cm wide, 40 cm deep, and 42 cm high at the rear were used. The cage provided 450 cm^2 with 12 cm feeder space and one water nipple per hen.

Large conventional cage (LC). Laying cages, 31 cm wide, 40 cm deep, and 42 cm high at the rear were used. The cage provided 600 cm^2 with 15 cm feeder space and two water nipples per hen.

Small furnished cage (SF). Laying cages, 65 cm wide, 46.5 cm deep, and 47 cm high at the rear were used. In accordance with Appleby and Hughes (1995), each cage was equipped with a nest, a dust bath, and a perch. The main cage area provided 604.5 cm^2 per hen, with a floor of 2.5 cm × 5.0 cm wire mesh. The nest box on one side of the cage was 25 cm wide, 46.5 cm deep, and 21 cm high at the rear. The nest area was 232.5 cm^2 per hen, so that total space allowance (excluding the dust bath) was 837.0 cm^2 per hen. The nest was constructed of and enclosed by wooden boards, with a floor of artificial turf. There was an 8 cm space under the front so that eggs would roll out, and an entrance 13 cm wide × 23 cm high (with a threshold 1.8 cm high so that eggs would not roll out of the side of the nest) so that hens readily stepped through. Above the nest was a dust bath 4.5 cm deep, which was supplied with wood shavings. All wood shavings were removed and replaced with fresh shavings every morning. Awooden perch was fitted across the width of the cage 10 cm from the cage floor and 18 cm from the rear of the cage. Perch, feeder, and drinker space per hen were 13.0 cm.

Large furnished cage (LF). Large commercially produced furnished cages (Meller, Melle, Germany) that were 240 cm wide, 62.5 cm deep, and 47 cm high at the rear were used. Each cage was equipped with a nest, a dust bath, and two perches. The main cage area provided

658.3 cm^2 per hen. On one side was the nest box, which was 60 cm wide, 35 cm deep, and 47 cm high at the rear. The nest area was 116.7 cm^2 per hen. A hanging red plastic sheet covered the entrance to the nest box; otherwise, the nest was solid walled and lined with artificial turf. Next to the nest box was a 30 cm wide and 35 cm deep dust bath, which was lined with artificial turf and supplied with wood shavings on the turf. All wood shavings were removed and replaced with fresh shavings every morning. The litter area was 58.3 cm^2 per hen, so that the total area of the litter and nest box was 150 cm^2 per hen, and the total cage area was 833.3 cm^2 per hen. Two perches were fitted across the width of the cage, 9 cm above the floor, one 20 cm and one 40 cm from the rear of the cage. Perch space per hen was 16.7 cm. Feeder space was 13.3 cm per hen, and each cage was equipped with six water nipples.

Single-tiered aviary (SA). The area of the single-tiered aviary was 360 cm ×360 cm, providing a total floor area of 7200 cm^2 per hen. Each aviary consisted of a litter area (180 cm × 360 cm; 3600 cm^2 per hen) in one-half of the area and a raised slatted platform (180 cm × 360 cm), which allowed droppings to accumulate underneath, in the other half of the area. Eight nest boxes (one nest per 2.3 hens) were provided at a height of 100 cm above the slatted floor, and two wooden perches (27 cm per hen) were placed in front of the nest boxes. The feeders (20 cm per hen) and drinkers (20 cm per hen) were placed along the length of the slatted platform.

Free-range (FR). The free-range system was a SA with an outdoor area. A passage hole (100 cm × 100 cm) was provided between the indoor and outdoor areas so that hens could readily go outside. The outdoor area consisted of a passage (100 cm × 850 cm) and three areas (300 cm × 400 cm/area) separated by net barriers. Clover was planted in these three areas, and hens were rotated through them; thus, the total outdoor area including all three areas was 1.1 hen/m^2. Hens were given access to pasture at 8:00 h and herded into the indoor area at 16:00 h to protect them from predators.

1.2.3. Behavioural observation

Observations were conducted at 22, 30, 38, 46, 54, and 63 weeks of age (3 days/housing system/week). The observations of the cage systems (SC, LC, SF, and LF) were conducted 3 days a week and of the non-cage systems (SA and FR) the other 3 days of the same week. The mean (±S.D.) temperatures in the henhouse for each week were 28.3 ± 2.9, 22.7 ± 2.0, 20.6 ± 3.1, 13.4 ± 2.5, 10.1 ± 0.8, and 15.2 ± 3.6 °C, respectively. Direct visual scans at 10 min intervals were conducted to record the behaviour of birds in each housing system for a total of 4 h/day, 2 h each in the morning (10:00–12:00 h) and afternoon (13:00–15:00 h). Each scan took about 20 s for the small groups (SC, LC, SF) and 60 s for the large groups (LF, SA, FR), and the number of hens performing behaviours using the beak was counted during the time. There were a total 1728 scans in each system, which would be large enough to offset the disadvantage of scan sampling (Martin and Bateson, 1993; Carmichael et al., 1999). Behaviours using the beak (grazing, eating, drinking, preening, aggressive pecking, gentle feather pecking, severe feather pecking, litter pecking, and object pecking) and dust bathing (Appleby et al., 2004) were recorded. Grazing was defined as pecking the ground in the outdoor area because it was impossible to judge whether the hen was actually eating the vegetation or just pecking it exploratively. Eating and drinking was recorded when a hen had her head in the feeder or when a hen pecked the water nipple, respectively. Preening was defined as when a hen pecked her own feathers with her beak. Pecking cage wires was classified as object pecking. Aggressive pecking was pecking the head of another hen, and excluded both severe feather pecking (forceful pecks, sometimes with feathers being pulled out and the recipient bird moving away) and gentle feather pecking (careful and explorative pecks, not resulting in feathers being pulled out and usually without reaction from the recipient bird). Dust bathing was recorded when one element of three (vertical wing-shaking, head-rubbing, scratching with one leg) was observed. All data were collected by the same two observers. Observation of FR hens was carried out at the same time by two observers, with one observer checking hens in the indoor area and the other in the outdoor area. No observations of FR were conducted on rainy days.

1.2.4. Statistical analyses

A non-parametric test was used because the normality and variance homogeneity were difficult to assume even if data transformations were conducted. The percentage of birds performing each behaviour was calculated in each housing system in each week. The weekly values were calculated by summing the counts in the morning and afternoon observations the mean taken of the 3 days. There were four replicate cages or pens in each housing system, giving four replications for each week. Because the data for each cage or pen in a housing system were not independent, Friedman's test with replication was used to evaluate the effects of housing system and week on the behaviour. Each behaviour therefore involved 144 data units in the analysis (six systems × 6 weeks × four replications). Significances of individual effects were evaluated by a non-parametric multiple comparison using the Steel-Dwass test.

1.3. Results

1.3.1. Effect of week

There was little effect of week, and the effect was confounded with the seasonal factor (see temperatures during each observation period given above). The mean total percentages combined for all the housing systems (±S.D.) of hens performing behaviours using the beak were 65.1 ± 5.2% at 22 weeks, 64.6 ± 5.4% at 30 weeks, 67.2 ± 5.1% at 38 weeks, 66.7 ± 5.6% at 46 weeks, 60.9 ± 4.4% at 54 weeks, and 59.6 ± 5.6% at 63 weeks. There were significant effects of week on the proportions of hens performing severe feather pecking ($\chi^2_6 = 22.11$, $P < 0.001$) and litter pecking ($\chi^2_6 = 16.83$, $P < 0.01$), and there was a tendency for these proportions to be higher at 46, 54, and 63 weeks. The proportions (±S.D.) at 22, 30, 38, 46, 54, and 63 weeks were 0.4 ± 0.4%, 0.6 ± 0.7%, 0.9 ± 0.8%, 1.6 ± 1.9%, 1.3 ± 1.4%, 0.8 ± 1.0%, respectively, for severe feather pecking, and 6.5 ± 5.2%, 6.8 ± 6.3%, 5.1 ± 4.9%, 8.9 ± 8.2%, 7.1 ± 5.7%, 7.6 ± 9.1%, respectively, for litter pecking. The proportion (±S.D.) of hens that performed gentle feather pecking was higher at 22 (2.5 ± 1.1%) than at 30 (1.3 ± 1.3%), 38 (1.0 ± 1.1%), 54 (1.1 ± 0.8%), and 63 (0.9 ± 0.9%) weeks (all $P < 0.05$).

1.3.2. Effect of housing system

The total proportion of hens performing behaviours using the beak and the breakdown of types across housing systems are shown in Figure 1. Significant effects of housing type were found on the proportion of hens eating ($\chi^2_6 = 94.86$, $P < 0.001$), drinking ($\chi^2_6 = 68.91$, $P < 0.001$), and preening ($\chi^2_6 = 67.41$, $P < 0.001$). The proportions were higher in SC, LC, SF, and LF than in SA, and higher in SA than in FR (all $P < 0.05$). The mean percentages (±S.D.) of hens eating, drinking, and preening in all four cage systems were 33.3 ± 5.8%, 4.3 ± 0.6%, and 16.9 ± 4.1%, respectively, while they were 20.9 ± 2.9%, 3.1 ± 0.7%, and 12.4 ± 4.0% in SA, and 13.5 ± 2.8%, 1.9 ± 0.8%, and 7.4 ± 1.9% in FR. In FR, 35.6 ± 11.7% of hens were grazing. Housing type had a significant effect on the percentage of hens performing object pecking ($\chi^2_6 = 60.08$, $P < 0.001$, Figure 1-1), and the percentages were higher in SC and LC than LF and SA, and higher in LF and SA than in SF and FR (all $P < 0.05$). A significant effect on the percentage of hens performing litter pecking was found ($\chi^2_4 = 46.03$, $P < 0.001$, Figure 1-1), and the proportion was higher in SA (17.1 ± 19.5%) than in SF, and higher in SF than in LF and FR (all $P < 0.001$). The proportion of hens performing gentle feather pecking was significantly affected ($\chi^2_6 = 18.48$, $P < 0.001$), and there were less in SA than in the other systems (all $P < 0.05$). The proportion of hens dust bathing was higher in SF (4.7 ± 2.9%) than in LF (2.1 ± 0.9%, $P < 0.01$) and FR (2.4 ± 1.8%, $P < 0.05$).

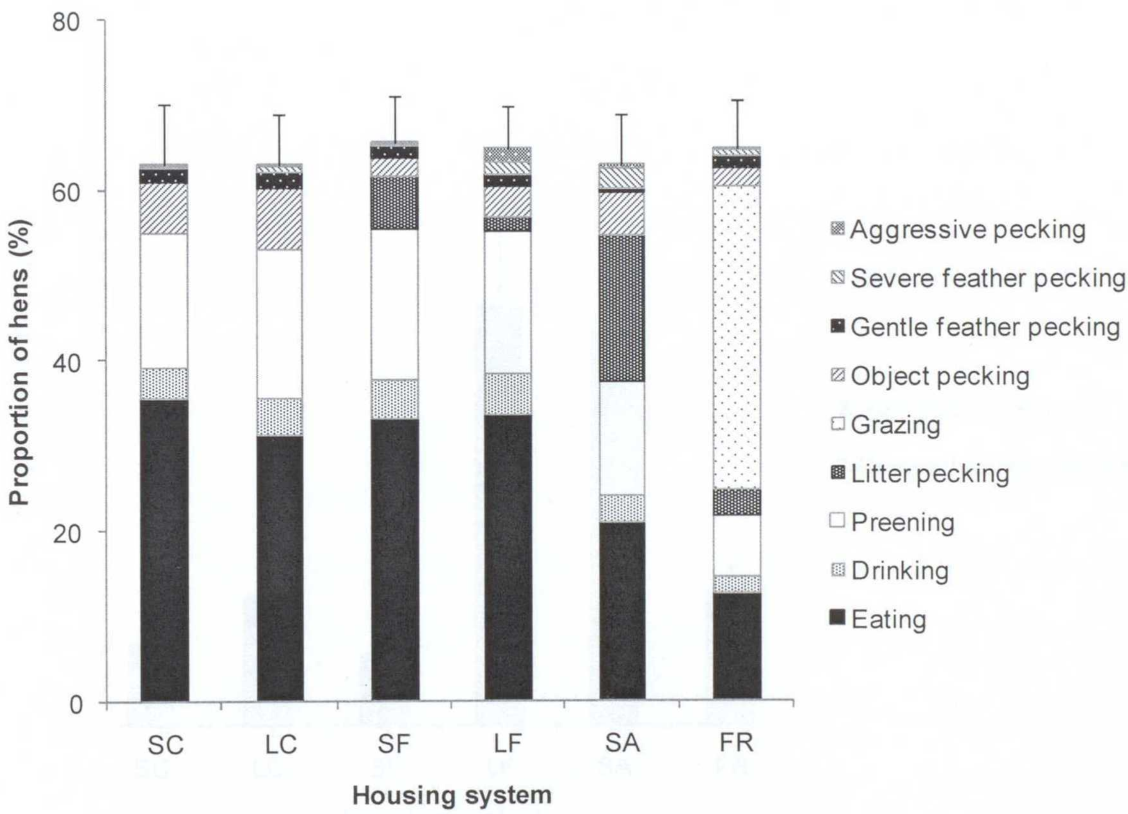

Figure 1-1. The mean total proportion (±S.D.) of hens performed pecking behaviour and the breakdown. SC, small conventional cage; LC, large conventional cage; SF, small furnished cage; LF, large furnished cage; SA, single-tiered aviary; FR, free-range.

Figure 1-2 shows the percentages of hens performing severe and aggressive pecking in the six housing systems. The proportion performing severe feather pecking ($\chi^2_6 = 60.12$, $P < 0.001$) was greater in LF and SA than in FR, and greater in FR than in SC, LC, and SF (all $P < 0.05$; Figure 1-2). The type of housing had a significant effect on the proportion of hens performing aggressive pecking ($\chi^2_6 = 66.93$, $P < 0.001$), and it was performed by more birds in LF than in SA, and by more in SA than in SC, LC, SF, and FR (all $P < 0.05$; Figure 1-2).

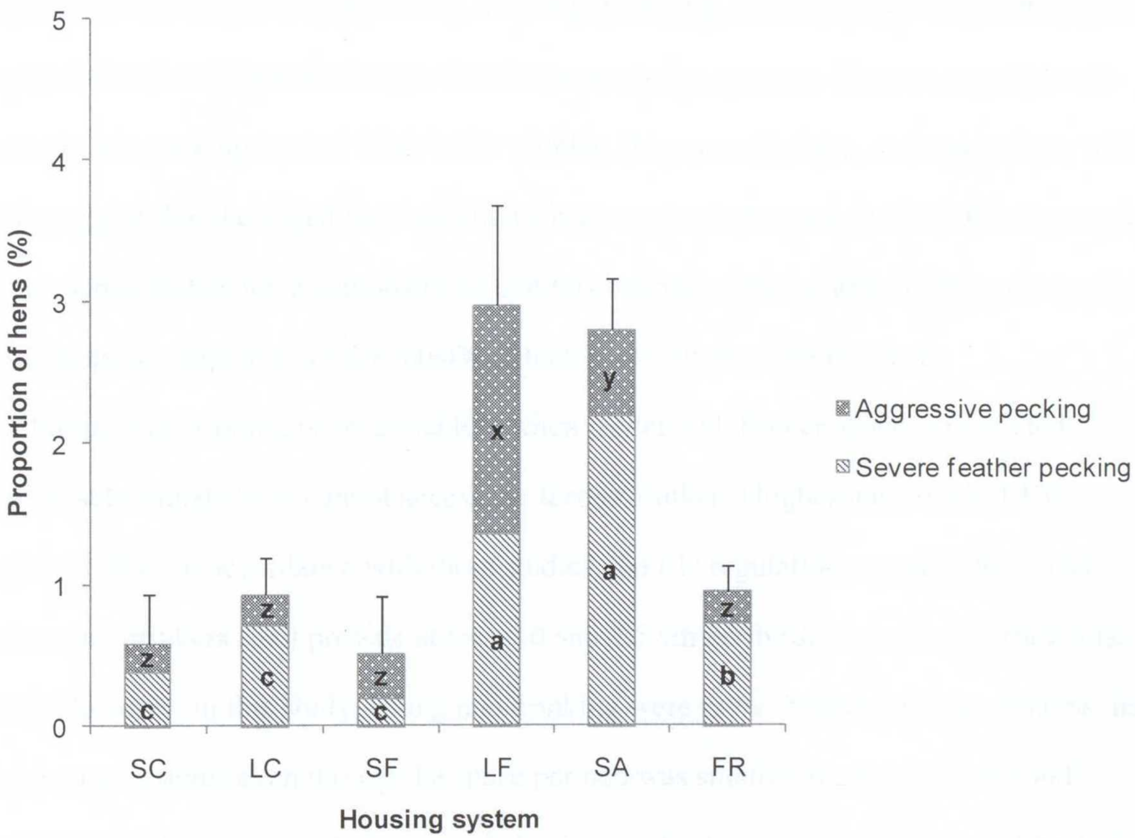

Figure 1-2. The mean total proportion (±S.D.) of hens performed aggressive pecking and severe feather pecking. SC, small conventional cage; LC, large conventional cage; SF, small furnished cage; LF, large furnished cage; SA, single-tiered aviary; FR, free-range. Different letters show the significant difference (a–b–c, x–y–z: $P < 0.05$).

The mean total percentages (±S.D.) of hens performing behaviours using beak in each system were almost the same (63.0 ± 7.1% in SC, 63.0 ± 5.9% in LC, 65.6 ± 5.4% in SF, 64.7 ± 5.0% in LF, 62.9 ± 5.9% in SA, 64.9 ± 5.6% in FR), and no significant effect of housing system was found ($\chi^2_6 = 5.50$, $P = 0.358$).

1.4. Discussion

The total proportion of hens performing behaviours using the beak was almost the same among the six housing systems, although the breakdown by type was different. The proportions of hens performing eating, drinking, preening, and object pecking were higher in conventional and furnished cages than in the non-cage systems. Hens in cage systems directed their pecking toward food, water nipples, their own feathers, and cage wires, which might suggest that the caged hens satisfied a high motivation to use their beak in these ways. In fact, some studies have demonstrated that this eating, preening, and object pecking of laying hens is compensatory for satisfying their motivation to use the beak.

Eating and drinking is observed less when feeder and drinker space is restricted because subordinate hens cannot access the feeder/drinker (Hughes and Black, 1976; Hughes, 1983). In accordance with these studies, the EU regulations specify that linear feeders and drinkers shall provide at least 10 and 2.5 cm per bird, respectively (Blokhuis, 2004). However, in this study, eating and drinking were more frequent in cage systems than in non-cage systems even though the space per hen was smaller in cages. Tanaka and Yoshimoto (1985) compared the eating behaviour and actual feed intake in caged hens, and indicated that hens housed in cages pecked feed both to consume it and as explorative pecking. There are also some demonstrations that the proportion of hens eating was higher in conventional cages than in a multiple-tiered aviary, while actual feed intake was almost same between the two housing systems (Tanaka and Hurnik, 1991, 1992). Considering these lines of evidence, it might be suggested that caged hens spend more time in explorative feed pecking, resulting in an increased total frequency of eating. Therefore, if sufficient feeder and drinker space is provided, the time spent by caged hens eating and drinking might be increased by explorative pecking at feed and water nipples.

Preening is reported to have two functions: preening to keep their plumage in good condition (Van Liere and Bokma, 1987) and preening as a displacement behaviour in frustrating situations (Duncan and Wood-Gush, 1971). I proposed that preening has a third function: compensation for satisfying the motivation to use the beak. This was actually confirmed in previous studies (Lee and Craig, 1990; Sandilands and Savory, 2002; Shimmura et al., 2008d). Sandilands and Savory (2002) reported that beak-trimming resulted in reduced feather pecking and more preening, and it was also confirmed in my study that preening increased after withdrawal of feed for forced moulting (Shimmura et al., 2008d). The amount of time spent preening was larger in the cages than in the non-cage systems in the present study, and similar results have been reported in studies that compared behavioural frequencies between housing systems: conventional and furnished cages (Shimmura et al., 2008c), and floor housing and free-range (Shimmura et al., 2007a). These reports support the theory that caged hens direct pecking to the own feathers to fulfill the pecking motivation.

Object pecking has also been reported to be affected by supplying another material by a variety of studies of environmental enrichment. For example, Tanaka and Yoshimoto (1987) supplied hens with rolled paper and reported that the birds redirected their pecking from explorative pecking of food to the paper. Jones et al. (2004) concluded in their review that providing hens with bunches of plain white string promoted explorative pecking and resulted in a decrease in feather pecking in the laboratory and at a commercial farm. Redirected pecking from feathers to a supplied material was reported in studies in which hens were supplied with litter materials, and Huber-Eicher andWechsler (1998) confirmed the reduction of feather pecking due to the provision of wood shavings. Further, El-Lethey et al. (2000) investigated the effects of foraging material and food form and reported that rates of feather pecking were highest in the group housed without straw and fed pellets. These lines of evidences indicate that housing conditions affect pecking behaviour and that pecking to one object is decreased when pecking to another object is increased. In the present study, the time budget of object pecking was higher in conventional cages compared with other housing types supplied with litter materials, which suggests that caged hens that cannot peck litter compensated by pecking cage wires. On the other hand, the amount of litter pecking appeared to be affected by the amount of litter space per hen, and feather pecking and aggressive pecking were increased in the housing with large group size regardless of the time budget of litter pecking, whereas the studies cited above have demonstrated that aggressive behaviours were decreased due to increased litter pecking.

Severe feather pecking causing feather damage is correlated with cannibalism, which is a serious welfare problem in laying hens because it can cause high mortality (Savory, 1995). In the present study, significant effects of week were found on gentle and severe feather pecking; and more birds performed gentle feather pecking at a young age while at an older age more performed severe pecking. These changes agree with the general consensus that gentle feather pecking develops into severe feather pecking (Savory, 1995; Zimmerman et al., 2006). However, a number of studies have demonstrated that the effect of the housing system is more important than age. The most important causes in this study would be group size and stocking density because the environmental factors such as light and food were the same in all six housing systems and the resources, such as perches and nest boxes were also sufficient to fulfil the EU regulation and not result in an increase in aggressive interactions (Blokhuis, 2004). In small groups of up to 10 or 12 birds, there is evidence of a positive correlation between group size and the rate of aggressive interaction (Hughes et al., 1997).

Aggression is also generally increased with increased stocking density (Hansen and Braadstad, 1994; Huber-Eicher and Audige, 1999). However, the incidence of aggressive interaction was low, and no effect of group size and stocking density was found when the group sizes were even larger, reaching hundreds or thousands, such as in commercial large-scale non-cage systems (Craig and Guhl, 1969; Carmichael et al., 1999; Zimmerman et al., 2006). Compared to these previous studies, the group size of the non-cage systems in this study was very small and, as expected, aggressive pecking and severe feather pecking were more frequent in the systems that housed the larger groups (LF and SA, 18 hens/cage or pen) compared with the small groups (SF, five hens/cage) with a similar stocking density to LF. On the other hand, the effect of stocking density seemed to be reduced in this study because the amounts of severe feather pecking and aggressive pecking were similar in LF and SA, although these two systems had the same group size but a very different stocking density (LF, 833 cm^2/hen; SA, 7200 cm^2/hen). Whereas some studies have shown that severe feather pecking and aggressive pecking can be restrained by enrichment devices or litter material (Jones et al., 2004; Huber-Eicher and Wechsler, 1998), these behaviours were increased in the current study in larger groups. Thus, it is difficult to say simply that aggressive pecking and feather pecking are decreased when enrichment material is supplied. On the other hand, these aggressive behaviours by free-range hens were fewer in spite of the large group size. Even if the group size is large, the risk of aggressive pecking and feather pecking is decreased when an outdoor area is available, which might be due to the lower density and/or environmental enrichment by providing an outdoor area (Mahboub et al., 2004; Shimmura et al., 2008c). Therefore, it is suggested that aggressive pecking and feather pecking might be affected mainly by group size and decreased when an outdoor area is provided.

As many studies have demonstrated, pecking behaviour is varied in different housing systems (e.g. Shimmura et al., 2008c). Together, much of the evidence indicated that pecking to one object is decreased when pecking to another is increased. These results were confirmed in the present study. More striking is the fact that the total amounts of all pecking behaviours were almost the same in the six housing systems, which suggests that hens have the same motivation to use their beak, and to fulfill it, they direct their pecking toward different materials in their environment. However, feather pecking appeared not to be decreased simply by the pecking redirected at other things.

Hens in a free-range system given commercial feed ad libitum were found to consume a considerable quantity of grass from the pasture (Hughes and Dun, 1983). Red Junglefowl also spent most of their time in explorative pecking of the ground in a zoo (Dawkins, 1989). If FR is the most natural of the six housing systems, caged hens that are unable to peck grass or litter may satisfy their motivation to use the beak by pecking other materials such as food, water nipples, their own feathers, and the wires of their cages. I think this is a 'compensatory behaviour' that domesticated laying hens use to adapt to the caged environment. Here, the word 'compensatory behaviour' does not means compensation for even behavioural function but compensation to perform behaviour (compensation of time). In this sense, SA may not be a 'natural' environment compared with FR because compensatory preening and object pecking were more frequent in SA.

Such compensatory oral behaviours have also been confirmed in domesticated cattle. Ishiwata et al. (2008) reported that cattle kept in a pen under a restricted feeding regime might compensate for the lower time spent feeding or finding food by performing other oral behaviours. More striking evidence is that the amount of time Red Junglefowl, the ancestor of the domestic fowl, in a semi-wild environment spent using the beak in a day (Dawkins, 1989) is almost the same as that of the domesticated hens in this study. Therefore, compensatory oral behaviours are found in other domestic animals, and it is possible that the motivation for using the mouth is genetically ''fixed'' and inherited unchanged from ancestors. However, further studies are needed on the species-specificity and evolution of oral behaviours.

In conclusion, the total proportions of hens performing behaviours using the beak were almost the same regardless of the housing system, although the breakdown of types of beak use was different. Therefore, caged hens might compensate for their motivation to use the beak by directing their pecking toward materials such as food, water nipples, their own feathers, and the wires of their cages, although feather pecking appeared not to be decreased simply by redirected pecking. These results suggested that pecking behavior such as eating, drinking, preening, and litter pecking may be difficult to be used as welfare parameter, while aggressive pecking and feather pecking have high usefulness as indicator.

1.5. Summary

In order to verify the hypothesis that hens in different housing systems have the same time budget for different beak-related behaviours, comparison of the pecking behaviour of hens in following six housing systems was conducted: small (SC) and large (LC) conventional cages, small (SF) and large (LF) furnished cages, single-tiered aviary (SA), and free-range (FR). At the age of 16 weeks, 284 medium hybrid layers were randomly divided into six groups and moved to the six housing systems. The number of hens performing behaviours using the beak (grazing, eating, drinking, preening, aggressive pecking, gentle feather pecking, severe feather pecking, litter pecking, and object pecking) was recorded at various ages up to 63 weeks of age. Grazing by a large proportion of hens was observed in FR, and litter pecking by a large proportion of hens in SA. The proportions of hens eating, drinking, and preening were higher in SC, LC, SF, and LF than in SA, and higher inSA than inFR (all $P < 0.05$). The proportion of hens performing object pecking was higher in SC and LC than in the other systems (all $P < 0.05$). The proportion of hens performing severe feather pecking was higher in LF and SA than in FR, and more in FR than in SC, LC, and SF (all *P*

< 0.05). The percentages of hens performing all pecking behaviours were almost identical among the six housing systems (63.0 ± 7.1% in SC, 63.0 ± 5.9% in LC, 65.6 ± 5.4% in SF, 64.7 ± 5.0% in LF, 62.9 ± 5.9% in SA, and 64.9 ± 5.6% in FR), indicating that the total frequency of beak use was almost the same regardless of the housing system, although the breakdown of types of beak use was different. It was concluded that caged hens may express a motivation for beak-related behaviour by directing it at food, drinking nipples, their own feathers, and the cage wires, although feather pecking appeared not to be decreased simply by the redirected pecking. These results suggested that pecking behavior such as eating, drinking, preening, and litter pecking may be difficult to be used as welfare parameter, while aggressive pecking and feather pecking have high usefulness as indicator.

CHAPTER 2

Multi-factorial investigation of six housing systems

2.1. Introduction

In Chapter 1, comparison of beak-related behaviours in six housing systems for selection of welfare parameter was reported. In Chapter 2, based on the result of Chapter 1, welfare indicators were selected, and the overall evaluation of welfare levels, egg production, and immune response in the six housing systems was conducted.

Animal welfare has progressed rapidly from a concept to laws or guidelines around the world. The World Organisation for Animal Health (OIE) proposed two new missions, animal welfare and food safety, at the 70th General Session of the OIE in May 2002, where permanent working groups for these missions were inaugurated (OIE, 2002). The Animal Welfare working group presented a 'world animal welfare guideline' at the Global Conference on Animal Welfare in February 2004. In the European Union (EU) countries, in which animal welfare is well developed, regulation of animal welfare has been enforced by law (European Union, 1999). In the EU, conventional cages will be banned from 2012, and a variety of housing systems that consider animal welfare have been developed, e.g., furnished cages, aviaries, and free-ranges (Tauson, 2005). In the United States of America, an animal husbandry guideline has been prepared by the United Egg Producers (UEP), and the cage space per hen was increased, although conventional cages will not be banned (United Egg Producers, 2006). A guideline for farm animal welfare is also being developed in Japan.

In such circumstances, fundamental information about the advantages and disadvantages of various housing systems is needed. For example, it is essential for a producer to know what effect these housing systems have on productivity and immunity. In addition, to differentiate and sell the stock farm products produced in the systems, it would be also important to inform consumers of what added values are attached to the systems (Eurobarometer, 2005). The added values of the housing systems are high welfare level. To clarify these advantages and disadvantages, comparisons between the housing systems are effective and have high scientific validity. However, almost all studies compare two housing systems: conventional and furnished cages (e.g. Appleby and Hughes, 1995), different types of furnished cages (e.g. Abrahamsson et al., 1995), conventional cage and non-cage systems (e.g. Tanaka and Hurnik, 1992), and different types of non-cage systems (e.g. Odén et al., 2002). Because there are many differences such as hybrid and rearing condition between these studies, it is difficult to compare simply the studies, and therefore, advantages and disadvantages in various housing systems has not been clarified completely. Moreover, parameters for welfare evaluation are limited, and so clarifying the advantages and disadvantages are difficult.

Therefore, following six housing systems were selected: two types of conventional cages (small and large), furnished cages (small and large), and non-cage systems (single-tiered aviary, free-range). Some systems were not commercial (e.g. small-scale non-cage system was equipped for behavioural observation) and in this sense, this study might be a pilot for work in commercial condition. Then the welfare levels, egg production, and immune response of these housing systems built in the same location were evaluated for one and a half year. For welfare evaluation, considering the result of Chapter 1, pecking behavior such as eating, drinking, preening, and litter pecking was excluded from welfare indicator, while aggressive pecking and feather pecking was included. And, to evaluate overall, multi-factorial investigation by ethology, physiology, anatomy, production, and physical condition was conducted. In addition, I attempted to clarify the advantages and disadvantages on welfare of the housing systems from the viewpoint of the five freedoms by weighting each measurement.

2.2. Materials and Methods

2.2.1 Animals and housing

In total, 300 chicks were prepared and 284 medium hybrid laying hens (a White Leghorn/Rhode Island Red cross-breed) were used for the trial. All chicks had their beaks trimmed at 1-d-old, were grouped by 100 chicks, and raised in pens with wood shavings on the floor. Lighting was adjusted to give an intensity of 10 lux at the food troughs. At the age of 16 weeks, 284 of 300 birds were randomly choose and introduced into one of six housing systems at the same location as the rearing facility. Forty-eight birds were housed in 12 small and 12 large conventional cages with two birds per cage. To obtain four replicates, three cages were grouped (these cages were treated as independent and these averaged value were treated as one data), resulting in a total of four groups in each type of conventional cage; 20 birds in four small furnished cages with five birds per cage; and 216 birds in four large furnished cages, four single-tiered aviaries, and four free-range pens with 18 birds per cage or pen. There were four replicates of each system. The experimental period was by 86 weeks of age.

The building was ventilated with three ceiling fans. The average daytime temperature (±SD) during the observation period was 18.3±6.7°C at the centre of the building. Lighting was provided by miniature ceiling bulbs, adjusted to give an intensity of 10 lux at the food troughs at the height of 100 cm. The illumination cycle was 14 h of light and 10 h of darkness, with the light period from 05:00 to 19:00 h. The birds had *ad libitum* access to water and feed. The feed contained more than 185g CP and 11.88 MJ ME per kg. Feeding and other routine work, such as supplying wood shavings, was done between 09:00 and 09:30 h, and eggs were collected between 16:00 and 16:30 h.

2.2.2. Housing systems

The design and equipment of all systems, except the small conventional cages, fulfilled the regulations in the EU (European Union, 1999). Non-cage systems were not on a large commercial scale but small to facilitate behavioural observation. The conventional cages with two hens per cage were most popular management method in Japan. The details of all six housing systems were the same with Chapter 1.

2.2.3. Measurements

2.2.3.1 Welfare evaluation

To clarify the advantages and disadvantages in the housing systems, many-sided measurements (ethology, physiology, anatomy, production, and physical condition) were selected with reference to the LayWel Project (Blokhuis et al., 2007), and they are shown in Table 2-1. The hens used in each measurement were generally selected at random. For some focal measurements, the birds were individually marked, using a combination of coloured leg-rings, at 17 weeks of age. The details of each measurement were as follows.

Behaviour. Behavioural observations were conducted at 22, 30, 38, 46, 54, and 63 weeks of age (3 d/housing system/week). The mean (±SD) temperatures in the henhouse for each week were 28.3±2.9, 22.7±2.0, 20.6±3.1, 13.4±2.5, 10.1±0.8, and 15.2±3.6°C, respectively. Direct visual scans at 10 min intervals were conducted to record the behaviour of birds in each housing system for a total of 4 h/d, 2 h each in the morning (10:00 to 12:00 h) and afternoon (13:00 to 15:00 h). For behaviour, the following activities were recorded: comfort (dust-bathing, stretching, tail-flapping, wing-flapping), aggressive pecking, severe feather pecking, litter-scratching, sham dust-bathing, moving, and pre-laying (Appleby et al., 2004). Dust-bathing was recorded when one element of three (vertical wing-shaking, head-rubbing, scratching with one leg) was observed (Van Liere, 1992). Pre-laying sitting was recorded when a hen was sitting in the nest box. Aggressive pecking was pecking the head of another hen, and excluded both severe feather pecking (forceful pecks, sometimes with feathers being pulled out and the recipient bird moving away) and gentle feather pecking (careful and explorative pecks, not resulting in feathers being pulled out and usually without reaction from the recipient bird). Other behaviours using the beak (grazing, eating, drinking, gentle feather pecking, litter pecking, and object pecking) were excluded from evaluation because these behaviours have compensatory functions, and so quantitative comparisons make little sense (Shimmura et al., 2008e). All data were collected by the same two observers. Observation of FR hens was carried out at the same time by two observers, with one observer checking hens in the indoor area and the other in the outdoor area. No observations of FR were conducted on rainy days, because use of outdoor area of FR was affected significantly by it.

Tonic immobility test. To evaluate fearfulness, the tonic immobility (TI) test, a common test of fearfulness of laying hens (see Jones, 1986), was conducted on 72 birds (three birds/pen or cage; total 12 birds/housing system) at 45 weeks of age. The TI test was carried out in a separate noise-free room. In accordance with Jones (1986), TI was induced by restraining the bird on its back for 15 s in a U-shaped wooden cradle (32 cm wide, 21 cm deep, and 27 cm high) that was covered with a dark towel, with the head hanging outside. The operator held one hand gently over the bird's breast, while the other hand covered the first hand. After removing the hands of the operator from the bird, the duration of TI, latency to selfrighting, was recorded. The minimum and maximum durations of TI were set to 10 and 1200 s, respectively (The number of hens actually showed minimum and maximum durations was 0 and five hens, respectively). If the bird righted itself less than 10 s, it was captured again, and the restraining procedure was repeated.

Heterophil/lymphocyte ratio. The heterophil/lymphocyte (H/L) ratio was measured as an indicator of physiological stress response (see Jones et al., 1988). In accordance with Wall et al. (2004), blood samples were collected from the focal 48 birds (two birds/pen or cage; total eight birds/housing system) at 37, 60, and 76 weeks of age. Blood was drawn from the wing vein using a 2.5 ml syringe and 23 gauge needle. The blood, approximately 0.3 ml, was gently ejected into tubes with K_2EDTA, and then blood smears were prepared in the laboratory. After drying, the smears were stained using May-Giemsa stain. Heterophils and lymphocytes were counted at ×400 magnification until a total of 100 cells per slide were reached, and the H/L ratios were calculated. The count was carried out by two observers, and these values were averaged. Higher H/L ratio indicates higher physical stress.

Production. Egg production, mortality, and feed intake were selected to evaluate production. The number of eggs laid and the mortality were recorded daily, and the feed intake was also measured once every three weeks until 86 weeks of age.

Physical condition. Body weight, feather condition, foot condition, and claw length were measured on 72 focal birds at 35, 45, and 68 weeks of age (three birds /pen or cage; total 12 birds/housing system). In accordance with Bilčik and Keeling (1999), feather damage at six parts of the body (neck, breast, back, belly, wing, tail) was scored from 1 (no damage) to 6 (denuded), giving a total score from 6 to 36. Parts of the back and belly were also used for evaluation of risk for feather pecking and cannibalism (Savory, 1995). Foot condition was scored from 1 to 3 (1: no inflammation; 2: inflammation of one footpad; 3: inflammation of both footpads; Mahboub et al., 2004). The assessment of feather and foot damage was carried out by two or three people working together to ensure maximum consistency in scoring. The centre front and rear claws of the right foot were measured using a digital vernier calliper by recording the straight length from the claw root to the tip.

Bone density and keel bone deformity. Bone density and keel bone deformity were measured as anatomical evaluations. In accordance with Fleming et al. (2006), after slaughter by CO exposure (European Union, 1999), the right tibia and humerus and keel were excised from 48 birds (two birds/pen or cage; total eight birds/housing system) at 86 weeks of age. After drying, the tibia and humerus were radiographed in a X-ray apparatus (VPX-120A, Toshiba, Tokyo) using mammography film (CM-H for Mammography, Konica Minolta, Tokyo) in cassettes (MD-100 for Mammography, Konica Minolta) with a screen (MD-100 6F 1088, Konica Minolta,). Exposure was at 40kV and 8 mAs, and each exposed plate included an aluminium step wedge for calibration. The density of the centre of the bone on the resultant films was digitised using a densitometer (FD101, Fujifilm, Tokyo). The values were calculated by subtracting density at a determined floor of the aluminium step wedge from the bone density on a sheet of film. Higher permeability indicates weaker bone. The keel bone deformity was scored as: 1, normal; 2, twisted; 3, severe (Fleming et al., 2004).

2.2.3.2. Egg production

The number of eggs laid, including cracked eggs, at each location and hen mortality were recorded daily until the hens were 86 weeks of age. The feed intake and egg weight were also measured once every three weeks until 86 weeks of age, and the egg mass (g egg/hen/d) and feed efficiency (g egg/g feed) were calculated from these measurements. For egg quality, egg shell thickness, egg shell weight, egg shell deformation, Haugh unit, egg yolk weight, egg yolk colour, meat spot, ratio of egg yolk weight, ratio of egg shell weight, and egg shell colour of five eggs per cage or pen were determined at ages of 36, 42, 54, 66, and 76 weeks. The egg shell colour were measured by using a spectrophotometer (CM-2002, Minolta, Tokyo). The spectrophotometer shows the colour by four parameters (L, a, b, Δe), with the L-value indicating lightness, a-value red chromaticity, b-value yellow chromaticity, and Δe-value luminance.

2.2.3.3. Immune response

To evaluate humoral immune response, antigen response of hens to Newcastle disease (ND) antigen was measured by a hemagglutination inhibition test using microtiter plates (El-Lethey et al., 2003). At the age of 36 weeks, four focal hens per housing system received a cervical hypodermic injection of 0.5 ml oil vaccine including ND antigen (Oilvax 7, Chemo-Sero-Therapeutic Research Institute, Kumamoto). Blood samples were collected from a wing vein at 6, 23, and 40 weeks after the injection (at the age of 42, 59, and 76 weeks), and sera were collected after centrifugation (1500 rpm for 10 min). After adding these serum samples to the microtiters, the microtiters were serially diluted in sterile phosphate-buffered saline (PBS) in two-fold steps using a dilutor in v-shaped bottom microtiter plates, 25 μl ND hemagglutinin (Chemo-Sero-Therapeutic Research Institute), and 25 μl red blood cell of hens diluted with PBS were added. The microtiter plate was incubated for 60 min at 25°C. The agglutination antibody titer was expressed as the $\log_2$ of the reciprocal of the highest rate of dilution showing 50% agglutination.

To evaluate the cellular immune response, delayed hypersensitivity and chemotaxis and phagocytosis of macrophages were measured. In accordance with the previous study (Cook & Springer, 1983), the delayed hypersensitivity test was conducted using four hens per housing system at the ages of 37, 60, and 78 weeks, respectively. The wattle thickness of hens was measured on both sides using a digital vernier calliper, and a line was drawn on the wattle prior to intradermal injection of 0.1 ml 0.1% Phytohemagglutinin-P (Wako Pure Chemical Industries, Osaka) dissolved in the sterile PBS into the right wattle and 0.1 ml sterile PBS into the left wattle. The wattle thickness of both sides was measured at the previously drawn line 24 hours after the injection. The difference of wattle thickness was calculated by subtracting the difference before and after the injection of left wattle from the difference before and after the injection of right wattle.

The chemotaxis and phagocytosis of macrophages were evaluated using four and five hens, respectively, per housing system at the age of 80 weeks. Blood samples were collected from the wing vein, and mononuclear and polymorphonuclear leucocytes were separated by using mono-poly resolving medium (DS Pharma Biomedical Company, Osaka), followed by centrifugation (1800 rpm for 20 min). The mononuclear leucocyte layer was moved to a centrifuge tube using a Pasteur pipette. After adding Dulbecco's modified Eagle's Medium+ (DMEM+) culture medium for washing, the centrifuge tube was centrifuged (1500 rpm for 10 min), the serum was removed, and precipitated mononuclear leucocytes were placed in 1 ml DMEM+ culture medium. Cells were counted using a cell counting chamber at a concentration of 5×10^6 cells per ml, and mononuclear leucocytes were used for evaluation of functions of macrophage, chemotaxis and phagocytosis, as follows.

Chemotaxis of macrophages was assayed in chemotaxis chambers with 24 wells each containing 500 μl dead *Escherichia coli* at a concentration of 10 mg per ml in the bottom. After setting chemotaxicells with 5 μm bores on the membrane (CH5-24, Kurabo, Osaka) on each well and adding 200 μl mononuclear leucocytes prepared as above to each chemotaxicell, the chamber was incubated for 24 h at 37°C in 5% CO_2. The chemotaxicells were washed with sterilized PBS to remove floating cells, stained with Giemsa stain, and mounted on glass slides. The number of bores migrating to the bottom of all bores was counted in a visual field under the microscope (× 1,000) in four randomly selected visual fields. The chemotaxis was evaluated by calculating the ratio of transmigrated bores to all bores.

Macrophage phagocytosis was evaluated using a latex bead method. Phagocytosis assays were carried out on a chamber tray with eight slides (Lab-Tek 2, Nalge Nunc International, Naperville, IL, USA) containing 200 μl mononuclear leucocytes prepared as described above. The chamber tray was incubated for 24 h at 37°C in 5% CO_2, and serum was removed with DMEM+ culture medium. Latex beads (200 μl) were then added to each slide. After incubation for 1 h at 37°C in 5% CO_2, the slides were fixed with cold methanol and stained with Giemsa stain. The number of cells engulfing more than three beads out of all cells per visual field was counted under the microscope (× 1,000) in four randomly selected visual fields. The phagocytosis was expressed as the percentage of engulfed cells (% phagocytic).

2.2.6. Statistical analyses

The proportions of birds performing each behaviour were calculated in each cage or pen each week. There were four replicate cages or pens of each housing system, giving four replications each week. Because the behavioural data of each cage or pen in a system and the individual data of some focal measurements (e.g. humoral immune response) were linked, repeated measures ANOVA was used to evaluate the effects of housing system, week, and interactions between these effects on the measurements. Similarly, two-way ANOVA was used for the measurements without such a link (e.g. delayed hypersensitivity). For the other measurements without such repeats (e.g. TI test), one-way ANOVA was used to evaluate the effects of housing system. The significances of the effect of the housing system were evaluated by a multiple comparison using the Tukey-Kramer test. The correlations between immune response and welfare level (the total welfare score described below) were analyzed by using Spearman's correlation coefficients by rank test. The statistical software Statcel (version 2; Yanagii, 2007) was used for analyses.

2.2.7. Weighted scoring system

To clarify the advantages and disadvantages on welfare of housing system from the viewpoint of the five freedoms, five procedures were performed. First, the measurements were distributed among the five freedoms (Table 2-1), a concept of welfare widely accepted all over the world (Farm Animal Welfare Council (FAWC), 1992), with reference to the LayWel Project (Blokhuis et al., 2007). Second, only the measurements that showed a significant effect of housing system were focused (Table 2-1), and in the measurements in which a lower value represents a higher welfare level, the values were transformed to positive values if the welfare level was high. For example, in the case of the measurement "mortality due to cannibalism", SC was given a value of 6.9 while FR received a value of 0.0 (see Table 2-1). To avoid double counting, functional links between measurements were eliminated as far as possible, and similar measurements were grouped and averaged. For example, the value of the measurement "claw length" was calculated by averaging two measurements ("centre front" and "rear"; see Table 2-1). Third, to make the score of each measurement uniform before actually weighting it, the score in a measurement was set from 0 (worst level) to 1 (best level) by using the formula:

$$MS_{i,h} = \frac{MV_{i,h} - MV_{i,\min}}{MV_{i,\max} - MV_{i,\min}}$$

where $MS_{i,h}$ is the relative measurement score of the i-th measurement for a housing system (h, on a scale from 0 to 1); $MV_{i,h}$ is the actual measurement value of the i-th measurement for a housing system; $MV_{i,min}$ and $MV_{i,max}$ are the measurement values for the housing systems with the worst and best values in the i-th measurement. For example, in the case of the measurement "moving" (see Table 2-1), SC with the worst value was given a score of 0.0, and FR with the best value was 1.0, and the other housing systems were: LC, 0.06; SF, 0.21; LF, 0.29; SA, 0.55. All housing systems received a score of 1 in the measurements in which no significant effect of housing systems was seen. Fourth, weights of each measurement were calculated. The weighting method for each measurement was the same as Chapter 3 applied Bracke's method (Bracke et al.. 2002a), and here, the outlines are shown. A great number of scientific statements were first collected from scientific studies on the welfare of laying hens around the world, and weights of each measurement were set by using it. For setting weights, following 12 weighting categories (WC) were selected: pain, illness, survival, fitness, HPA (hypothalamic-pituitary-adrenocortical), SAM (sympathetic-adrenal-medullary), aggression, abnormal behaviour, frustration and avoidance, natural behaviour, preferences, and demand. Three categories are positive (natural behaviour, preferences, and demand), and the others are negative. These categories were weighted from -5 to +5, with positive weighting scores (WS) (+1 to +5) for the positive categories and negative WS (-5 to -1) for the negative ones. Then each collected scientific statements was attached to one weighting category and score on the basis of its intensity, duration, frequency and so on. For example, in the case of the measurement "moving", a declaration "A large time is spent moving under a semi-natural condition" was attached to the category "Natural behaviour" and the score was +3. At this attachment, the specific type (T) of the category type was also registered. Following the protocol above, all scientific statements were attached to one weighting category, one weighting score, and one type, respectively. The weight (W) of a measurement was finally determined by using the following calculation formula:

$$W_i = \left[\sum_{wc} \left\{ \underset{wcl}{Max}(WS_{wcl}) + 0.2 \times NT_{wc} \right\}\right] WL_{i,best} - \left[\sum_{wc} \left\{ \underset{wcl}{Min}(WS_{wcl}) - 0.2 \times NT_{wc} \right\}\right] WL_{i,worst}$$

where W_i is the weight of the i-th measurements; $WL_{i,best}$ is the best level, and $WL_{i,worst}$ is the worst level of the i-th measurements; WS_{wcl} is the weighting score; wc identifies the weighting category; wcl identifies the weighting category levels within one weighting category; and NT_{wc} is the number of unique type per weighting category. For example, the weight of the measurement "moving" was 5.4 (see Table 2-1). The weights of each measurement are shown in Table 2-1. The actually obtained score of each measurement was calculated by multiplying MS by the weight. For example, SF obtained a score of 1.134 (= 0.21 (MS) × 5.4 (weight)) in the measurement "moving" (see Table 2-1). Finally, the total scores for each freedom in each housing system were calculated, and the total scores of each housing system were then calculated. A high score is supposed to be good. All housing systems earned no score in the freedom from discomfort, because the housing systems were built in the same location and therefore there was no measurement in this freedom.

2.3. Results

2.3.1. Welfare evaluation

The average values of each housing system in each measurement are shown in Table 2-1. The results are described below by freedom.

For the freedom from pain, injury, and disease, a significant effect of housing system was found on the mortality due to cannibalism, and the mortality tended to be higher in the housing systems with a large group size (LF, SA, and FR). A significant effect of housing system on the permeability of the humerus bone was found, and the permeability was higher in the cage systems than in the non-cage systems (all $P < 0.05$). Housing system had also significant effect on foot damage, and the damage was greater in FR than the other systems (all $P < 0.05$). The total values achieved by the scoring method mentioned above were higher in the cage systems with small group size, and the FR had the lowest score (SC, 21.0; LC, 22.0; SF, 22.7; LF, 10.7; SA, 14.1; FR, 4.6).

For the freedom from hunger and thirst, there was no significant effect of housing systems on the measurements, resulting in a total score of 4.6 for all housing systems.

Table 2-1. Average values of each measurement in six housing systems.

Five freedoms	Measurement	Weight	Housing system†						Pooled	ANOVA
			SC	LC	SF	LF	SA	FR	SEM	*F*-value
Pain, injury , and disease	Mortality due to cannibalism (%)	16.8	0.0	0.0	0.0	2.8	5.6	6.9	0.82	3.9^{*}
	Bone	2.4								
	Bone permeability (humerus)		1.2^{a}	1.1^{a}	1.0^{a}	1.1^{a}	0.9^{b}	0.9^{b}	0.02	11.8^{***}
	Bone permeability (tibia)		0.7	0.6	0.5	0.6	0.5	0.5	0.02	2.2
	Keel bone deformity		1.5	2.0	1.3	1.5	1.1	1.3	0.17	1.6
	Foot damage	4.4	1.0^{b}	1.0^{b}	1.1^{b}	1.0^{b}	1.2^{b}	1.6^{a}	0.04	10.7^{***}
Hunger and thirs	Body weight (g)	2.2	1943.1	1915.8	2140.3	1917.1	1954.4	1958.9	24.93	1.9
	Feed intake (g)	1.2	109.3	108.9	111.7	109.6	113.4	113.0	0.56	2.7
	Egg production (%)	1.2	82.2	84.9	79.3	83.5	79.5	85.6	1.09	1.0
Normal behaviou	Comfort	5.4								
	Stretching (%)		0.0	0.2	0.2	0.3	0.2	0.2	0.02	2.7
	Wing-flapping (%)		0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.3^{b}	0.5^{a}	0.02	47.3^{***}
	Tail-flapping (%)		0.0	0.0	0.0	0.0	0.1	0.1	0.01	2.8^{*}
	Dust-bathing	14.2								
	Dust-bathing (%)		-	-	4.7^{a}	2.1^{b}	3.6^{ab}	2.4^{b}	0.28	6.2^{**}
	Sham dust-bathing (%)		2.8^{a}	2.8^{a}	0.3^{b}	1.3^{b}	-	-	0.18	13.8^{***}
	Moving (%)	5.4	0.1^{d}	0.8^{d}	2.5^{c}	3.5^{c}	6.5^{b}	11.8^{a}	0.38	108.7^{***}
	Pre-laying (%)	10.4	-	-	1.4^{b}	3.1^{b}	6.9^{a}	6.0^{a}	0.33	24.2^{***}
	Litter scratching (%)	14.2	-	-	0.1^{c}	0.1^{c}	0.7^{b}	2.3^{a}	0.13	172.6^{***}
Fear and distres	TI duration (s)	3.6	713.2^{a}	627.8^{a}	591.8^{a}	430.6^{a}	375.6^{ab}	157.8^{b}	42.25	4.9^{***}
	H/L ratio	3.2	0.3^{a}	0.3^{a}	0.2^{b}	0.2^{ab}	0.2^{b}	0.2^{b}	0.03	6.7^{***}
	Pecking	10.8								
	Feather pecking (%)		0.4^{b}	0.7^{b}	0.2^{b}	1.4^{ab}	2.2^{a}	0.7^{b}	0.10	6.4^{**}
	Feather damage (total of back and belly)		3.4^{b}	2.9^{b}	4.0^{ab}	4.2^{ab}	6.7^{a}	5.6^{a}	0.30	12.9^{***}
	Aggressive pecking (%)		0.2^{b}	0.2^{b}	0.3^{b}	1.6^{a}	0.6^{b}	0.2^{b}	0.05	21.0^{***}
	Feather damage (total of all regi	2.2	19.4	18.9	17.6	18.4	18.1	16.8	0.70	2.1
	Claw length	3.4								
	Center front (mm)		23.0^{a}	23.5^{a}	20.8^{a}	21.8^{a}	18.3^{b}	15.2^{b}	0.42	20.2^{***}
	Back (mm)		12.6^{a}	12.8^{a}	10.8^{b}	12.5^{a}	10.8^{b}	10.0^{b}	0.18	13.6^{***}
Discomfort	-									

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. -: no data. †SC: small conventional cages; LC: large conventional cages; SF: small furnished cages; LF: large furnished cages; SA: single-tiered aviary; FR: free-range. Different superscript letters within a measurement indicate significant difference (a-b-c-d: $P < 0.05$).

For the freedom to express normal behaviour, a significant effect of housing system was seen on the proportion of hens performing wing-flapping, and the proportion was higher in FR than in SA ($P < 0.05$), and in SA than in the other systems (all $P < 0.05$). Significant effects of housing system on the proportion of hens performing dust-bathing and sham dust-bathing were seen, and the former was higher in SF than in LF and FR (both $P < 0.05$), and the latter was higher in the conventional cages than in the furnished cages (all $P < 0.05$). A significant effect of housing system on the proportion of hens moving was found, and the proportion was higher in FR than in SA ($P < 0.05$), in SA than in the furnished cages (both $P < 0.05$), and in the furnished cages than in the conventional cages (all $P < 0.05$). Pre-laying showed a significant effect of housing system, and the proportion of hens performing pre-laying was higher in the non-cage systems compared with the furnished cages (all $P < 0.05$). A significant effect of housing system on the proportion of hens performing litter scratching was found, and the proportion was higher in FR than in SA ($P < 0.05$), and in SA than in the furnished cages (both $P < 0.05$). The freedom to express normal behaviour scored better in the non-cage systems, especially FR, than the cage systems. Among the cage systems, the score was higher in SF than in LF, and in LF than in the conventional cages (SC, 0.0; LC, 1.5; SF, 19.4; LF, 15.8; SA, 34.5; FR, 44.2).

For the freedom from fear and distress, the TI duration showed a significant effect of housing system; the duration was shorter in FR than in the cage systems (all $P < 0.05$). The housing system had a significant effect on H/L ratio, and the value was lower in FR, SA, and SF than in the conventional cages (all $P < 0.05$). Significant effects of housing system on feather pecking, feather damage, and aggressive pecking were found. Feather pecking was more common in SA than in all other systems excluding LF (all $P < 0.05$), and aggressive pecking was more frequent in LF compared with the other systems (all $P < 0.05$). Feather damage on the parts of back and belly was higher in the non-cage systems than in the conventional cages (all $P < 0.05$). Significant effects of housing system were seen on the claw at the centre front and rear, and these lengths were shorter in the non-cage systems than in the other cage systems, excluding the back claw in SF (all $P < 0.05$). The total scores of the freedom from fear and distress were similar among the housing systems (SC, 11.1; LC, 11.4; SF, 14.3; LF, 8.1; SA, 7.5; FR, 15.7).

The total scores of SF tended to be as high as the non-cage systems. On the other hand, LF scored the lower and was equal to the conventional cages (SC, 36.7; LC, 39.5; SF, 61.0; LF, 39.2; SA, 60.7; FR, 69.2).

2.3.2. Egg production

Egg production rates and egg quality are shown in Table 2-2. Although egg production was, as a whole, low, this is due to including the data until 86 weeks of age. Significant effects of housing system on feed intake and mortality were found, and these values tended to be higher in the two non-cage systems (SA and FR), although no significant difference between housing systems was found. There were significant effects of housing systems on egg yolk colour and egg shell colour: the egg shell colour was lighter (L-value), redder (a-value), yellower (b-value), and more vivid (Δe-value) in FR than in SA ($P < 0.05$), and in SA than in all the other cage systems (all $P < 0.05$). Significant effects of housing system was also found on egg shell thickness, egg shell weight, and ratio of egg shell weight, and the eggs of the non-cage systems were thicker and had a higher ratio of egg shell weight. Egg shell weight also showed a similar tendency, although no significant difference between housing systems was found.

Table 2-2. Average values of egg production and egg quality in six housing systems.

Measurement	Housing system†						Pooled	ANOVA
	SC	LC	SF	LF	SA	FR	SEM	*F*-value
Egg production (%)	82.2	84.9	79.3	83.5	79.5	85.6	1.09	1.0
Cracked egg (%)	1.2	1.6	4.5	1.2	2.1	0.9	0.41	2.3
Feed intake (g/hen per d)	109.3	108.9	111.7	109.6	113.4	113.0	0.56	2.9 *
Egg weight (g/egg)	60.7	60.9	61.8	60.6	61.1	61.4	0.21	0.7
Egg mass (g egg/hen per d)	49.8	51.7	48.8	50.5	48.3	52.5	0.69	0.9
Feed efficiency (g of egg/g of feed)	2.3	2.1	2.3	2.2	2.4	2.2	0.03	1.6
Egg shell thickness (mm)	0.36 ^{ab}	0.35 ^{b}	0.34 ^{b}	0.35 ^{ab}	0.37 ^{a}	0.37 ^{a}	0.002	8.4 ***
Egg shell weight (g)	5.7	5.6	5.6	5.5	5.9	5.9	0.05	3.6 *
Egg shell deformation (kg/cm^2)	3.8	3.6	3.6	3.7	4.1	3.9	0.06	1.6
Haugh units	74.8	73.5	79.4	79.3	78.3	78.5	0.78	2.2
Egg yolk weight (g)	18.1	17.9	18.7	17.9	18.3	18.1	0.14	1.0
Egg yolk color	10.1 ^{b}	10.2 ^{ab}	10.4 ^{ab}	10.4 ^{ab}	10.5 ^{a}	10.4 ^{ab}	0.04	4.4
Meat spot (%)	5.0	12.0	9.0	11.0	11.0	0.0	1.34	2.8 *
Ratio of egg york weight	29.3	29.2	29.5	29.3	29.0	28.4	0.15	1.2
Ratio of egg shell weight	9.2 ^{ab}	9.1 ^{ab}	8.8 ^{b}	9.1 ^{ab}	9.4 ^{a}	9.3 ^{a}	0.05	4.4 **
Egg shell color								
L-value	80.6 ^{c}	80.4 ^{c}	79.8 ^{c}	81.2 ^{c}	84.3 ^{b}	87.2 ^{a}	0.58	32.8 ***
a-value	5.2 ^{a}	5.0 ^{a}	5.7 ^{a}	4.8 ^{a}	3.0 ^{b}	1.2 ^{c}	0.34	42.3 ***
b-value	16.5 ^{a}	17.1 ^{a}	18.0 ^{a}	15.9 ^{a}	13.9 ^{b}	9.7 ^{c}	0.60	38.5 ***
Δe-value	25.5 ^{a}	26.0 ^{a}	27.2 ^{a}	24.8 ^{a}	20.8 ^{b}	15.6 ^{c}	0.86	40.0 ***
Mortality due to cannibalism (%)	0.0	0.0	0.0	2.8	5.6	6.9	0.82	3.9 *

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. †SC: small conventional cages; LC: large conventional cages; SF: small furnished cages; LF: large furnished cages; SA: single-tiered aviary; FR: free-range. Different superscript letters within a measurement indicate significant difference (a-b-c: $P < 0.05$).

2.3.3. Immune response

The immune response results are shown in Table 2-3. The ND antibody titer showed a significant effect of housing system, and tended to be lower in LF compared with the other housing systems (SC, 9.4; LC, 9.5; SF, 10.0; LF, 7.8; SA, 9.2; FR, 10.4). Significant effects of housing system and week were found on delayed hypersensitivity. It was higher at 60 (2.2) and 78 (2.1) than at 37 (1.0) weeks of age (both $P < 0.01$; 37 wk, 1.0; 60 wk, 2.2; 78 wk, 2.1), and tended to be higher in the non-cage systems and furnished cages than in the conventional cages (SC, 1.3; LC, 1.3; SF, 2.1; LF, 1.9; SA, 1.9; FR, 1.9), although no significant difference between housing systems was found. There was no significant effect of housing systems on chemotaxis and phagocytosis of macrophages.

For the correlation between immune response and welfare level (the total welfare score), a significant strong-positive correlation between delayed hypersensitivity and welfare level was found ($rs = 0.89$, $P < 0.05$), while no significant correlation between ND antibody titer and welfare level was found ($rs = 0.77$, $P = 0.110$).

Table 2-3. Average values of immune response of laying hens in six housing systems.

Measurement	Housing system†						Pooled SEM	ANOVA F-value
	SC	LC	SF	LF	SA	FR		
ND antibody titer								
42 wk	8.8	8.3	9.5	7.3	9.5	10.3		
59 wk	9.8	10.3	10.3	7.8	9.0	10.5	0.19	4.5 **
76 wk	9.8	10.0	10.3	8.3	9.0	10.5		
Delayed hypersensitivity								
37 wk	0.8	0.8	0.9	1.1	1.2	1.3		
60 wk	1.3	1.7	3.0	2.4	2.5	2.1	0.12	2.8 *
78 wk	1.8	1.5	2.4	2.2	2.1	2.4		
Macrophage								
chemotaxis	45.3	56.1	58.8	60.8	49.3	61.6	2.39	1.4
phagocytosis	24.0	24.2	31.4	33.8	28.7	22.1	1.41	2.2

*$P < 0.05$; **$P < 0.01$ †SC: small conventional cages; LC: large conventional cages; SF: small furnished cages; LF: large furnished cages; SA: single-tiered aviary; FR: free-range. ‡Significant effect of week on delayed hypersensitivity was also found ($F = 19.5$, $P < 0.001$), and it was higher in 60 and 78 than in 37 weeks of age (both $P < 0.01$).

2.4. Discussion

2.4.1. Welfare evaluation

For the freedom from pain, injury, and disease, the permeability of hens in the non-cage systems was lower, which indicate that the bone is stronger. This is due to a larger movement and behavioural repertoire such as litter scratching, which has been reported in the previous studies (Fleming et al., 2004, 2006), and was confirmed in this study. While the non-cage systems have the advantage of producing stronger bone, the systems have the disadvantage of higher risk of mortality and bumble foot. In this study, the mortality due to cannibalism tended to be higher in the systems with large group sizes (LF, SA, and FR), and foot damage was higher in FR. As mentioned below, the mortality due to cannibalism is generally higher with increase of group size. In fact, the LayWel Project (Blokhuis et al., 2007), a large-scale project that assessed the welfare of housing systems for laying hens, reported that mortality was higher in large-scale housing systems (e.g., large furnished cages, non-cage systems) compared with cages with small group sizes (e.g., conventional cages, small furnished cages), which is similar to this study. The LayWel Project also reported that bumble foot was especially increased in non-cage systems including free-range, which is due to increased movement and litter scratching of hens or to the complex structure of the housing systems.

For the freedom from hunger and thirst, no significant effect of housing systems was found in any measurement. The *ad libitum* access to water and feed is common for laying hens (Appleby et al., 2004), and therefore, no difference would naturally result from the freedom from hunger and thirst.

For the freedom to express normal behaviour, the behaviour was, as a whole, more diversified in the non-cage systems, especially FR, than in the cages. Among the cages, the behavioural diversification was higher in SF than in LF, and behaviour was most restricted in the conventional cages. As confirmed in this study, supplying a larger space encourage comfort behaviour (e.g. Nicol, 1987) and movement (e.g. Appleby et al., 2002), and supplying more litter space and nest box leads to the increased litter exploring (e.g. Shimmura et al., 2008a) and pre-laying (Appleby, 2004). It is, therefore, undoubted that these behaviours were more common in the non-cage systems and furnished cages, especially the former with a more enriched environment. On the other hand, the behaviour was remarkably restricted in conventional cages, with little space and no resources, and sham dust-bathing, an expression of frustration (see Keeling, 2004), was also observed. Among the furnished cages, the behavioural diversification was higher in SF than in LF.

This result supports the importance of the design of furnished cages, as demonstrated by a large number of previous studies (e.g. Appleby and Hughes, 1995; Appleby et al., 2002). Moving and comfort behaviour are enhanced in furnished cages with large spaces, which lead to stronger bone and a higher welfare level (Wall et al., 2004). However, in the cases without enough resources such as litter area such as large furnished cages in this study, competition for the resource is increased and results in increased aggressive interactions and decreased litter-related behaviours (Shimmura et al., 2008a, 2008b, 2009a).

For the freedom from fear and distress, the TI durations were shortest in FR and the H/L ratios were lower value in the non-cage systems compared with the conventional cages. Increased H/L ratios were associated with prolonged TI after corticosterone infusion (Jones et al., 1988) and ACTH injection (Beuving et al., 1989), indicating that H/L ratios and TI duration are closely related to physical stress. The usefulness of these measurements was also evaluated (Siegel and Gross, 1980; Gross and Siegel, 1983), and they are actually used as stress indicators (e.g. Mahboub et al., 2004; Wall et al., 2004). The result of the present study, therefore, might suggest that physical stress level that hens received is especially low in FR. Also, a large litter space and outdoor area promote litter scratching, which restrained claw overgrowth (Shimmura et al., 2007b), although it sometime caused footpad inflammation, as mentioned above. The short claw is one of the advantages of the non-cage systems with a large litter area because overgrowth leading to claw breakage is frequently observed in conventional cages (Hills, 1975; Tauson, 1986). On the other hand, large group size is one of the disadvantages of the non-cage systems. A number of studies have demonstrated that feather pecking and aggressive pecking were increased with increments of group size (e.g., Hughes and Wood-Gush, 1977; Hughes et al., 1997), and these behaviours were reported to be more common in housing systems with large group sizes (e.g. Appleby et al., 2002), although little aggressive interaction was observed when group size is still lager: the incidence of agonistic interaction was low and similar in groups of 100, 200 and 400 (e.g. Hughes et al., 1997). On this point, the risk for these aggressive behaviours was lower in cage systems with small group size. Among the housing systems with large group size, feather pecking was performed less in FR than in SA and LF. It has been reported that the risk for feather pecking is lower when an outdoor grazing area is provided because the motivation to peck is redirected to grass or because the distances between individual hens is greater (Mohboub et al., 2004; Shimmura et al., 2008c).

In the evaluation by weighted scoring system, the freedom to express normal behaviour scored higher in the non-cage systems, while the freedom from pain, injury, and disease was not secured, although the bone was stronger. The reverse situation was found in the conventional cages. These results were, on the whole, agree with a number of evidences mentioned above. Duncan (2001) concluded in his review that while conventional cages have the disadvantage of extremely restricted behaviour, the cages excel over alternative systems especially in the points of increased hygiene and low incidence of social friction. Considering that the freedom from discomfort (e.g., hygiene status) is most secured in conventional cages, the welfare level, as a whole, might differ little between conventional cages and non-cage systems. More striking result is that the total score of SF was comparable to those of both the non-cage systems, and the LF score was similar to those of the conventional cages. This result supports the importance of the design of furnished cages as mentioned above, and suggests the welfare level of furnished cages might stand comparison with non-cage systems if the cage design is good, and if not, little difference might be found between furnished cages and conventional cages. It also suggests that large furnished cages may offer a higher welfare level than small ones if enough resources supplied. However, this weighted scoring system is an attempt and has some problems such that indicators were not provided with equal coverage of all five freedoms. Further revision would be needed, and therefore, the results might be not enough meaningful at the present time.

2.4.2. Egg production

The feed intake and mortality both tended to be increased in the non-cage systems in this study. The non-cage systems have the advantages of higher behavioural diversification and increased movement, but conversely it can lead to energy loss. Therefore, feed intake generally is increased in the systems with large floor areas, as reported in previous studies (e.g. Abrahamsson and Tauson, 1995; Abrahamsson et al., 1996, 1998). Some studies reported decreased egg production and feed efficiency (e.g. Tauson et al., 1999; Michel and Huonnic, 2003), although these tendencies were not found in this study.

For egg quality, egg shells were thicker, but egg shell colour was paler in the non-cage systems, especially FR, compared with the cage systems. The ultraviolet (UV) radiation in sunlight has various effects on the body, and above all, an increase of vitamin D_3 production is most important in producing eggs. It is known that absorption of calcium is promoted by increased vitamin D_3 in many species, including laying hens (see Ameenuddin et al., 1985). It was also confirmed that exposure of laying hens to UV radiation produces thicker egg shells (Hughes et al., 1925; Hart et al., 1925). On the other hand, the increase of vitamin D_3 caused pale egg shells. In fact, paleness of egg shells in housing systems with exposure to sunlight, such as free-range, has been reported (Ryan, 2007). In his study investigating the relationship between the level of vitamin D_3 and UV radiation, Ryan (2007) demonstrated that egg shell colour was paler due to excess vitamin D_3 when vitamin D_3 was included in the feed and was increased due to exposure to UV radiation. Therefore, in this study, vitamin D_3 would be increased by sunlight, which resulted in increased egg shell sickness and pale egg shells in the non-cage systems, especially FR. Considering that there was no significant difference in the number of cracked eggs, the increased egg shell thickness is not advantageous, and therefore, housing systems that allow exposure to sunlight, especially with an outdoor area, might have the disadvantage of pale egg shells.

2.4.3. Immune response

The ND titer tended to be lower in LF, and the delayed hypersensitivity was lower in SC and LC. The result that SF has higher immunity like the non-cage systems suggests a relationship with stress rather than just hygiene status. Stress decreases the number of lymphocytes, and the decline white blood cell response to viruses is largely measured by lymphocytes (Siegel, 1980). It consequently results in an increase in the heterophil/lymphocyte ratio (H/L ratio), an indicator of the physiological stress response, and a decline in the immune response, such as the established antibody titer to diseases of viral origin and the delayed hypersensitivity that lymphocytes were multiplied by injection of phytohemagglutinin-P (Siegel, 1980). In fact, it has been reported that these indicators are decreased by various stressors: ACTH injection (Puvadolpirod and Thaxton, 2000), heat (Thaxton and Siegel, 1970; Thompson and Lippman, 1974; Gillis et al., 1979), cold (Brown and Nestor, 1973; Subba Rao and Glick, 1977), and behaviours such as aggressive interaction (e.g. Gross and Siegel, 1965, 1973). As mentioned above, H/L ratios were higher in SC, LC, and LF, and that welfare levels seemed to be lower in these systems compared with SF, SA, and FR. Considering that the immune response was lower in SC, LC, and LF systems in the present report, the immune response of hens in housing systems with higher stress levels might be lower. Conversely, it also suggests that the immune response of hens living in housings with higher welfare levels might be higher. The suggestion might be supported by the result of a strong-positive correlation between delayed hypersensitivity and welfare level. However, few studies have investigated the relationship between physiological stress and immunity in housing systems for laying hens. The present results are not fully conclusive, and further investigation of the relationship is needed.

2.4.4. Conclusion

The present study aimed to clarify the advantages and disadvantages of various housing systems for laying hens and was a pilot study for work in commercial condition. The non-cage systems, especially FR, have some low evaluation for the freedom from pain, injury, and disease, and some disadvantages, such as pale eggs and increased feed intake for production. On the other hand, the evaluation for the freedom to express normal behaviour was high and immune response was high in the non-cage systems. The reverse situation was found in the conventional cages. Among the furnished cages, behaviour was more diversified in SF than in LF, and immune response of SF was comparable with the non-cage system. The total welfare score of SF was comparable to those of both the non-cage systems, and the LF score was similar to those of the conventional cages. This result supports the high potential value of furnished cage. However, in large furnished cages, competition for a restricted number of resources was frequently observed due to increased group size, while mobility and comfort behaviour are enhanced by providing a larger total cage area.

2.5. Summary

The present study aimed to clarify the advantages and disadvantages of various housing systems for laying hens and was a pilot study for work in commercial condition. We selected six housing systems: two types of conventional cages (small: SC; large: LC), furnished cages (small: SF; large: LF), and non-cage systems (single-tiered aviary: SA; free-range: FR). We evaluated the welfare, egg production, and immune response of these

housing systems built in the same location through one and a half year. The non-cage systems, especially FR, have some low evaluation for the freedom from pain, injury, and disease, and some disadvantages, such as pale eggs and increased feed intake for production. On the other hand, the evaluation for the freedom to express normal behaviour was high and immune response was high in the non-cage systems. The reverse situation was found in the conventional cages. Among the furnished cages, behaviour was more diversified in SF than in LF, and immune response of SF was comparable with the non-cage system. The total welfare score of SF was comparable to those of both the non-cage systems, and the LF score was similar to those of the conventional cages. This result supports the high potential value of furnished cage. However, in large furnished cages, competition for a restricted number of resources was frequently observed due to increased group size, while mobility and comfort behaviour are enhanced by providing a larger total cage area.

CHAPTER 3

Development of overall welfare assessment

3.1. Introduction

In Chapter 1, comparison of beak-related behaviours in six housing systems for selection of welfare parameter was reported. In Chapter 2, based on the result of Chapter 1, welfare indicators were selected, and the overall evaluation of welfare levels, egg production, and immune response in the six housing systems was conducted. In Chapter 3, new assessment was devised and its usefulness was evaluated by using the animal-based values of Chapter 2.

Animal welfare has progressed rapidly from a concept to laws or guidelines around the world. In the European Union (EU), in which animal welfare standards are well developed, conventional cages will be banned from 2012, and only furnished cages and non-cage systems (e.g. aviary, free-range housing systems) will be allowed. The farm products produced in these housings are sold in many EU supermarkets. However, consumers are not able to fully understand what the welfare level of a flock is without any label on the food package. According to a consumer survey by the European Commission, the inability of EU consumers to actually find this information has reduced the interest of consumers in farm animal welfare (Eurobarometer, 2005), and so a system of grading eggs by an integrated welfare assessment is needed. The Welfare Quality Project (WQ Project) plays a key role in developing welfare assessment in the EU. In this project they are attempting to differentiate farms by welfare level. Such attempts have also been made in Japan, and it was decided to evaluate welfare from the viewpoint of the five freedoms, a concept of welfare widely accepted all over the world (Farm Animal Welfare Council (FAWC), 1992). Since then, welfare assessment has been a central subject in studies of farm animal welfare.

Many welfare assessment systems now exist, and the methods are varied (Botreau et al., 2007a). Among the assessments used in practice at the farm animal level, the Animal Needs Index (ANI) designed by Bartussek (1999) is the best known. It has a version for each farm animal species, including one for laying hens, ANI 35-L/2001 – laying hens (Bartussek, 2001). In Austria, ANI is used officially mainly to regulate organic farming and in connection with animal welfare legislation (Bartussek, 1999). This assessment system is based mainly on environment-based measurements (e.g., group size, litter area). Mollenhorst et al. (2005) reported that the ANI score correlated well with some animal-based measurements (e.g., feather condition, behaviour), which would suggest the usefulness of ANI. However, no correlations with animal-based assessments have been reported (Zaludik et al., 2007), and criticisms for relying on environment-based parameters to assess welfare are not rare (Sandøe et al., 1997; Sundrum, 1997; Whay et al., 2003). It was also pointed out that the weighting method of the measurements in ANI is not based on scientific studies and/or experts and overrates the importance of outdoor access (Bracke et al., 2002a; Kohari et al., 2006; Seo et al., 2007).

The decision support system developed by Bracke et al. (2002a) is based on the literature and therefore called science-based or information-based assessment. It includes a definite selection of measurements including multiple parameters (animal-, environment-, and management-based) and the weighting method is based on scientific statements from studies on farm animal welfare. Being based on scientific studies would imply validity, which is important in animal welfare studies because they are continuously changing. Bracke picked the pregnant sow as the model animal and developed a system for sows, so-called SOWEL (SOw WELfare: Bracke et al., 2002a, 2002b), and they then provides decision support systems for other farm animals (COWEL: Ursinus et al., 2008; FOWEL: De Mol et al., 2006). However, FOWEL remains making and no evaluation of the usefulness, although SOWEL has been evaluated from many sides (e.g. Bracke et al., 2002b). Therefore, the evaluation of the welfare assessment for laying hens is uncertain.

Here, I propose a science-based assessment for laying hens that applies Bracke's modelling principles. The protocol of this study comprised the construction and evaluation of welfare assessment. To increase the validity of the evaluation and facilitate expansion and maintenance of the assessment system, I planned a basic strategy that used many accumulated studies on animal welfare and created a database of studies on the welfare of laying hens around the world. On the basis of it, a science-based overall assessment for laying hens was devised. The usefulness of the assessment was also evaluated by comparing it with the environment-based Animal Needs Index (ANI) and animal-based assessment.

3.2. Materials and Methods

3.2.1. Housing systems

Six types of housing systems were evaluated using this model, ANI, and animal-based assessment: two types of conventional cages (small and large), furnished cages (small and large), and non-cage systems (aviary and free-range). For precise comparison with animal-based assessment, these housing types were built in the same location, and the same hybrid (total 284 birds) was introduced. The design and equipment of all systems, except the small conventional cages, fulfilled the regulations in the EU (European Union, 1999). Non-cage systems were not on a large commercial scale but small to facilitate behavioural observation. The conventional cages with two hens per cage were most popular management method in

Japan. There were four replicates of each system. The details and management of the six housing systems were the same as Chapter 1.

3.2.2. Construction of welfare assessment

This model is a science-based overall welfare assessment system that applies Bracke's model (Bracke et al., 2002a) and accepts a bottom-up approach. Scientific studies and declarations on the welfare of laying hens around the world were first collected, and 28 measurements were selected and their levels and weights were set. These measurements were then distributed among the five freedoms, and finally one overall assessment was completed. Therefore, the completed assessment included the measurements, levels and weightings based on the scientific studies and can be clarified the advantages and disadvantages of housing systems from the view point of the five freedoms. This hierarchical bottom-up approach consisted of six procedures:

1) Collection of scientific information. Scientific information was defined as published information. This database included as many studies of the welfare of laying hens around the world as we could find. The information was collected mainly from the guidelines of each country (e.g., Scientific Veterinary Committee Report, 1996), guidelines of welfare protection groups (e.g., RSPCA, FAWC, DEFRA), books (e.g., Welfare of the Laying Hen, Perry, 2004; Poultry Behaviour and Welfare, Appleby et al., 2004), large-scale projects (e.g., the LayWel project, Blokhuis et al., 2007), reviews (e.g., World's Poultry Science Journal), and other publications (e.g., Applied Animal Behaviour Science, British Poultry Science, Animal Welfare).

2) Collection of scientific declarations. A scientific declaration was defined as the common information shared by some papers. The scientific information obtained from a paper is limited and not fully certain. A total of 498 scientific declarations, or 17.8 scientific declarations per measurement, were collected.

3) Selection of measurements. To make overall assessment possible, the set of measurements must be exhaustive, minimal, practical, and independent (Botreau et al., 2007b). We selected measurements that fulfill these requirements, referring especially to the LayWel project (Blokhuis et al., 2007) and the WQ project (Butterworth et al., 2007). First, measurements were selected on the basis of each of the five freedoms (pain, injury, and disease; hunger and thirst; discomfort; normal behaviour; and fear and distress) and evaluation bases (animal, environment and management) to make it exhaustive. Second, to minimize the number of items, only necessary measurements (excluding redundant or irrelevant measurements) were picked up based on scientific declarations. Minimizing the number of measurements assures it will be practical. To increase the practicality, animal-based measurements that needed behavioral observations were excluded because they are affected by various factors (e.g., season, weather, time of day, age of animal) and take time (Mollenhorst et al., 2005). We evaluated using animal-based (without behavioural observation) or environment-based measurements if it can compensate for behavioral studies (Bracke, 2007). For example, feather pecking can be evaluated by the feather and skin condition (e.g. Bilčik and Keeling, 1999). In laying hens, an approach like this is possible and effective because many full studies of the effects of each resource on the related behaviour are included. Third, the measurements must be independent of each other, and to avoid double counting, there should be no functional links between measurements, as far as possible. Bracket et al. (2002a) selected measurements based on behavioural/motivational systems and to keep functions separate. Following their method, measurement "litter floor" in this study is separated into the two measurements of "litter for foraging" and "litter for dust-bathing" because of different behavioural/motivational systems, and scientific declarations are also separated and attached to the measurements separately, which mentioned below. However, there are no small numbers of scientific declarations that such separation is difficult. For example, scientific declaration "aggressive interactions was increased by decreased litter area" cannot be separated but should be attached one measurement "litter floor". Therefore, in this study, measurements were not separated by behavioural/motivational systems. Finally, the set of measurements shown in Table 3-1 was accepted.

Table 3-1. Measurements distributed to the five freedoms and three bases and number of levels (L) and weight. The actual scoring method of free-range system is illustrated on the right side from applicable level (AL), and the AL, measurement score (MS), and final score (MS × Weight) of free-range is shown.

No.	Five freedoms	Base	Measurement	L	AL	MS	Weight	Point
1	Pain, injury, and disea	Animal	Feather condition	5	4	0.75	10.8	8.1
2			Foot condition	4	3	0.67	4.4	2.9
3		Environment	Red mite	2	2	1	3.2	3.2
							Subtotal:	14.2
4	Hunger and thirst	Management	Molting	4	4	1	17.2	17.2
5			Feeding level	3	3	1	6.6	6.6
6			Drinking level	2	2	1	4.4	4.4
							Subtotal:	28.2
7	Discomfort	Environment	Food agonism	4	3	0.67	10.8	7.2
8			Exposure to heat	3	3	1	8.6	8.6
9			Air quality	4	4	1	7.6	7.6
10			Rearing condition	4	2	0.33	7.4	2.4
11			Light	4	4	1	6.0	6.0
12			Exposure to cold	3	3	1	5.4	5.4
13			Separation from ma	4	1	0	5.2	0.0
14			Movement comfort	3	3	1	3.2	3.2
15			Water agonism	2	2	1	2.2	2.2
16			Hygiene	3	3	1	1.2	1.2
17			Litter quality	3	3	1	1.2	1.2
							Subtotal:	45.1
18	Normal behaviour	Environment	Group size	3	2	0.5	15.6	7.8
19			Litter floor	6	6	1	14.8	14.8
20			Density (space/hen	9	9	1	14.6	14.6
21			Perch	3	3	1	11.6	11.6
22			Nest	4	4	1	10.4	10.4
23			Total floor space	4	4	1	5.4	5.4
24			Floor level	5	2	0.25	4.4	1.1
25			Food type	2	2	1	2.2	2.2
							Subtotal:	67.9
26	Fear and distress	Animal	Fear	3	3	1	3.6	3.6
27		Management	Beak trimming	4	3	0.67	14.2	9.5
28			Handling	3	3	1	5.6	5.6
							Subtotal:	18.7
							Total:	174.1

4) Setting levels in each measurement. On the basis of scientific declarations, more than two levels were set for each measurement (Table 3-1). For example, measurement #21 "perch" has three levels: level 1 (worst), no perch; level 2, < 14 cm perch per hen; level 3 (best), ≥ 14 cm perch per hen. For scoring, a measurement score (MS) was then attached to each level in each measurement. To make the weight of each measurement uniform before actual weighting it, the score in each measurement was set from 0 (worst level) to 1 (best level) by using the formula:

$$MS_{i,j} = \frac{NL_i - RL_{i,j}}{NL_i - 1}$$

where $MS_{i,j}$ is the measurement score of the j-th level of the i-th measurement; NL_i is the total number of levels of measurement i; and $RL_{i,j}$ is the rank number of the j-th level of the i-th measurement. For example, in the case of the measurement #21 "perch" with three levels, level 1 was given a score of 0, level 2 was 0.5, and level 3 was 1. A measurement with four levels, such as #22 "nest", has MS of 0, 0.33, 0.67, and 1. The score of level applied to the system was obtained. For example, free-range housing has 27 cm perch per hen, which fulfils the best level of the measurement #21 "perch" (level 3, ≥ 14 cm perch per hen), and so the free-range obtained a score of 1 for the measurement "perch" (see Table 3-1). Finally, the obtained score 1 (MS) was multiplied by the score weight 11.6, which is explained below.

5) Weighting each measurement. The weighting method for each measurement was almost the same as Bracke's (Bracke et al., 2002a). First, following 12 weighting categories (WC) were selected: pain, illness, survival, fitness, HPA (hypothalamic-pituitary-adrenocortical) axis activity, SAM (sympathetic-adrenal-medullary) axis activity, aggression, abnormal behaviour, frustration and avoidance, natural behaviour, preferences, and demand. Three categories are positive (natural behaviour, preferences, and demand), and the others are negative. These categories were weighted from -5 to +5, with positive weighting scores (WS) (+1 to +5) for the positive categories and negative WS (-5 to -1) for the negative ones. Then each collected scientific declaration was attached to one weighting category (average 17.2 scientific declarations per measurement) and scored on the basis of its intensity, duration, frequency and so on. In the case of the measurement #19 "litter floor", a declaration "A large time is spent pecking the ground under a semi-natural condition" was attached to the category "Natural behaviour" and the score was +3 (see Table 3-2). At this attachment, the specific type (T) of the category was also registered. Following the protocol above, all scientific declarations were attached to one weighting category, one weighting score, and one type, respectively. The weight (W) of a measurement was finally determined by using the following calculation formula:

$$W_i = \left[\sum_{wc} \left\{ \underset{wcl}{Max}(WS_{wcl}) + 0.2 \times NT_{wc} \right\} \right] WL_{i,best} - \left[\sum_{wc} \left\{ \underset{wcl}{Min}(WS_{wcl}) - 0.2 \times NT_{wc} \right\} \right] WL_{i,worst}$$

where W_i is the weight of the i-th measurements; $WL_{i,best}$ is the best level, and $WL_{i,worst}$ is the worst level of the i-th measurements; WS_{wcl} is the weighting score; wc identifies the weighting category; wcl identifies the weighting category levels within one weighting category; and NT_{wc} is the number of unique types per weighting category. For example, the case of measurement #19 "litter floor" is illustrated in Table 3-2, which shows only for the positive weighting categories (the left side of the formula above). First, the maximum weighting scores per weighting category are determined. In Table 3-2, as marked with superscript a, the maximum score was 3 for natural behaviour, 3 for preferences, and 3 for demand. Second, the number of unique types per weighting category is counted. In Table 3-

2, as marked with superscript b, the number was 1 for natural behaviour, 3 for preferences, and 2 for demand. Third, following the Brake's algorithm (Bracke *et al.* 2002a), these maximum weighting scores are added to the score that multiplies the number of a unique type by 0.2. Thus, the weighting point was 3.2 for natural behaviour ($3 + 1 \times 0.2$), 3.6 for preferences ($3 + 3 \times 0.2$), and 3.4 for demand ($3 + 2 \times 0.2$). Fourth, these weighting points are summed, that is, the total of the weighting points was 10.2 ($3.2 + 3.6 + 3.4$) for positive weighting categories of the measurement "litter floor". Similarly, the total of the weighting points for negative weighting categories (the right side of the formula above) was calculated (-4.6 in the case of the measurement "litter floor"). Finally, these two weighting points were substituted in the formula above. This resulted in a score 14.8 ($= 10.2 - (-4.6)$), which is the finally attached weight to the measurement 'litter floor'.

Table 3-2. Positive weighting categories used to calculate weighting of measurement #20 "litter floor". The scientific declaration picked up from scientific information was attached to one weighting category (WC), weighting score (WS), and type, and the weight was calculated by using it.

WC	WS	Type	Scientific	Scientific information
Natural behaviour	3[a]	Duration[b]	Much time is spent pecking ground under semi-natural conditions.	· Hens in a zoo spent 60% of time pecking the ground (Dawkins 1989). · The proportion of hens grazing and pecking ground was 38.6% in a free-range environment (Shimmura et al. 2008).
	2	Duration	Dust-bathing on ground is observed	· 2.4% of hens perform dust-bathing in a free-range environment (Shimmura et al. 2008).
Preferences	3[a]	Duration[b]	Much time is spent pecking litter material.	· The proportion of hens foraging litter was 25% in the litter area of an aviary (Appleby et al. 1989). · The proportion of hens pecking litter was 17.1% in an aviary (Shimmura et al. 2008).
	2	Duration	A set amount of time is spent on dust-bathing.	· The duration of dust-bathing was 30 min after one week deprivation of sand (Liere 1992). · The duration of dust-bathing was 24 min after nine days deprivation of sand (Lundberg & Keeling 2003). · Dust-bathing was performed every two days (Hogan & van Boxel 1993). · The proportion of hens performing dust-bathing was 3.6% in an aviary (Shimmura et al. 2008).
	2	Duration	Litter floor is preferred to wire floor.	· In a choice test, the time spent on a litter floor was much longer than on a wire mesh floor (Dawkins 1981). · In a choice test, hens preferred enclosures with litter substrate than with a wire mesh floor (Hughes 1976). · In a choice test, hens preferred a small cage with litter to a large cage with a wire mesh floor (Dawkins 1981). · In a choice test, hens preferred enclosures with litter than without litter (Dawkins & Beardsley 1986).
	2	Rhythm[b]	Dust-bathing is observed frequently in afternoon.	· The peak of dust-bathing occurred in early afternoon (Vestergaard 1982). · Dust-bathing was observed more frequently in afternoon than in morning in an aviary (Tanaka & Hurnik 1992; Shimmura et al. 2006).
	1	Function[b]	Dust-bathing removes excess fat and improves feather condition.	· Dust-bathing decreased fat on feathers and made them fluffier (Van Liere & Bokma 1987).
Demand	3[a]	Motivation[b]	Laying hens have high motivation to dust-bathe.	· Hens were willing to work to gain access to a dusty substrate (Widoski & Duncan 2000). · Laying hens deprived of litter tried to reach hens performing dust-bathing, even if blocked (Olsson et al. 2002).
	3	Vaccuum[b]	Laying hens without litter materials performed sham dust-bathing.	· Laying hens without litter materials performed sham dust-bathing (Vestergaard, 1981, 1991; Vestergaard et al. 1990, 1993; Lindberg & Nicol 1997; Shimmura et al. 2007). · Sham dust-bathing did not reduce motivation to dust-bathe in litter (Vestergaard et al. 1997; van Liere & Wiepkema 1992; Olsson et al. 2002).
	2	Motivation	Preference to litter material is high, though its priority is not so.	· In an operant test, all hens work to litter material (Gunnarsso et al. 1993). · In an operant test, hens found litter reinforcing (Dawkins & Beardsley 1986). · In an operant test, hens preference for litter was not high (Lagadic & Faure 1987; Faure & Lagadic 1994; Petherick et al. 1993).

[a]The maximum weighting scores per weighting category. [b]Unique types per weighting category.

6) Assignment of measurements to the five freedoms. To clarify the advantages and disadvantages of housing systems, each measurement was assigned to one of the five freedoms (Table 3-1). The five freedoms is standard concept of welfare accepted widely (FAWC 1992), and in Japanese welfare assessment, it was decided to evaluate from the viewpoint of five freedoms in any farm animal species. The assignment to the five freedoms also assures that the measurements are related to animal welfare.

3.2.3. Evaluation of welfare assessment

To evaluate the usefulness of this science-based assessment, we compared it with the environment-based Animal Needs Index (ANI) and animal-based assessment. There were four replicates of each housing system, and the score of a system was the average of the four replicates. The other details are:

Comparison with Animal Needs Index (ANI). The six housing systems described above were scored by using ANI35-L/2001 – laying hens (Bartussek, 2001). This assessment consisted of five categories: 1) locomotion, 2) social interaction, 3) flooring, 4) light, air, and noise, and 5) stockmanship. To measure "condition of skin" in the sheet on stockmanship, two parts of the body (back and vent), which are important for evaluation of severe feather pecking and/or risk for cannibalism (Savory, 1995), were scored using the scoring method of Bilčik and Keeling (1999), which is same with the evaluation of the measurement #1 "feather condition" in this model. The measurement of "bird health" on the sheet of stockmanship was based on mortality. The evaluations were conducted at the beginning of December, 2006 (winter). The six sets of data evaluating the six housing types by ANI were compared with the data obtained by this model.

Correlation with animal-based assessment. During construction of this model and its evaluation of the six housing systems, we assessed these housing systems using animal-based measurement (Chapter 2). This animal-based assessment was a multi-factorial investigation measuring ethology, physiology, anatomy, physical condition, and production, and scoring and evaluating from the viewpoint of the five freedoms was conducted using it. To investigate the correlation with this animal-based evaluation, the measurements of this model and ANI were first distributed to the five freedoms, respectively. The six housing systems were then scored for each freedom in each assessment. Finally, correlations between the scores and this model or between ANI and the scores from animal-based measurements were analyzed for each freedom by using Spearman's correlation coefficients by rank test, and these values were compared. The statistical software 'R' (version 2.4.1; R Development Core Team, 2006) was used for the analyses. The freedoms from hunger and thirst and from discomfort were excluded from the analyses because the values of the six housing systems by animal-based assessment were the same in both freedoms (see Chapter 2).

3.3. Results

3.3.1. Construction of model

The values for evaluation of the six housing systems by using this model are shown in Figure 3-1. The freedom from pain, injury, and disease was better secured in the conventional cages. For the freedom from hunger and thirst, the score was same among six housing systems. For the freedom from discomfort, the score was similar among the six housing system, although LC and FR tended to have higher scores and SC a lower score. The freedom to express normal behaviour scored better in the non-cage systems than the cage systems, and among the cage systems, the score was higher in SF than in LF, and in LC than in SC. This tendency was similar in the freedom from fear and distress and the total score.

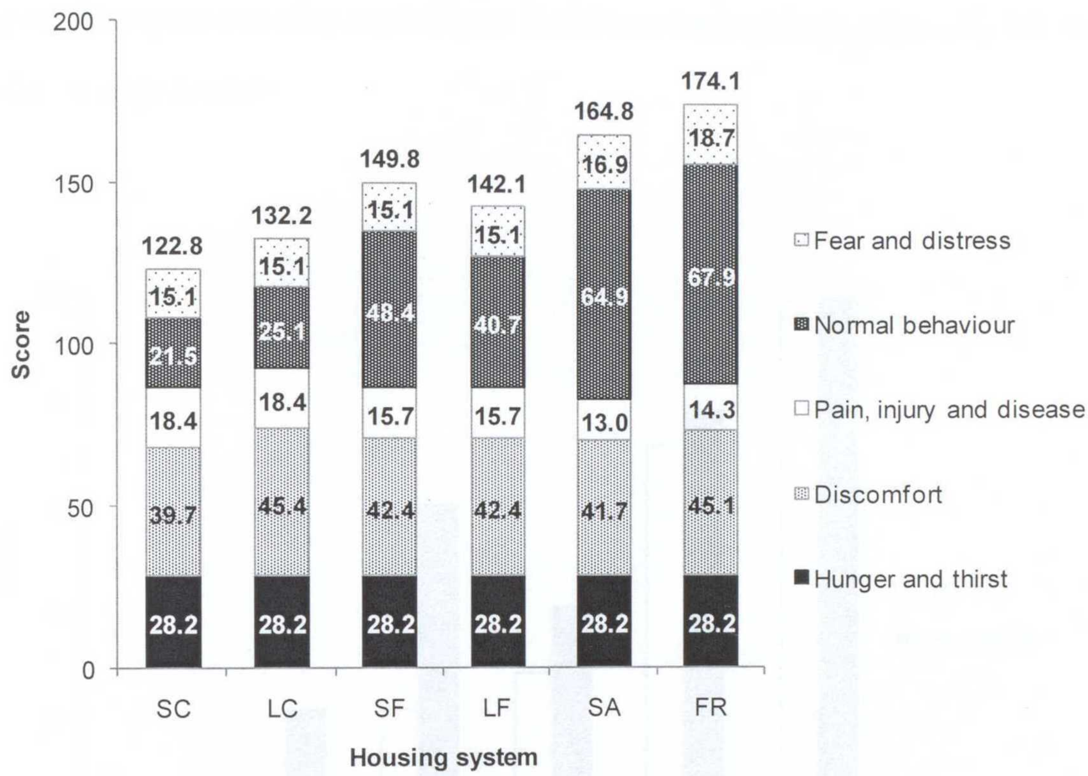

Figure 3-1. Comparison of assessed scores of six housing systems from the viewpoint of the five freedoms by this model. SC: small conventional cage; LC: large conventional cage; SF: small furnished cage; LF: large furnished cage; SA: single-tiered aviary; FR: free-range.

3.3.2. Evaluation of model

Comparison with Animal Needs Index (ANI). The scores evaluated with ANI were: SC, 3.5; LC, 3.5; SF, 8.0; LF, 9.5; SA, 18.5; FR, 24.5. The scores for SC and FR calculated with this model and ANI were respectively lowest and highest in both assessments. For comparison of these assessments, data transformation on a scale from 0 to 10 was conducted with SC receiving a score of 0 and FR a score of 10 (Figure 3-2). In this model, conventional cages ranked highly compared with ANI, and the LC score was higher than SC, and SF higher than LF. On the other hand, in ANI, the scores of the conventional cages were the same regardless of whether they were small or large, and the LF score was higher than the SF score. The score was also much higher in the non-cage systems, especially FR, compared with the cage systems.

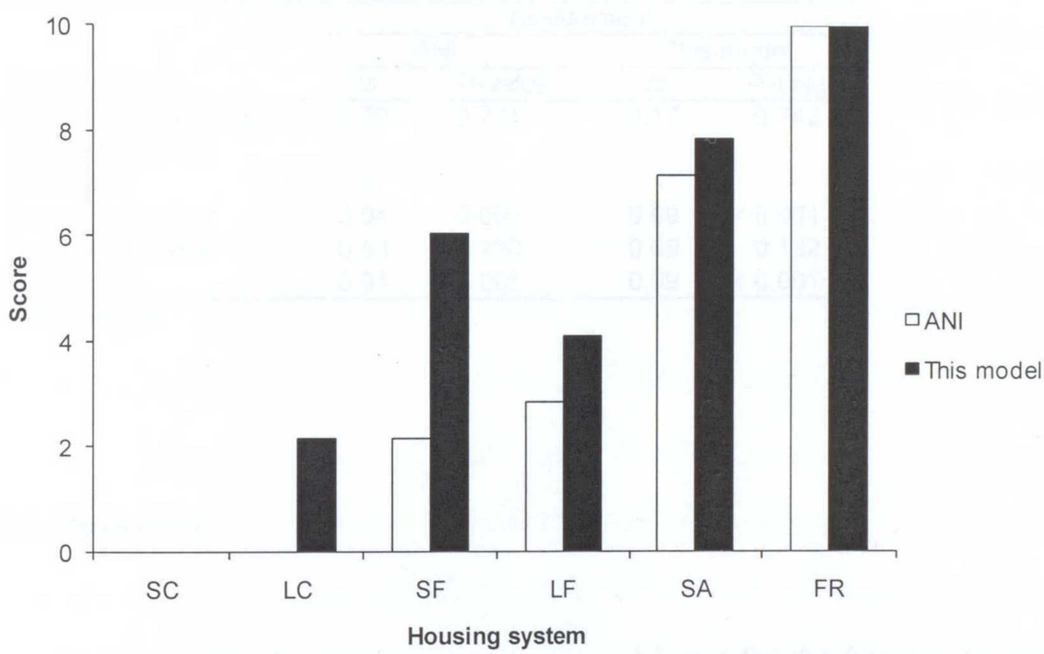

Figure 2. Comparison of assessed scores of six housing systems by ANI and this model. The score shows the values after data transformation on a scale from 0 to 10 with SC receiving a score of 0 and FR a score of 10. SC: small conventional cage; LC: large conventional cage; SF: small furnished cage; LF: large furnished cage; SA: single-tiered aviary; FR: free-range.

Correlation with animal-based assessment. The correlations between the scores from this model or ANI and the scores from animal-based measurements are shown in Table 3-3. For the freedom from pain, injury, and disease, no significant correlation with animal-based assessment was found for both assessments. On the other hand, a significant strong-positive correlation was found in both this model and ANI for the freedom to express normal behaviour (this model: $rs = 0.99$, $P < 0.001$; ANI: $rs = 0.94$, $P < 0.01$) and the total score (this model: $rs = 0.99$, $P < 0.001$; ANI: $rs = 0.94$, $P < 0.01$). The freedom from fear and distress score on this model also inclined towards a tendency of beign correlated with that of animal-based assessment (this model: $rs = 0.69$, $P = 0.132$; ANI: $rs = 0.53$, $P = 0.280$).

Table 3-3. Correlation between ANI or our model and animal-based assessment for each freedom.

	Assessment			
	ANI		This model	
Five freedoms	*rs*	*P*-value	*rs*	*P*-value
Pain, injury, and disease	-0.20	0.704	-0.17	0.742
Hunger and thirst	-	-	-	-
Discomfort	-	-	-	-
Normal behaviour	0.94	0.005	0.99	< 0.001
Fear and distress	0.53	0.280	0.69	0.132
Total score	0.94	0.005	0.99	< 0.001

-: no analysis.

3.4. Discussion

In this model, the non-cage systems scored better for the freedom to express normal behaviour and the freedom from fear and distress, while the freedom from injury, pain, and disease was better secured in the cage systems. The risk of injury due to feather pecking is generally low in conventional cages because the group size is small (e.g., Tauson, 2005; Blokhuis et al., 2007). The risk increases with increments in group size (Hughes and Wood-Gush, 1977; Hughes et al., 1997), which is similar with the result that the freedom from injury, pain, and disease was not secured in the systems with larger group size, such as SA and FR. FR tended to have a slightly higher score than SA, but this is due to the outdoor access in FR. It has been reported that the risk for feather pecking is lower when an outdoor grazing area is provided because the motivation to peck would be directed to grass or because the distances between individual hens would be greater (Mohboub et al., 2004; Shimmura et al., 2008c). On the other hand, a cage has the disadvantage of behavioural restriction. As expected, the score for freedom to express normal behaviour was higher in the non-cage systems, especially FR, than in the cage systems. Among the cages, LC scored better than SC and LF worse than SF. The behavioural restriction is a somewhat reduced even in conventional cages when the density is lower or total floor area is larger (Freeman, 1983; Hurnik and Lewis, 1991; Dawkins and Hardie, 1989). In furnished cages, the qualities of the resources are more important than the cage floor area. Limited resources lead to competition, as well as behavioural restriction, which results in increased aggressive interactions and mortality (Shimmura et al., 2007a, 2008a, 2009c). As mentioned above, these scores as a whole were supported by previous studies.

For the freedom from hunger and thirst and the freedom from discomfort, the scores for the six housing systems were similar. The ad libitum access to water and feed is common for laying hens (Appleby et al., 2004), and therefore, no difference in scores would be expected for the freedom from hunger and thirst. Few differences were found in the freedom from discomfort, though many measurements were included, because, firstly, similar scores were obtained for several measures. Because all housing systems evaluated in this study were built in the same location, there was no difference in some factors such as "exposure to cold". Secondly, advantages and disadvantages were compensatory. For example, the non-cage systems scored better on "movement comfort", but worse for "separation from manure". Taking this into consideration, the small difference in scores might also be an appropriate result in the freedom from discomfort.

A significant strong-positive correlation was found for the total scores of animal-based assessment, this science-based model, and ANI, although small sample size might result in such strong correlation. This result indicates that the welfare of housing systems can be assessed by both models, which also agrees with previous studies of ANI (Bokkers and Koene, 2001; Alban et al., 2001; Mollenhorst et al., 2005) and Bracke's science-based model (Bracke et al., 2002a). The correlation between ANI and animal-based measurements has been reported for both laying hens (Mollenhorst et al., 2005) and other farm animals (Alban et al., 2001; Bokkers and Koene, 2001). Comparing this model with ANI in detail, LC scored better than SC and SF better than LF in this model, while LC had the same score as SC and SF was worse than LF in ANI (Fig 2). As mentioned above, the welfare level is higher in LC than in SC, with its smaller floor space, and higher in SF than in LF, with its insufficient resources per hen. This result suggests that this science-based model more sensitively evaluates the welfare level than ANI. This is because ANI targets the assessment of non-cage systems and excludes cage systems from the evaluation (Bartussek, 1999; Kohari et al., 2006; Seo et al., 2007). In fact, ANI includes many assessments of the outdoor area or pasturage, which may result in a targeted evaluation of non-cage systems, especially free-range systems. However, there is no denying that ANI overemphasizes pasturage (Kohari et al., 2006; Seo et al., 2007) and insufficiently evaluates cage systems, including conventional and furnished cages. These limitations would suggest that ANI is inadequate as an international prototype that is applicable in Japan, where cage systems constitute the majority, though its application could be appropriate in the EU, where conventional cages will be banned. Therefore, this science-based assessment is useful and sensitive in evaluating housing systems, including cage systems.

As mentioned in the section on Materials and Methods, the six housing systems were assessed in the same location, which is not sufficient for a full for evaluation of this science-based assessment. Further studies at various farms are needed. There is also room for improvement in the set of measurements and its methods on the basis of more expert opinions (Bracke et al., 1999a, 1999b, 2002b). However, this is the first time that a semantic model for overall welfare assessment of farm animal housing systems has been empirically validated, i.e. compared with animal-based measurements.

I constructed a science-based system for assessment of the welfare of laying hens applying Bracke's model that included measurements and weightings based on scientific information, and clarified the advantages and disadvantages of six housing systems from the viewpoint of the five freedoms. The science-based assessment also has the advantage of facilitating maintenance and expansion. This science-based model seemed to evaluate the welfare level more sensitively than ANI, although these systems can be assessed by both this model and ANI.

3.5. Summary

To increase the validity of evaluations and facilitate expansion and maintenance of assessment systems, we started a database of studies on the welfare of laying hens around the world. On the basis of it, we devised a science-based welfare assessment. This model includes the measurements, level and weighting based on the scientific studies and can be clarified the advantages and disadvantages of the housing systems from the view point of the five freedoms. We also evaluated the usefulness of this model by comparing it with environment-based Animal Needs Index (ANI) and animal-based measurements. This model showed that freedom from injury, pain, and disease and from discomfort was more secured in the cage system, while non-cage systems scored better for natural behavior and freedom from fear and distress. A significant strong-positive correlation was found between the animal-based assessment and the total scores of ANI ($rs = 0.94$, $P < 0.01$) or this model ($rs = 0.99$, $P < 0.001$), which indicate that housing systems can be evaluated by both assessments. However, assessment using this model was more sensitive than ANI and can be applied to cage systems, which suggest that this model have higher usefulness.

CHAPTER 4

Relation between social order and use of resources in new modified cage

4.1. Introduction

The results of both Chapters 2 and 3 indicated the high potential value of furnished cages. However, in large furnished cages, competition for a restricted number of resources was frequently observed due to increased group size, while mobility and comfort behaviour are enhanced by providing a larger total cage area. Based on these results and my previous studies, a medium-sized furnished cage with resources on both sides of the cage ('separated' resources) was designed. In Chapter 4, relation between social order and use of resources in this modified cage is reported.

Conventional cages for laying hens will be banned in the European Union (EU) in 2012. It is in the EU that most development of alternative housing systems for laying hens has occurred. These alternatives comprise furnished cages and non-cage systems such as deep litter, aviaries and free-range. Furnished cages contain a perch, nest box and littered area, and provide more height and area per hen than conventional cages, and will be the only legal form of cage in the EU from 2012 (Blokhuis, 2004). Furnished cages provide most of the economic advantages of conventional cages while removing many behavioural restrictions (Appleby et al., 2002). Today, about 40% of layers in Sweden are kept in furnished cages (Tauson, 2005). Other countries in the EU where this system exists are the United Kingdom, Norway, Germany and Denmark. Attention is also being given to furnished cages in Asia including Japan, because they can increase welfare while maintaining good performance. Some farmers use furnished cages in Japan.

An early model of furnished cages, the Edinburgh Modified Cage, was for groups of four birds (Appleby and Hughes, 1995). Small group size has the benefit of a low incidence of aggressive interactions, but if group size is increased this reduces egg production cost per hen. Therefore, more recently the size of furnished cages has increased, e.g. 16 hens (Wall et al., 2004) and 40 hens per cage (Weitzenburger et al., 2005). Large furnished cages would benefit the birds by providing a larger total cage area, leading to more exercise and probably, in turn, improved bone strength. However, increased group size means that more hens share a restricted number of resources such as the nest box and litter area, which results in increased competition for these resources.

The occurrence of competition for a concentrated resource is one of the disadvantages of the newly-developed furnished cages. Some researchers have demonstrated that competition for a dust bath occurs in furnished cages and even in non-caged systems (Van Rooijen, 1999; Shinmura et al., 2006a,b). Van Rooijen (1999) investigated dust-bathing of all 42 hens in a furnished cage and reported that 18.7% of the 75 dust-bathing was disturbed by aggression, resulting in a short duration of dust-bathing compared to durations reported in a number of previous studies. In later research, it was found that dominant hens had priority using the dust bath (Shimmura et al., 2007c). In large furnished cages with a small allowance of dust bath per hen (58.3 cm^2 per hen), higher-ranked hens used the dust bath and performed dust-bathing more than lower-ranked hens. These results indicated that competition for a small dust bath would occur. Competition was observed even in furnished cages with a large dust bath area (232.5 cm^2 per hen; Shimmura et al., 2008a). Therefore, in large furnished cages, only a small number of hens (those that are high-ranking) may have priority using resources such as the dust bath, even if those resources seem to be used fully by many hens. It would be difficult to conclude, in these conditions, that furnished cages have an unequivocal advantage in removing behavioural restrictions.

From these previous studies, it seemed that the problem was that a resource was placed on one side of the cage ('localised'). Therefore, a medium-sized furnished cage with dust baths and nest boxes on both sides of cage ('separated', MFS) was designed. The aim of this design was to reduce competition for the dust bath and to increase the use of the dust bath by lower-ranked hens. To evaluate this new cage design, we compared the behaviour of high-, medium- and low-ranked hens in MFS cage with that of hens in small (SF) and medium furnished cages (MFL) with a 'localised' dust bath and nest box on one side of the cage.

4.2. Materials and Methods

4.2.1. Animals and housing

In total, 150 White Leghorn layers were used. All birds had their beaks trimmed at 1-day-old and were raised in conventional cages. At the age of 17 weeks, the birds were randomly divided into three groups and moved to furnished cages in a laying house. One group was housed in six small furnished cages (5 hens per cage) and the others in two types of medium furnished cages with the dust bath and nest box on both sides of the cage (separated; six cages and 10 hens per cage) and the dust bath and nest box on one side of the cage (localised; six cages and 10 hens per cage).The total dust bath and nest box areas per hen were same for the three cage designs.

The house was ventilated with six ceiling fans. Average daytime temperature (±S.D.) during the observation period was 21.4 ± 4.5 ºC at the centre of the house. Lighting was provided by two fluorescent lights (37 W), adjusted to give an intensity of 10 lux at the food troughs. The illumination cycle was 14 h of light and 10 h of darkness, with the light period from 05:00 to 19:00 h. The hens had ad libitum access to water and feed. The feed contained at least 16% crude protein and 2900 kcal metabolic energy per kg. Feeding and any other routine work, such as supplying wood-shavings to the dust bath, was done between 08:00 and 09:00 h and eggs collected between 16:00 and 17:00 h.

4.2.2. Furnished cage

The designs, locations and space per hen of each resource were same for SF, MFL and MFS. Designs and equipment of the three furnished cages fulfilled the regulations in the EU (Blokhuis, 2004). Small furnished cages (SF) were made by using laying cages that were 65 cm wide, 46.5 cm deep and 47 cm high at the rear. In accordance with Appleby and Hughes (1995), each cage was equipped with a nest, a dust bath and a perch. The main cage area was 604.5 cm^2 per hen with a floor of 2.5 cm × 5.0 cm wire mesh. The nest box was added to one side of the cage and was 25 cm wide, 46.5 cm deep and 21 cm high at the rear. The nest area was 232.5 cm^2 per hen, so that total space allowance (excluding the dust bath) was 837.0 cm^2 per hen. The nest was enclosed and constructed from wooden board, with a floor lined with artificial turf. There was an 8 cm space under the front so that eggs would roll out, and an entrance 13 cm wide × 23 cm high (with a threshold 1.8 cm high so that eggs would not roll out of the side of the nest) which hens readily stepped through. Above the nest was a dust bath 4.5 cm deep, which was supplied with wood-shavings. All wood-shavings were removed and replaced with fresh shavings in the morning. A wooden perch (4 cm deep and 3 cm high with a chamfered top edge) was fitted across the width of the cage with its centre 10 cm from the cage floor and 18 cm from the rear of the cage. AV-shaped feeder was located in front of the cage, and a U-shaped drinker was rear. Perch, feeder and drinker space per hen were 13.0 cm.

The medium furnished cage with a localised resource (MFL) was the cage that was used for SF but doubled. The cage was 130 cm wide, 46.5 cm deep and 47 cm high at the rear. The nest box was added to one side of the cage, and was 50 cm wide, 46.5 cm deep and 21 cm high at the rear. Above the nest was a dust bath 4.5 cm deep. Awooden perch was fitted across the width of the cage. The feeder was located in front of the cage, and the drinker was at the rear.

The medium furnished cage with separated resources (MFS) was the cage design used for MFL but with the dust bath and nest box separated to both sides of the cage. Each nest box was 25 cm wide, 46.5 cm deep and 21 cm high at the rear. Above the nests were dust baths 4.5 cm deep. A wooden perch was fitted across the width of the cage. The feeder was located in front of the cage, and the drinker was at the rear.

4.2.3. Measurements

For focal sampling, all 150 birds were individually marked, using a combination of coloured leg-rings, at 17 weeks of age.

Dominance hierarchy. Observations of aggression were conducted for a total of 10 days when the hens were between 23 and 25 weeks of age. Aggressive interactions were counted in all cages, for a period of 10 min per cage in SF and of 20 min per cage in MFL and MFS. The observations were repeated twice a day, morning (10:00–12:30 h), and afternoon (13:00–15:30 h). The total observation time was therefore 100 min per cage in SF (10 min/day × 10 days) and 200 min per cage in MFL and MFS (20 min/day × 10 days). The aggressive behaviours recorded were aggressive pecking, displacing, chasing and threatening, with both winner and loser noted (Appleby et al., 2004). Aggressive pecking was to the head of recipient, and excluded both severe feather pecking (forceful pecks, sometimes with feathers being pulled out and with the recipient bird moving away) and gentle feather pecking (careful pecks, not resulting in feathers being pulled out and usually without reaction from the recipient bird). Threatening was defined as the pecker attempting to peck the recipient but the recipient moved away before it was pecked. From the data of aggressive interactions observed, the dominance index of individual hens was calculated by using the index of Clutton-Brock (ICB; Clutton-Brock et al., 1979, 1986) expressed as the following formula:

$$\text{Dominance index} = (B + \Sigma b + 1) / (L + \Sigma l + 1)$$

where B = number of hens that an individual beat; □b = total number that those hens beat excluding the subject; L = number of hens that the individual lost to; □l = total number that those hens lost to excluding the subject. Winners and losers were determined from all aggressive interactions, with the hen giving the pecks being the winner if the other hen showed escape behaviour. When a hen pecked at another but it did not escape, the interaction was excluded from the record. All hens were compared against each other and ranked according to the numbers of wins and losses (those with most wins being classed as dominant). This index takes into account the success of opponents, so that the score of an individual is determined by the score of the individuals it dominated and of those dominating the individual. The formula is especially effective in the case of a linear and fixed hierarchy such as for domestic hens (Boyd and Silk, 1983) and is useful when it is difficult to observe most of the interactions between two individuals because of its large group size (Mateos and Carranza, 1999). The linearity in each cage was also calculated, using Landau's index of linearity (Lehner, 1996). Normalised index values (h) range from 0 (nonlinear) to 1 (perfectly linear), and $h \geq 0.9$ would be a reasonable (although arbitrary) cutoff criterion for 'strong', nearly linear hierarchies.

The highest, middle and lowest ranked hen was determined in each cage using the dominance index. In MFL and MFS, rank 1 was the highest-ranked hen, rank 5 or 6 was the middle-ranked hen, and rank 10 was lowest-ranked hen. The hen that had a dominance index closest to the average of the 10 hens in a cage was chosen as the middle-ranked hen. In SF, hens ranked 1, 3 and 5 were used. The observations of aggressive interactions were also conducted for 3 days when hens were 32 weeks of age, in order to determine whether the dominance hierarchies remained the same as before.

Behavioural observation. Observations were conducted at 25, 30, 33 and 37 weeks of age (3 days/week). Direct visual scans at10 min intervals were conducted to record the location and behaviour of all birds in all cages for 6 h/day, 2 h in each of the early morning (06:00–08:00 h), the late morning (10:00–12:00 h) and the afternoon (13:00–15:00 h). The location was scored as either the nest, dust bath, perch, cage floor (front, rear) or feeder. The location ''feeder'' was recorded when a hen had her head in the feeder whether her head through the front bars or not. For behaviour, the following activities were recorded: eating, drinking, resting, dust-bathing, exploring (litter pecking, litter scratching, gentle feather pecking (mate pecking), object pecking), aggression (aggressive pecking, escaping), severe feather pecking, sham dust-bathing, moving, and pre-laying sitting (Appleby et al., 2004). Eating or drinking was recorded when a hen had her head in the feeder or drinker. Resting was defined as when the hen lowered and ''tucked-in'' her head, or closed her eyes and was still. Dust-bathing was recorded when one element of three (vertical wing-shaking, head-rubbing, scratching with one leg) was observed. Pre-laying sitting was recorded when a hen was sitting in the nest box. All data were collected by the same observer.

Physical condition. Body weight, feather damage and claw length were recorded when hens were 25 and 37 weeks of age, before and after the behavioural observation. In accordance with Bilčik and Keeling (1999), feather damage was scored from 0 (no damage) to 5 (denuded) for eight parts of the body (head, neck, breast, back, leg, belly, wing, tail), giving a total score from 0 to 40. Slightly different criteria were used for scoring flight feathers than for the rest of the plumage, because of the different types of feathers and damage. The assessment of feather damage was carried out by 2–4 people working together to ensure maximum consistency when scoring. The centre front and rear claws of the right foot were measured with a digital vernier calliper, recording the straight length from the claw root to the tip.

4.2.4. Statistical analyses

The proportions of time spent by each individual hen at each location and in each behaviour were calculated. The value of physical condition was calculated by the score at 37 weeks minus the score at 25 weeks. Rank categories (high, middle and low) are hereafter referred to as ''ranks''. There were six replicate cages of each design, giving six replications for each rank in SF, MFL and MFS. As the data of each rank in a cage were linked, repeated measure ANOVA was used to evaluate the effects of the social rank (high, medium, low), cage design (SF, MFL, MFS) and interactions between them on the use of facilities, behaviour and physical condition. Each measurement therefore involved 54 data units in the analysis (three social ranks × three cage designs × six replications). A parametric test was used, as the normality of the distributions was confirmed in all data using the statistic software Statcel (Yanagii, 2007). Significances of individual effects were evaluated by a multiple comparison using the Tukey test. When significant interactions between social order and cage design were found, the dual data were unified and then compared using one-way ANOVA followed by the Tukey test.

4.3. Results

4.3.1. Dominance hierarchy

The average total number (±S.D.) of aggressive interactions observed per cage was 38.8 ± 21.7 in SF, 120.2 ± 52.1 in MFL and 87.5 ± 27.8 in MFS.

The mean (±S.D.) index values of linearity (h) were 0.87 ± 0.16 in SF, 0.90 ± 0.13 in MFL and 0.97 ± 0.08 in MFS, confirming that hierarchies within cages were nearly linear.

Mean (±S.D.) dominance index of high-, middle- and low-ranked hens were 7.8 ± 2.4, 1.0 ± 0.5 and 0.2 ± 0.1 in SF, 21.9 ± 8.8, 1.3 ± 0.8 and 0.1 ± 0.0 in MFL and 20.9 ± 10.2, 0.8 ± 0.3 and 0.1 ± 0.0 in MFS, respectively. As expected, there was significant variation in dominance index among rank categories (Friedman's test with replication: $P < 0.001$) and significant differences were found between each category (Steel–Dwass' multiple comparison test: all $P < 0.01$).

4.3.2. Use of facilities

The proportions of time spent by hens in each facility are shown in Table 4-1. No significant difference was found in use of the feeder and perch. A significant effect of social order ($P < 0.001$) and cage design ($P < 0.05$) on the use of the rear of the cage floor was found. The proportion of time spent at the rear of the cage was lower in high- than medium-ranked hens, and lower in medium- than in low-ranked hens (both $P < 0.05$). A significant interaction between social order and cage design was found in the proportion of time spent in the dust bath ($P < 0.001$), and there was a tendency for the proportion to be higher in the high-ranked SF (23.9%) and MFL (18.9%) hens than in the high-ranked MFS hens (12.1%). Additionally, in SF and MFL, the proportion was higher in the high-ranked hens (23.9%, SF; 18.9%, MFL) than the low-ranked hens (5.6%, SF; 4.7%, MFL). Conversely, the MFS low-ranked hens (15.4%) tended to use the dust bath more frequently than the SF and MFL low-ranked hens. There was a significant interaction between social order and cage design in the proportion of time spent in the nest box ($P < 0.01$), and the proportion was higher in the low-ranked MFS and MFL hens than in the high-ranked hens, though not statistically significant. In contrast, no difference was found in the SF hens.

The mean proportions (±S.D.) of eggs laid in the nest were higher in MFL (98.3 ± 0.6%) than MFS (93.5 ± 3.9%, $P < 0.05$). MFS hens laid eggs in the dust bath (0.9 ± 1.2%, $P = 0.20$) and on the cage floor (5.6 ± 2.8%, $P < 0.01$), more than MFL. For MFS eggs, 74.1 ± 13.9% were laid in one of the two nest boxes ($\chi^2 = 50.8$, $P < 0.001$).

Table 4-1. Mean proportion ± standard deviation of time spent by high-, medium- and low-ranked hens in each location in a small and two medium furnished cages.

Location	Cage design†	Social order			Repeated-Measure ANOVA F‡		
		High	Medium	Low	Cage design	Social order (S)	C×S
Feeder	SF	29.5 ± 7.1	39.7 ± 10.3	33.8 ± 7.1			
	MFL	36.8 ± 6.8	31.9 ± 6.2	31.4 ± 9.3	0.1	0.7	2.0
	MFS	39.2 ± 5.8	33.1 ± 11.0	31.7 ± 7.6			
Cage floor							
Front	SF	6.5 ± 2.0	13.2 ± 6.9	14.2 ± 5.2			
	MFL	10.8 ± 3.6	7.2 ± 2.7	7.2 ± 5.1	2.4	1.3	5.5 **
	MFS	12.6 ± 2.5	15.0 ± 6.3	7.7 ± 3.0			
Rear	SF	1.6 ± 1.6	2.9 ± 2.6	3.6 ± 1.9			
	MFL	1.8 ± 1.6	3.5 ± 1.7	5.3 ± 3.1	4.0 *	12.2 ***	1.1
	MFS	2.4 ± 1.7	5.2 ± 1.7	8.3 ± 4.6			
Perch	SF	29.7 ± 8.1	32.3 ± 6.0	35.2 ± 12.0			
	MFL	24.0 ± 9.1	29.4 ± 6.3	34.0 ± 8.6	2.8	1.9	0.9
	MFS	24.5 ± 5.4	29.9 ± 10.3	23.9 ± 6.7			
Dust bath	SF	23.9 ± 5.4	7.6 ± 5.7	5.6 ± 4.4			
	MFL	18.9 ± 7.0	17.0 ± 4.0	4.7 ± 2.9	0.3	15.1 ***	9.4 ***
	MFS	12.1 ± 3.6	10.5 ± 4.4	15.4 ± 6.8			
Nest box	SF	8.9 ± 4.2	4.4 ± 3.1	7.7 ± 1.9			
	MFL	7.6 ± 3.2	11.0 ± 4.5	17.5 ± 8.0	4.5 *	13.8 ***	4.7 **
	MFS	9.1 ± 2.9	6.3 ± 2.8	13.1 ± 0.9			

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. †SF: small furnished cage with localised dust bath and nest box; MFL: medium furnished cage with localised dust bath and nest box; MFS: medium furnished cage with separated dust bath and nest box. ‡df of the effect of cage design (C) was 2, social order (S) was 2 and S×C was 4 in each location. n was 54 in each location.

4.3.3. Behaviour

The proportions of time spent by hens in each behaviour are shown in Table 4-2. A significant interaction between social order and cage design was found in the proportion of time spent dust-bathing ($P < 0.001$), as well as the use of the dust bath. The MFS low-ranked hens tended to spend more time dust-bathing (2.2%) than the SF (1.0%) and MFL (0.5%) low-ranked hens. No significant difference was found in sham dust-bathing. Significant effects of cage design ($P < 0.05$) and social order ($P < 0.001$) on litter pecking were found. More time was spent litter pecking in MFL than SF ($P < 0.05$), and was also observed more frequently as social rank increased (all $P < 0.05$). There was a significant effect of social order on litter scratching ($P < 0.001$), with more time spent by high-ranked hens compared to the medium- ($P < 0.05$) and low-ranking hens ($P < 0.01$).

Table 4-2. Mean proportion ± standard deviation of time spent by high-, medium- and low-ranked hens in performing each behaviour in a small and two medium furnished cages.

Behaviour	Cage design†	Social order: High	Social order: Medium	Social order: Low	Repeated-Measure ANOVA F‡: Cage design	Repeated-Measure ANOVA F‡: Social order (S)	Repeated-Measure ANOVA F‡: C×S
Comfort							
Dust bathing	SF	5.6 ± 3.0	0.8 ± 0.9	1.0 ± 0.8			
	MFL	5.0 ± 1.8	2.8 ± 1.1	0.5 ± 0.4	0.9	24.7 ***	5.1 **
	MFS	2.7 ± 1.7	1.0 ± 0.8	2.2 ± 1.9			
Exploring							
Litter pecking	SF	5.0 ± 1.4	2.0 ± 1.1	0.6 ± 0.5			
	MFL	8.6 ± 4.7	5.3 ± 3.0	1.7 ± 1.1	6.2 *	23.6 ***	1.2
	MFS	5.2 ± 1.8	3.5 ± 1.6	1.8 ± 1.2			
Litter scratching	SF	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0			
	MFL	0.2 ± 0.4	0.1 ± 0.1	0.0 ± 0.0	1.3	6.1 **	0.9
	MFS	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0			
Gentle feather pecking	SF	0.5 ± 0.5	0.1 ± 0.1	0.8 ± 1.0			
(Mate pecking)	MFL	0.0 ± 0.0	0.6 ± 0.9	1.2 ± 2.7	0.1	1.2	1.7
	MFS	0.2 ± 0.4	0.9 ± 1.0	0.1 ± 0.3			
Aggression							
Aggressive pecking	SF	1.9 ± 0.7	0.4 ± 0.2	0.0 ± 0.0			
	MFL	4.4 ± 2.3	0.7 ± 1.3	0.0 ± 0.0	5.8 *	43.6 ***	2.8 *
	MFS	4.7 ± 2.2	0.5 ± 0.5	0.0 ± 0.0			
Escaping	SF	0.0 ± 0.0	1.2 ± 0.6	2.2 ± 0.6			
	MFL	0.0 ± 0.0	3.0 ± 2.8	8.2 ± 3.5	6.2 *	56.6 ***	6.4 ***
	MFS	0.0 ± 0.0	1.9 ± 1.3	5.2 ± 2.2			
Severe feather pecking	SF	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0			
	MFL	1.2 ± 2.7	0.5 ± 0.9	0.0 ± 0.0	1.4	2.1	0.4
	MFS	0.9 ± 1.3	0.4 ± 0.5	0.0 ± 0.0			
Sham dust-bathing	SF	0.0 ± 0.0	0.1 ± 0.1	0.8 ± 1.3			
	MFL	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.6	0.7	2.3	1.2
	MFS	0.1 ± 0.1	0.3 ± 0.7	0.2 ± 0.3			
Moving	SF	1.9 ± 0.5	2.6 ± 1.0	4.4 ± 1.4			
	MFL	2.0 ± 1.1	6.2 ± 1.0	5.2 ± 0.8	16.7 ***	17.6 ***	1.8
	MFS	3.4 ± 1.6	6.6 ± 1.9	7.3 ± 3.5			
Pre-laying	SF	8.7 ± 4.1	3.8 ± 2.6	4.7 ± 1.1			
	MFL	7.3 ± 3.5	7.3 ± 4.1	5.8 ± 1.7	0.4	8.7 **	1.5
	MFS	9.0 ± 2.9	4.8 ± 2.3	4.4 ± 3.1			

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. †SF: small furnished cage with localised dust bath and nest box; MFL: medium furnished cage with localised dust bath and nest box; MFS: medium furnished cage with separated dust bath and nest box. ‡df of the effect of cage design (C) was 2, social order (S) was 2 and S×C was 4 in each location. n was 54 in each location.

A significant interaction between social order and cage design was found for aggressive pecking ($P < 0.05$). The proportion of time spent on aggressive pecking increased with social rank (all $P < 0.05$), and was higher in MFL and MFS compared with SF (both $P < 0.05$). While only 4.0% (3/75) of aggressive pecking by MFS high-ranked hens was observed in the dust bath, there was 27.5% (22/80) in MFL high-ranked hens ($P < 0.05$). Escaping also showed a significant interaction ($P < 0.001$). The proportion of time spent escaping was greater as social rank decreased (all $P < 0.01$), and was especially high in MFL compared with SF ($P < 0.01$). No significant difference was found in gentle feather pecking (mate pecking) and severe feather pecking.

Cage design and social order had significant effects on moving (both $P < 0.001$). Moving was observed more frequently in MFS than in MFL, and more in MFL than in SF (both $P < 0.05$). The proportion of time spent moving was also greater in the medium- and low- than in the high-ranked hens (both $P < 0.01$).

Social order had a significant effect on pre-laying sitting ($P < 0.001$), and more time was spent in this behaviour in the high- than in the medium- and low-ranked hens (both $P < 0.05$). The proportion of time spent performing each behaviour in the nest is shown in Table 4-3. More than 90% of the time in the nest box was spent in pre-laying sitting by the high-ranked hens in all cages. Conversely, the proportion was lower as social rank decreased (all $P < 0.01$). There was a significant effect of social order on the proportion of time spent standing ($P < 0.001$), and moving ($P < 0.01$) in the nest. These proportions were higher in the low- than in the medium-ranked hens, and higher in the medium- than in the high-ranked hens (all $P < 0.01$). A significant interaction between social order and cage design was found for escaping in the nest box ($P < 0.05$). The proportion of time spent escaping was greater as social rank decreased (all $P < 0.05$), and tended to be higher in MFL and MFS compared with SF.

Table 4-3. Mean proportion ± standard deviation of time spent by high-, medium- and low-ranked hens in each behaviour in nest box in a small and two medium furnished cages.

Behaviour	Cage design†	Social order			Repeated-Measure ANOVA $F^{‡}$		
		High	Medium	Low	Cage design	Social order (S)	C×S
Pre-laying sitting	SF	97.9 ± 1.7	79.3 ± 39.3	63.1 ± 13.9			
	MFL	97.2 ± 4.5	75.3 ± 8.0	37.5 ± 27.4	3.4	33.0 ***	1.0
	MFS	94.4 ± 6.8	64.5 ± 15.4	37.9 ± 10.2			
Standing	SF	2.1 ± 1.7	9.4 ± 20.0	17.8 ± 12.3			
	MFL	0.0 ± 0.0	8.7 ± 7.1	27.0 ± 11.8	2.1	26.3 ***	0.9
	MFS	3.5 ± 6.7	18.1 ± 8.1	30.1 ± 8.9			
Escaping	SF	0.0 ± 0.0	0.0 ± 0.0	4.0 ± 5.4			
	MFL	0.0 ± 0.0	4.8 ± 4.7	18.1 ± 10.6	3.1	19.4 ***	2.8 *
	MFS	0.0 ± 0.0	10.5 ± 16.0	17.4 ± 11.6			
Moving	SF	0.0 ± 0.0	10.7 ± 19.7	12.8 ± 4.7			
	MFL	1.5 ± 2.7	11.2 ± 7.5	10.7 ± 10.8	0.4	6.5 **	0.3
	MFS	2.1 ± 3.5	6.9 ± 2.3	8.1 ± 4.4			

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. †SF: small furnished cage with localised dust bath and nest box; MFL: medium furnished cage with localised dust bath and nest box; MFS: medium furnished cage with separated dust bath and nest box. ‡df of the effect of cage design (C) was 2, social order (S) was 2 and S×C was 4 in each location. n was 54 in each location.

4.3.4. Physical condition

Body weight gain, worse feather condition and claw growth from 25 to 37 weeks of age are shown in Table 4-4. The total feather scores at 37 weeks of age were 15.9 ± 1.5 in SF, 17.7 ± 3.2 in MFL and 17.4 ± 1.4 in MFL. A significant effect of social order on worse feather condition was found ($P < 0.05$), with a tendency toward a worse score among the low- and medium- than high-ranked hens, though no significant effect on the feather score of each region of body was found. No significant differences were found regarding other measures of physical condition.

Table 4-4. Mean increament ± standard deviation of physical conditions from 25 to 37 weeks of high-, medium- and low-ranked hens in a small and two medium furnished cages.

Measurement	Cage design†	Social order: High	Social order: Medium	Social order: Low	Repeated-Measure ANOVA F‡: Cage desgin	Social order (S)	C×S
Body weight gain (g)	SF	150.0 ± 80.2	135.0 ± 253.8	175.0 ± 257.1			
	MFL	246.7 ± 292.1	63.3 ± 95.2	181.7 ± 60.5	0.5	0.7	0.7
	MFS	178.3 ± 319.1	228.0 ± 58.5	266.7 ± 125.2			
Worse feather (total)	SF	1.5 ± 0.5	3.3 ± 1.5	2.8 ± 1.7			
	MFL	0.3 ± 2.2	2.7 ± 0.8	3.0 ± 2.7	0.5	3.7 *	0.5
	MFS	1.5 ± 3.1	2.4 ± 1.9	2.0 ± 2.3			
Claw growth (mm)							
front	SF	1.8 ± 1.5	3.4 ± 0.9	1.9 ± 3.2			
	MFL	2.6 ± 1.6	2.1 ± 1.9	2.2 ± 1.4	3.2	1.0	0.8
	MFS	0.4 ± 0.9	1.7 ± 0.8	1.5 ± 1.9			
rear	SF	-0.2 ± 3.0	-1.0 ± 4.0	-1.6 ± 2.6			
	MFL	2.9 ± 4.1	0.1 ± 2.8	2.3 ± 1.0	3.0	1.1	0.6
	MFS	1.7 ± 3.1	1.4 ± 0.7	0.4 ± 4.0			

*$P < 0.05$. †SF: small furnished cage with localised dust bath and nest box; MFL: medium furnished cage with localised dust bath and nest box; MFS: medium furnished cage with separated dust bath and nest box. ‡df of the effect of cage design (C) was 2, social order (S) was 2 and S×C was 4 in each location. n was 54 in each location.

4.4. Discussion

Moving is more frequent as cage area increases (Keeling, 1994), which is one advantage of large furnished cages. It was also found in this study that movement was greater in the medium- than small-sized cages, indicating that they provide an advantage. However, as cage size and group size increase, feather pecking is more frequently observed, which is a disadvantage of larger furnished cages (Shimmura et al., 2008a). Gentle feather pecking develops into severe feather pecking causing feather damage and severe feather pecking is also correlated with cannibalism, which is a serious welfare problem in laying hens which can cause high mortality (Savory, 1995). However, in this study no differences between cage designs were found in the frequency of gentle or severe feather pecking, feather condition and body weight gain. This suggests that the group size might be sufficiently small for hens not to perform feather pecking frequently. Also, there were no significant effects of cage design on use of the perch and feeder. According to the 1999 EU-directive, all laying cage must be enriched by 2012, providing at least following: 600 cm^2

usable area, 150 cm^2 for nest and litter facilities, 15 cm of perch and 12 cm of feeder per hen (Blokhuis, 2004). If sufficient perch and feeder space is provided then group size and cage design may not affect their usage. However, for the use of dust bath and nest box, there were significant effects and these are discussed below.

The fact that dust-bathing, litter pecking and litter scratching tended, on the whole, to be higher in higher-ranked hens than in lower-ranking hens in SF and MFL, suggests that the higher-ranked hens had priority in using the dust bath in SF and MFL. In fact, high-ranked MFL hens performed a lot of aggressive pecking in the dust bath, which induced some of the lower-ranked hens to leave the dust bath. In contrast, the incidence of aggressive pecking in the dust bath by MFS high-ranked hens was rare, and the MFS low-ranked hens used the dust bath more than the low-ranked SF and MFL hens. Lundberg and Keeling (2003) conducted a study on the social effects of video imagery on dust-bathing behaviour. They observed that the high-ranked birds were stimulated to dust-bathing by viewing a video image of a dust-bathing bird ranked medium, but the low-ranked birds did not react to the same video image. Thus, it was suggested that low-ranked birds might not be stimulated to dust-bath when higher-ranked birds are in the dust bath. This phenomenon that dust-bathing by low-ranked hens was restrained by higher-ranked hens was confirmed and the reason was also revealed by several later studies that used furnished cages for laying hens. Van Rooijen (1999) reported that the average duration of dust-bathing was lower among the low- than in the high-ranked group of hens and that dust-bathing was disturbed by aggressive pecking. Similar results were reported in my previous studies, showing that aggressive pecking occurred frequently in the dust bath area in furnished cages relative to the number of hens at the dust bath (Shinmura et al., 2006a, 2006b). The reason aggression was performed frequently in the dust bath was found, in a later study, to be due to the priority of use by higher-ranked hens. This priority of use was observed in a large furnished cage with a small allowance of dust bath per hen (58.3 cm^2 per hen); high-ranked hens performed dust-bathing more frequently in the dust bath and low-ranked hens performed sham dust-bathing on the cage floor (Shimmura et al., 2007c). This priority of use was also confirmed in small furnished cages with a large dust bath area per hen (232.5 cm^2 per hen; Shimmura et al., 2008a). Therefore, dominant hens have priority in using a dust bath, whether the dust bath size per hen is small or large. As a result, aggression occurs frequently in the dust bath, and lower-ranked hens that are pecked by higher-ranked hens are restricted in their use of the dust bath. In the present study, the competition for the dust bath was reduced by separating the dust bath on each side of the cage instead of localising it on one side. This resulted in a more equal use of the dust bath by hens from each rank. Compared to studies of aviaries, which have reported that litter areas are well used by hens' pecking, scratching and dust-bathing (Odén et al., 2002), in the furnished cages dust baths seem to be less attractive and frequently remain unused by hens (Lindberg and Nicol, 1997). This might be due to competition, such as we found in SF and MFL cages. Separation of the dust bath to both sides of the cage might be an effective arrangement so that the dust bath is frequently used by all hens.

Pre-laying sitting was performed more frequently among the high-ranked hens in all types of furnished cages. More than 90% of the time in the nest box was spent performing pre-laying sitting by the high-ranked hens, with the proportion of time being lower with lower social rank. Laying hens usually search for dark and enclosed place and sit there before laying egg (Appleby et al., 2004). Mills et al. (1985) recorded the heart rates of hens sitting pre-laying and concluded that birds are calm during this phase. Dominant birds occupying a nest occasionally peck other birds entering the nest, resulting in subordinate birds leaving the nest box and being unable to sit calmly pre-laying (Appleby et al., 2004). A study by Freire et al. (1997) showed that hens made more attempts to find alternative routes to the nest box during the searching phase of pre-laying behaviour when a dominant or unfamiliar stimulus bird was present. This result also indicates that subordinate hens will try to avoid dominant hens in the pre-laying phase and therefore may avoid a nest box occupied by a dominant bird. In the present study, the proportion of time spent in pre-laying sitting was lower in low-ranked compared to high-ranked hens, which agrees with my previous findings (Shimmura et al., 2008a). Further, about 75% of MFS eggs were laid only in one box. Taken together, all of these findings suggest that, with both localised and separated nest boxes, there was competition and high-ranking hens had priority of access. This resulted in the lower ranking hens being able to sit calmly pre-laying.

While the frequency of pre-laying sitting was lower among the low-ranked hens, the proportion of time spent in the nest box was higher among low- than high-ranking hens, especially in the two medium furnished cages (MFL and MFS). This result indicates that the low-ranked hens used the nest box for reasons other than laying. It was reported that low-ranked hens occasionally escaped to the rear of the cage (Appleby et al., 2002) or nest box (Shimmura et al., 2007c) in small furnished cages when they were pecked by other hens. Escaping to the nest box suggests that it functions as a refuge for lower-ranked hens. The frequency of escaping to the nest box is more pronounced in larger furnished cages (Shimmura et al., 2008a), because aggressive pecking occurs more frequently with an increase in the number of birds (Hughes and Wood-Gush, 1977). As expected, aggressive pecking by the higher-ranked hens was observed more frequently, especially in the two medium furnished cages (MFL and MFS) and escaping and moving in the cage by the lower-ranked hens was observed more frequently in the MFL and MFS cages. Also, the low-ranked hens spent more time at the rear of the cage and more time escaping in the nest box. These results agree with those mentioned above. Thus, the nest box was not used only for laying eggs but also as a refuge by the lower-ranked hens. Our findings suggest that it is possible that the frequency of use of the nest box as a refuge might be higher with an increased group size, as a result of increased aggressive interactions.

In conclusion, it was suggested that a separation of the nest box and dust bath in furnished cages would be an effective arrangement to reduce competition for them and promote equal use of them by all hens. This was achieved for the dust bath but not for the nest box. It was also confirmed that the nest box was used for not only laying eggs but also as a refuge by the lower-ranked hens. As furnished cages become larger, there is a possibility that large group size could have large effects on welfare, because of a high level of competition for the dust bath. Therefore, alternative design of separating the dust bath in this study could offer advantages to hen welfare and the future of furnished cages through the greater use of dust baths by more hens. However, the issue of reducing competition for the nest box and allowing low-rank hens to sit calmly pre-laying remains to be resolved.

4.5. Summary

In my previous studies, it was demonstrated that dominant hens had priority in using the dust bath, resulted in increased competition for the resource. It seemed that the problem was that the resource was placed on one side of the cage ('localised'). Therefore, a medium-sized furnished cage with a dust bath and nest box on both sides of the cage ('separated', MFS) was designed. To evaluate the effects of separation of these resources, the behaviour of high-, medium- and low-ranked hens in MFS cage was compared with that in small (SF) and medium furnished (MFL) cages with a localised resource. In total, 150 White Leghorn layers were used. At the age of 17 weeks, the hens were randomly divided into three groups and moved to small furnished cages (SF, 90 cm wide; five birds per cage) and two types of medium furnished cages (180 cm wide; 10 birds per cage) with a nest box and dust bath on both sides (MFS) and a nest box and dust bath on one side of the cage (MFL). The total dust bath and nest box areas per hen were same for the three cages. The dominance hierarchy was determined by observing the aggressive interactions and by this high-, medium- and low-ranked hens in each cage were identified. The behaviour, use of facilities and physical

condition of these hens were measured. Data were analysed by using repeated measure ANOVA. A significant interaction between social order and cage design was found in the proportions of time spent in the dust bath and on performing dust-bathing (both $P < 0.001$), and these proportions tended to be higher in higher-ranked hens in SF and MFL. Conversely, the MFS low-ranked hens tended to use the dust bath more than the SF and MFL low-ranked hens. Thus, hens from each rank used the dust bath equally in MFS, though the MFS high-ranked hens tended to use the resource less than the SF and MFL high-ranked hens. While the frequency of pre-laying sitting was lower among low-ranked hens ($P < 0.05$), the proportion of time in the nest box was higher among low- than high-ranked hens ($P < 0.01$). The low-ranked hens spent more time performing escaping, moving and standing in the nest box. In conclusion, it was suggested that separation of the dust bath to two locations would be an effective arrangement to promote more equal usage of the dust bath by hens from each rank in the furnished cages. It was also confirmed in the present study that nest boxes were not only used for laying eggs but also as a refuge by lower ranked hens.

CHAPTER 5

Overall evaluation of new modified cage by behaviour, physical condition and production

5.1. Introduction

Based on the results of previous chapters (Chapter 2 and 3) and my previous studies, a medium-sized furnished cage with resources on both sides of the cage ('separated' resources) was designed. In Chapter 4, relation between social order and use of resources in the new modified cage was reported. In Chapter 5, usefulness of the modified cage was evaluated overall by measuring behaviour, physical condition and production.

Conventional cages for laying hens will be banned in the European Union (EU) in 2012. It is in the EU that the most development of alternative housing systems for laying hens has occurred. These alternatives comprise furnished cages and non-cage systems such as deep litter, aviaries and free-range systems (Tauson, 2005). Furnished cages contain a perch, nest box and litter area, and they provide more height and area per hen than conventional cages (Appleby and Hughes, 1995). They will be the only legal form of cage in the EU from 2012 (Blokhuis, 2004). Furnished cages provide most of the economic advantages of conventional cages while removing many behavioural restrictions (Appleby et al., 2002). Today, about 40% of egg layers in Sweden are kept in furnished cages (Tauson, 2005) and other countries in the EU where this system is used are the United Kingdom, Norway, Germany and Denmark. Attention is also being given to furnished cages in Asia, including Japan, because these cages can increase welfare while maintaining good performance (Shimmura et al., 2007a, 2007b). The number of farmers using furnished cages has been increasing little by little in Japan.

An early model of furnished cages, the Edinburgh Modified Cage, was used for groups of four birds (Appleby and Hughes, 1995). A small group size offers the benefit of a low incidence of aggressive interactions, but if the group size is increased, this reduces the egg production cost per hen. Therefore, more recently, the size of furnished cages has increased, e.g. 16 hens (Wall et al., 2004) and 40 hens per cage (Weitzenburger et al., 2005). Large furnished cages would benefit the birds by providing a larger total cage area, leading to more exercise and probably, in turn, improved bone strength. However, an increased group size implies that more hens share a restricted number of resources such as the nest box and litter area, which could result in increased competition for these resources.

The occurrence of competition for a concentrated resource is one of the disadvantages of the newly developed furnished cages. Some researchers have demonstrated that competition for a dust bath occurs in furnished cages (Van Rooijen, 1999). Van Rooijen (1999) investigated dust-bathing of 42 hens in a furnished cage and reported that 18·7% of 75 dust-bathing events was disturbed by aggression, resulting in shorter duration of dust-bathing bouts as compared to those reported in previous studies (Vestergaard, 1982). Later, it was observed that dominant hens had priority when using the dust bath (Shimmura et al., 2007c). In large furnished cages with a small allowance of dust bath area per hen (58·3 cm^2 per hen), higher-ranked hens used the dust bath and performed more dust-bathing than lower-ranked hens, indicating that competition for a small dust bath would occur. And competition was observed even in furnished cages with a large dust bath area (232·5 cm^2 per hen; Shimmura et al., 2008a). Therefore, in large furnished cages, only a small number of hens (those that are high-ranking) may have the priority while using resources such as a dust bath, even if those resources seem to be used fully by many hens. It would be difficult to conclude, in these conditions, that furnished cages have an unequivocal advantage in removing behavioural restrictions.

From these previous studies, it seemed that the problem was that a resource was placed on one side of the cage ('localised'). Therefore, a medium-sized furnished cage with dust baths and nest boxes on both sides of the cage ('separated', MFS) was designed, which proved an effective arrangement to promote more equal usage of the dust bath by hens of differing rank (Shimmura et al., 2008b). However, this evaluation was not sufficient, because the behavioural sampling was limited to a few hens. To evaluate this new cage design thoroughly, the behaviour of all birds using a scanning technique, their physical condition and egg production in MFS were measured, and they were compared with those of hens in conventional cages (CC), small furnished cages (SF) and medium furnished cages (MFL) with a 'localised' dust bath and nest box on one side of the cage.

5.2. Materials And Methods

5.2.1. Animals and housing

The experiment was conducted from 17 to 72 weeks of age. In total, 180 White Leghorn layers were used. All birds had their beaks trimmed when they were 1 day old and they were raised in conventional cages. At the age of 17 weeks, the birds were randomly introduced into one of the four cage designs in a laying house. Sixty birds were housed in six conventional (CC) and six small furnished cages (SF) with five birds per cage, and 120 birds in two types of medium furnished cages with the dust bath and nest box on both sides of the cage (separated, MFS; six cages and 10 hens per cage) and the dust bath and nest box on one side of the cage (localised, MFL; six cages and 10 birds per cage). The house was ventilated with six ceiling fans. The average daytime temperature (±SD) during the observation period was 17.6 ± 4.3°C at the centre of the house. Lighting was provided by two fluorescent lights (37 W), adjusted to provide an intensity of 10 Lux at the food troughs. The illumination cycle was 14 h of light and 10 h of darkness, with the light period from 0500 to 1900. The hens had ad libitum access to water and feed. The feed contained at least 16% crude protein and 2900 kcal metabolic energy per kg. Feeding and any other routine work such as supplying wood-shavings to the dust bath was done between 0800 and 0900 and eggs were collected between 1600 and 1700.

5.2.2. Cage design

Adding a conventional cage to three types of furnished cages in the Chapter 4, total four cage systems were accepted in this study. Conventional cage (CC) was standard laying cage 65 cm wide, 46.5 cm deep and 47 cm high at the rear. The main cage area was 604.5 cm^2 per bird with a floor of 2.5 × 5.0 cm wire mesh. A V-shaped feeder was located externally at the front of the cage, and a U-shaped drinker was placed at the rear. Feeder and drinker space per bird was 13.0 cm each. Following three furnished cages were same with Chapter 4: small furnished cage (SF), two types of medium furnished cages with the dust bath and nest box on both sides of the cage (separated, MFS) and the dust bath and nest box on one side of the cage (localised, MFL). The cage floor, feeder and drinker space per bird, and the shape and location of feeder and drinker were same for all cage designs. The total space of perch, dust bath and nest box per bird were equal for all furnished cage designs. Designs and equipment for these cages fulfilled the regulations laid down by the EU (Blokhuis, 2004).

5.2.3. Measurements

Behaviour. Observations were conducted at 35, 55 and 71 weeks of age (3 d/week). Direct visual scans at 10 min intervals were conducted to record the location and behaviour of all birds in all cages for 4 h/day, 2 h each in the morning (1000 to 1200) and the afternoon (1300 to 1500). The birds were not individually identifiable. The location was scored as either the nest, dust bath, perch, cage floor or feeder. The location 'feeder' was recorded when a hen had her head in the feeder, whether or not her head was through the front bars. For behaviour, the following activities were recorded: eating, drinking, resting, comfort (dust-bathing, preening, head-scratching, stretching, body-shaking, tail-flapping, wing-raising), aggressive pecking, severe feather pecking, exploring (litter-pecking, litter-scratching, gentle feather pecking (mate-pecking), object-pecking), sham dust-bathing, and moving (Appleby et al., 2004). Eating and drinking was recorded when a hen had her head in the feeder or drinker. Resting was defined as the time when the hen lowered and 'tucked in' her head or closed her eyes and was still. Dust-bathing was recorded when one element of three (vertical wing-shaking, head-rubbing, scratching with one leg) was observed. Aggressive pecking was on the head of the recipient and excluded both severe feather pecking (forceful pecks, sometimes with feathers being pulled out and with the recipient bird moving away) and gentle feather pecking (careful pecks, not resulting in feathers being pulled out and usually without reaction from the recipient bird). Pecking behaviour was defined as all behaviours that involved the use of the beak (eating, drinking, preening, aggressive pecking, feather pecking, litter pecking, object pecking, and mate pecking). Because litter-scratching was performed with litter-pecking and the duration was short, both were grouped as litter exploring. All data were collected by the same observer.

Physical condition. For focal sampling, all 180 birds were individually marked, using a combination of coloured leg rings, at 17 weeks of age. Body weight, feather damage and claw length were recorded on three focal birds per cage when the birds were 36 and 72 weeks of age. In accordance with Bilčik and Keeling (1999), feather damage was scored from 1 (no damage) to 6 (denuded) for six parts of the body (neck, breast, back, belly, wing, tail), giving a total score from 6 to 36. Slightly different criteria were used for scoring flight feathers than for the rest of the plumage, because of the different types of feathers and damage (Table 5-1). The assessment of feather damage was carried out by two or three people working together to ensure maximum consistency when scoring. The centre front and rear claws of the right foot were measured using a digital vernier calliper, (± 0.01 mm) by recording the straight length from the claw root to the tip.

Table 5-1. Description of scoring method used to evaluate the feather condition. A different scale was used for flight feathers (wing, tail) compared to feathers on the rest of the body (neck, breast, back, belly).

Score	Body	Flight feathers
1	Intact feathers	Intact feathers
2	Up to 5 damaged feathers	Up to 5 separated feathers, none damaged or broken feathers
3	More damaged feathers, up to 5 missing feathers, none bald patch	> 5 separated feathers, up to 5 damaged or broken feathers
4	Bald patch < 50% of area	Almost all feathers separated, > 5 damaged or broken feathers
5	Bald patch > 50% of area	Almost all feathers damaged or broken feathers, > 3 missing feathers
6	Completely denuded area	Almost all feathers missing

Production. The number of eggs laid, including cracked eggs, at each location and mortality was recorded daily by 72 weeks of age. Feed intake, egg weight, egg mass, feed efficiency, egg shell thickness, egg shell deformation and Haugh unit were measured for one egg per cage at ages of 35, 44, 55 and 63 weeks for 3 days each. Egg production was shown as hen day's average (total number of eggs/total number of hens × 100). The egg mass (g egg/hen per d) and feed efficiency (g of egg: g of feed) were calculated by the values of feed intake (g/hen per d) and egg mass (g/egg). The eggshell thickness (NFN380, FHK, Tokyo), eggshell deformation (NFN388, FHK, Tokyo) and Haugh unit (NFN381, NFN 382, NFN 383, FHK, Tokyo) were measured by using measuring instruments.

5.3.4. Statistical Analyses

The proportions of hens at each location and performing each behaviour were calculated for each cage, in the case of CC as (the total number of hens observed to perform a behaviour / 1080) × 100. The value of behaviour, physical condition and egg production at different ages was averaged for the number of replicate cages. Therefore, each cage involved one data point for each variable. A one-way ANOVA was used to evaluate the effects of cage design (CC, SF, MFL, MFS) on behaviour, physical condition and production. Significances of differences between cage designs were evaluated by multiple comparisons using Tukey-Kramer's test. Before these tests, the normal distribution and homogeneity of variance were confirmed both by a visible method (plot, box plot) and a statistical test (test for difference of mean, Bartlett test; Quinn and Keough, 2002). When not confirmed an arcsine- square-root transformation was carried out (Martin and Bateson, 1993). The data of dust bath usage and egg mass were analyzed using the Kruskal-Wallis' test followed by Steel-Dwass' multiple comparison test, because the normal distribution and homogeneity of variance were not present even after transformation). Because a high mortality was found in the two cages of CC, they were excluded from the analyses.

To determine where aggressive interaction (e.g. aggressive pecking, feather pecking) was performed frequently, the relationships between the number of hens at each location and the number of aggressive interactions at the location were analysed by a Chi-square test for independence. The selection of nest box for laying hens in MFS was also analyzed by a Chi-square test.

5.3. Results

5.3.1. Behaviour

The proportions of hens at each location are shown in Table 5-2. The proportion of hens in the dust bath was higher in MFL than in SF and MFS ($P < 0.05$). No significant difference was found for the other locations.

Table 5-2. The mean percentage (±standard deviation) of hens being each location in four cage designs. The data for dust bath are values after arcsine-square root transformation.

Location	Cage design†			Statistical value‡
	SF	MFS	MFL	
Feeder	34.1 ± 3.8	33.6 ± 4.5	31.0 ± 1.6	1.3
Cage floor	10.8 ± 2.9	13.6 ± 2.7	10.4 ± 1.2	3.2
Perch	38.8 ± 3.5	36.0 ± 1.8	36.8 ± 2.9	1.6
Dust bath	12.8 ± 4.5 ^{b}	12.2 ± 2.2 ^{b}	16.5 ± 1.2 ^{a}	7.0 *
Nest box	3.5 ± 2.3	4.5 ± 2.5	5.3 ± 1.5	1.1

*$P < 0.05$. †SF: small furnished cages; MFS: medium furnished cages with separated resources; MFL: medium furnished cages with localised resources. ‡Degrees of freedom was two. N was six in each cage type in each location. The data was compared using Kruskal-Wallis test followed by Steel-Dwass' multiple comparison test for the dust bath and one-way ANOVA for the other locations. The statistical value was Kruskal-Wallis' *H* for the dust bath and *F* for ANOVA. Different superscript letters in the same row indicate significant difference (a-b: $P < 0.05$).

The proportions of hens performing each behaviour are shown in Table5-3. Preening was observed more frequently in SF and MFS than in CC and MFL ($P < 0.01$), object-pecking was more in CC than the other cages ($P < 0.05$) and litter-exploring was more in MFL than in SF ($P < 0.01$). The total proportion of hens performing pecking behaviour was almost the same among the four cages (mean±SEM; CC, 56.9 ± 3.3%; SF, 58.9 ± 2.2%; MFS, 59.4 ± 4.6%; MFL, 55.3 ± 1.4%).

No significant difference was found in head-scratching, stretching, body-shaking, tail-flapping and wing-raising. Moving was performed more frequently in MFS and MFL than in CC and SF ($P < 0.01$) and resting was more in CC than in MFS ($P < 0.01$).

The proportion of hens performing aggressive pecking and severe feather pecking was higher in MFL than in CC and SF ($P < 0.05$). While the proportion of observation points when aggressive pecking and severe feather pecking were observed in the dust bath in all points observed these behaviours was 22.1% (15/68) in MFS, it was 43.2% (57/132) in MFL. The aggressive interaction occurred frequently in the dust bath area relative to the number of hens in the dust bath in MFL ($\chi^2 = 58.9$, $P < 0.001$), although this tendency was not found in MFS ($\chi^2 = 5.2$).

Table 5-3. The mean percentage (±standard deviation) of hens performing each behavior in four cage designs. The values for drinking, moving and sham dust-bathing are those after arcsine-square root transformation.

Behaviour	Cage design†				ANOVA
	CC	SF	MFS	MFL	F-value‡
Eating	36.6 ± 3.3	34.1 ± 3.8	33.6 ± 4.5	31.0 ± 1.6	2.1
Drinking	0.2 ± 0.0 ^{ab}	0.2 ± 0.0 ^{a}	0.2 ± 0.0 ^{b}	0.2 ± 0.0 ^{ab}	3.7 *
Resting	24.8 ± 5.0 ^{A}	18.6 ± 3.2 ^{AB}	15.1 ± 4.2 ^{B}	18.6 ± 3.1 ^{AB}	5.2 **
Comfort					
Dust-bathing	-	2.1 ± 1.1	2.7 ± 0.7	2.0 ± 0.5	1.4
Preening	10.8 ± 1.3 ^{B}	14.6 ± 1.3 ^{A}	14.8 ± 2.6 ^{A}	11.6 ± 1.4 ^{B}	6.6 **
Head-scratching	0.7 ± 0.3	1.1 ± 0.4	1.1 ± 0.2	0.9 ± 0.3	1.4
Stretching	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.2	0.3 ± 0.1	0.9
Body-shaking	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.2	0.4
Tail-flapping	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	1.4
Wing-raising	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.1	0.5
Aggressive pecking	0.2 ± 0.1 ^{b}	0.2 ± 0.3 ^{b}	0.4 ± 0.2 ^{ab}	0.7 ± 0.3 ^{a}	5.3 **
Severe feather pecking	0.0 ± 0.0 ^{b}	0.1 ± 0.1 ^{b}	0.2 ± 0.2 ^{b}	0.4 ± 0.2 ^{a}	9.8 ***
Exploring					
Litter-exploring	-	2.7 ± 1.1 ^{B}	4.3 ± 1.6 ^{AB}	5.3 ± 1.1 ^{A}	6.0 *
Object-pecking	4.8 ± 1.6 ^{a}	2.5 ± 1.1 ^{b}	2.5 ± 1.0 ^{b}	2.2 ± 0.4 ^{b}	6.1 **
Mate-pecking	0.7 ± 0.4	0.3 ± 0.1	0.5 ± 0.3	0.6 ± 0.3	2.2
Sham dust-bathing	0.1 ± 0.0 ^{A}	0.0 ± 0.0 ^{B}	0.0 ± 0.0 ^{B}	0.0 ± 0.0 ^{B}	16.0 ***
Moving	0.1 ± 0.0 ^{B}	0.1 ± 0.0 ^{B}	0.2 ± 0.0 ^{A}	0.2 ± 0.0 ^{A}	25.7 ***

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. †CC: conventional cages; SF: small furnished cages; MFS: medium furnished cages with separated resources; MFL: medium furnished cages with localised resources. ‡ Degrees of freedom was three. N was four in CC and six in the other cage types in each behaviour. The data was compared using one-way ANOVA followed by Tukey's multiple comarison test. Different superscript letters in the same row indicate significant difference (A-B: $P < 0.01$; a-b: $P < 0.05$).

The proportion of observations where a single hen was dust-bathing was high in all furnished cages (SF, 100.0%; MFS, 99.4%; MFL, 94.2%) but dust-bathing by more than two hens at the same time was rare. The proportion of hens performing sham dust-bathing was higher in CC than in the three furnished cages ($P < 0.01$).

5.3.2. Physical condition

Body weight, feather condition and claw length are reported in Table 5-4. No significant difference was found in the body weight. The total feather damage was higher in

MFS than in CC and SF ($P < 0.05$). The damage of the neck and breast was higher in SF and MFS than in MFL ($P < 0.05$), and the damage of the back and belly was higher in MFS than in CC ($P < 0.05$). The rear claw length was longer in CC than in the three furnished cages ($P < 0.05$).

Table 5-4. The mean value (±standard deviation) of physical condition in four cage designs.

Measurement	Cage design†				ANOVA
	CC	SF	MFS	MFL	F-value‡
Body weight (g)	1779.2 ± 93.9	1672.2 ± 75.0	1688.9 ± 109.5	1601.5 ± 147.0	2.1
Feather score					
Total	9.5 ± 0.2 ^{b}	9.6 ± 0.4 ^{b}	10.5 ± 0.4 ^{a}	10.0 ± 0.8 ^{ab}	4.4 *
Neck	2.4 ± 0.4 ^{A}	2.3 ± 0.2 ^{A}	2.0 ± 0.2 ^{A}	1.4 ± 0.3 ^{B}	13.6 ***
Breast	1.9 ± 0.6 ^{ab}	2.6 ± 0.2 ^{a}	2.0 ± 0.4 ^{a}	1.4 ± 0.3 ^{b}	9.5 ***
Back	2.0 ± 0.3 ^{b}	2.7 ± 0.7 ^{b}	4.1 ± 0.3 ^{a}	3.1 ± 1.1 ^{ab}	7.9 **
Belly	1.2 ± 0.3 ^{b}	2.0 ± 0.6 ^{ab}	2.3 ± 0.4 ^{a}	1.6 ± 0.3 ^{b}	6.1 **
Wing	4.3 ± 0.3	4.1 ± 0.2	4.2 ± 0.2	3.9 ± 0.2	2.7
Tail	5.0 ± 0.1	5.0 ± 0.0	5.0 ± 0.1	4.8 ± 0.2	2.2
Claw length					
Front	24.2 ± 0.8	25.5 ± 1.0	23.9 ± 2.0	25.1 ± 0.7	2.0
Rear	11.8 ± 0.7 ^{a}	9.8 ± 0.9 ^{b}	9.5 ± 1.3 ^{b}	9.9 ± 0.9 ^{b}	4.8 *

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. †CC: conventional cages; SF: small furnished cages; MFS: medium furnished cages with separated resources; MFL: medium furnished cages with localised resources. ‡ Degrees of freedom was three. N was four in CC and six in the other cage types in each behaviour. The data was compared using one-way ANOVA followed by Tukey's multiple comparison test. Different superscript letters in the same row indicate significant difference (A-B: $P < 0.01$; a-b: $P < 0.05$).

5.3.3. Production

Production traits and egg quality traits are shown in Table 5-5. Egg production and egg mass were lower in MFL than in SF ($P < 0.05$). The feed intake was higher in CC than MFL, and the feed efficiency was worse in CC than in SF (both $P < 0.01$). Mortality by cannibalism was 5 hens in CC, 0 hens in SF, 1 hen in MFS and 3 hens in MFL.

The mean proportions (±S.D.) of eggs laid in the nest were higher in MFL (98.3 ± 0.6%) than in MFS (93.5 ± 3.9%, $P < 0.05$). MFS hens laid eggs in the dust bath (0.9 ±

1.2%, P = 0.20) and on the cage floor (5.6 ± 2.8%, $P < 0.01$), more than MFL. In the case of MFS, 74.1 ± 13.9% of eggs were laid in the more heavily used of the two nest boxes (χ^2 = 50.8, $P < 0.001$).

Table 5-5. The mean value (±standard deviation) production traits and egg quality traits in four cage designs. The data of egg production and mortality was shown as the one after arcsine-squart transformation.

Measurement	Cage design†				Statistical value‡
	CC	SF	MFS	MFL	
Production (%)	1.2 ± 0.2 ab	1.2 ± 0.0 a	1.1 ± 0.1 ab	1.0 ± 0.0 b	4.3 *
Cracked egg (%)	0.9 ± 0.7	1.1 ± 0.6	0.7 ± 0.3	0.9 ± 0.4	0.5
Feed intake (g/hen per d)	129.9 ± 16.5 a	117.9 ± 6.2 ab	114.8 ± 11.7 ab	107.3 ± 5.0 b	4.2 *
Egg weight (g/egg)	62.3 ± 0.9	61.0 ± 2.0	62.1 ± 1.6	62.1 ± 1.6	0.7
Egg mass (g egg/hen per d)	51.2 ± 10.7 ab	54.6 ± 1.3 a	48.1 ± 5.7 ab	46.3 ± 2.1 b	8.1 *
Feed efficiency (g of egg: g of feed)	2.6 ± 0.3 a	2.2 ± 0.1 b	2.4 ± 0.2 ab	2.3 ± 0.2 ab	3.7 *
Egg shell thickness (mm)	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.2
Egg shell deformation (kg/cm^2)	3.4 ± 0.2	3.2 ± 0.4	3.5 ± 0.3	3.4 ± 0.5	0.6
Haugh unit	97.6 ± 0.9	98.1 ± 1.7	97.8 ± 3.6	97.5 ± 1.8	0.1
Mortality by cannibalism (%)	0.3 ± 0.4	0.1 ± 0.2	0.1 ± 0.1	0.1 ± 0.2	0.8

*$P < 0.05$. †CC: conventional cages; SF: small furnished cages; MFS: medium furnished cages with separated resources; MFL: medium furnished cages with localised resources. ‡Degrees of freedom was three. N was four in CC and six in the other cage types in each behaviour. The data was compared using Kruskal-Wallis' test followed by Steel-Dwass' multiple comarison test for the egg mass and using one-way ANOVA followed by Turkey's multiple comarison test for the other measurements. The statistical value was Kruskal-Wallis' *H* for the egg mass and was ANOVA's *F* for the other locations. Different superscript letters in the same row indicate significant difference (a-b: $P < 0.05$).

5.4. Discussion

The total proportion of hens using their beak was almost the same among the four cages. Object-pecking was observed more frequently in CC; litter-exploring, in MFL; and preening, in SF and MFS. This result suggests that the total frequency of pecking behaviour was similar regardless of the housing system, although the breakdown of types of beak use was different, which is in agreement with the results of Chapter 1. According to the previous study, it is guessed in this study that CC hens might peck the cage wire in a redirected way associated with not being able to peck the litter material. This redirected behaviour has been reported in many studies, among which studies on environmental enrichment form the majority. Blokhuis (1986) observed the pecking behaviour of hens in pens with and without litter and concluded that feather pecking is considered as redirected litter pecking. In the present study, aggressive interactions including severe feather pecking and aggressive pecking were observed more frequently in MFL with litter substrate. Therefore, that these aggressive behaviours are decreased by supplying litter would not be true in every situation, as stated in the SVC report (Commission of the European Communities, 1996). The probable cause why the aggression was increased in MFL is discussed below.

Sham dust-bathing was observed less frequently and the claw length was shorter in the three types of furnished cages. Moving was also performed more frequently in two medium furnished cages and resting was less in MFS. One of the advantages of furnished cages is the improvement of physical condition (Appleby et al., 2002). The excess of claw growth was also restrained by performing litter-scratching and dust-bathing (Shimmura et al., 2007b). Another merit was the larger space, resulting in higher bone strength due to increased activity (Vits et al., 2005), which is considerable if the size of the furnished cages is increased (Shimmura et al., 2008a). The furnished cages in this study retained these advantages, and for locomotory activity, medium furnished cages appeared to have more merit.

The frequency of dust bath usage and litter-exploring was high in MFL, while no significant difference was found in dust-bathing. Aggressive pecking and feather pecking were observed frequently in MFL. The space per dust bath result in the increment of dust bath usage and litter-exploring, which is one of the advantages in MFL with larger space per dust bath. Conversely, it is one of the disadvantages that MFL has a localised dust bath despite large group size, because such design of the resource leads to increased competition to dust bath. In fact, in this study, aggression was observed more frequently and the aggressive behaviour was performed in the dust bath in MFL, while the competition to dust bath was rare in MFS. The competition to dust bathe has been observed in previous studies as well. Van Rooijen (1999) reported that the average duration of dust-bathing was lower among low-ranked individuals than among high-ranked individuals and dust-bathing was disturbed by aggressive pecking. Similar results was reported in my previous studies, showing that aggressive pecking occurred frequently in the dust bath area in furnished cages relative to the number of hens in the dust bath (Shimmura et al., 2007a, 2007b). The reason aggression was observed frequently in the dust bath was found, in a later study, to be due to priority use of the dust bath by higher-ranked hens (Shimmura et al., 2007c, 2008a). Likewise, the priority usage of dust baths by dominant hens in MFL was reported, while such results were not found in MFS (Shimmura et al., 2008b). Taken together, the competition to dust bathe occurred frequently in MFL, by which aggressive interaction and cannibalism might be increased in the cage.

The feather score on the part of neck, breast and belly was higher in MFS than in MFL. Several methods for scoring feather have been presented during the year. Among of those, the method scored the feather on each body part is general and useful, because it can explain and describe possible reasons (Bilčik and Keeling, 1999; Tauson et al., 2005). For example, a high feather score on the belly would be due to feather pecking and have possibility of high mortality. Although the high score (e.g. score 5: bald patch > 50% of area) can indicate the risk of feather pecking and increased heat loss, this is unlikely for a low score. In the present study, a statistical significant difference was found on some parts between MFL and MFS, but the difference was less than 1 and the actual score value is very low (about score 1-2). It is unlikely this indicates an increased risk of feather pecking and heat loss, and therefore is of no biological significant difference.

The feed efficiency was better in SF than in CC, and egg production and egg mass was poorer in MFL than in SF. The production of MFS was similar to that in SF. Generally, a high frequency of aggressive interaction affects egg production (Candland et al., 1969;

Hughes and Duncan, 1972; Shimmura et al., 2007a). Therefore, the reason for the decreased egg production in MFL might lie in the higher frequency of aggressive pecking and feather pecking compared with SF, and the competition to dust bathe, as discussed above, may be the fundamental problem. A similar result that egg production is decreased by competition for the dust bath has also been found (Shimmura et al., 2007b). However, the most developed model of small furnished cages generally resulted in egg production similar to conventional cages (Abrahamsson et al., 1995; Abrahamsson and Tauson, 1997; Appleby et al., 2002; Shimmura et al., 2007b), as was found in medium (Wall et al., 2002) and even large furnished cages (Vits et al., 2005). Furthermore, the proportion of hens performing aggressive pecking and feather pecking in MFL is less than 1·0%, and it is uncertain that this value is sufficiently high to decrease production as compared with values obtained in the above previous studies (Candland et al., 1969; Hughes and Duncan, 1972; Shimmura et al., 2007a). Therefore, because this study is on a small scale, larger-scale retest would be required for confirming the relationship between production and aggression in furnished cages.

Although the production of MFS was similar in CC and SF, more eggs were produced outside the nest in MFS than in MFL, and about 75% of MFS eggs were laid only in one of the two nest boxes. Laying hens search for dark and enclosed place before laying their eggs and generally prefer to the nest box that another bird stay than the empty nest box (Appleby et al., 2004). In another study, it was reported in my previous report that dominant birds occupying a nest occasionally peck other birds entering the nest, resulting in subordinate birds leaving the nest box and being unable to sit calmly before laying in MFS (Shimmura et al., 2008b). Therefore, one of the two nest boxes in MFS was used intensively and consequently, and so hens that did not enter the nest might lay eggs out of the box. This resulted in an increased egg number outside the nest. Although the production of MFS was not poor on the whole, an improvement in cage design would be required in this sense.

In conclusion, a relatively high frequency of aggressive interaction and low production were found in MFL. However, in MFS, aggressive pecking and feather pecking decreased and egg production was similar to that in CC and SF, while the advantage of a higher activity remained. These results might indicate the usefulness of the MFS design. However, some inconsistent results and points for improving the MFS design were also found. A larger-scale study and further trials for cage design would therefore be required.

5.5. Summary

Based on my previous studies, a medium-sized furnished cage with a dust bath and nest box on both sides of the cage (MFS) was designed and its usefulness was evaluated. In total, 180 White Leghorn layers were used. At the age of 17 weeks, the birds were randomly introduced into one of the four cage designs: conventional cages (CC; six cages and five hens per cage), small (SF; six cages and five hens per cage) and medium furnished cages (MFL; six cages and 10 hens per cage) with a 'localised' dust bath and nest box on one side of the cage, and MFS (six cages and 10 hens per cage). The total spaces of resources per bird were same for all furnished cage designs. Behaviour, physical condition and production were measured in each cage. Moving was more frequent in MFS and MFL than in CC and SF. The proportion of hens performing aggressive pecking and sever feather pecking was higher in MFL than CC and SF. These aggressive interactions occurred frequently in the dust bath area in MFL; however, these tendencies were not found in MFS. Egg production and egg mass were lower in MFL than in SF, while the production in MFS was similar to those in CC and SF. MFS hens laid eggs on the cage floor more often than in MFL. In conclusion, these results might indicate the usefulness of MFS. However, some inconsistent results and points for improving MFS design were also found.

GENERAL DISCUSSION

In this study, the advantages and disadvantages of various housing systems were clarified from various points, and on the basis of it, a newly modified housing system resolving the disadvantages and an overall welfare assessment system evaluating various housing systems at a farm level were also developed. In this chapter, first, the discussions of these advantages and disadvantages with the previous commercial-scale reports are deepened because in this study small-scale housing systems were designed, especially the non-cage systems, for behavioural observation. For welfare, the advantages and disadvantages were discussed from the viewpoint of the five freedoms. The usefulness of furnished cages and the welfare assessment is then discussed.

For the freedom from pain, injury, and disease, the non-cage systems offer poor welfare, while cage systems, as a whole, provide high welfare. While the non-cage systems have the advantage of producing stronger bones by encouraging a larger movement and behavioural repertoire, such as litter scratching (Fleming et al., 2004, 2006), the systems have the disadvantage of high risks of mortality and bumble foot. As mentioned below, the mortality due to feather-pecking/cannibalism is generally higher with the increase in group size. In fact, the LayWel Project (Blokhuis et al., 2007), a large-scale project that assessed the welfare of housing systems for laying hens, reported that mortality due to feather-pecking/cannibalism in non-beak trimmed flocks was higher in large-scale housing systems without an outdoor area (e.g., large furnished cages, aviary) compared with cages with small group sizes (e.g., conventional cages, small furnished cages), which agrees with the results of Chapter 2. The non-cage systems, especially free-range systems, also carry high risks of infectious disease and internal parasites, because the hygiene status is poor, due to contact with the outdoor environment. In the investigation of 25 free-range systems by Van Emous and Van Fiks (2004), the average mortality of free-range chickens was 14.3%, the highest mortality rate was 28.5%, and even the lowest was still 8.0%. Considering that even the higher mortality rate is less than 10% in conventional cages (Tauson, 2005), the cost of free-ranges is extremely high. The main causes of death were *E. Coli* or Coccidiosis, which are most common infectious diseases in the non-cage systems (Van Emous and Van Fiks, 2004). The results of both the LayWel Project (Blokhuis et al., 2007) and Chapter 2 also indicated that bumble foot was especially increased in non-cage systems, due to increased movement and litter scratching of hens or to the complex structure of the housing systems. In contrast to the non-cage systems, the conventional and small furnished cages have the advantages of a small group, separation from droppings by cage wires, and restricted movement (or simple structure), which result in low risk of the mortality due to feather-pecking/cannibalism, infectious diseases, and bumble foot. Large furnished cages have variable risks for the freedom from pain, injury, and disease, because the risk of feather-pecking/cannibalism is increased by large group size (Chapter 2), although, similar to conventional and small furnished cages, the low risk of infectious diseases and bumble foot remained.

For the freedom from hunger and thirst, there is no difference in risk. The *ad libitum* access to water and feed is common for laying hens (Appleby et al., 2004), and therefore, no difference would naturally result in the freedom from hunger and thirst. In reality, feed intake had no significant difference among housing systems (Chapter 2), while the proportion of hens eating was different (Chapter 1).

In the category of the freedom to express normal behaviour, behaviour is, as a whole, more diversified in the non-cage systems, especially free-range, than in the cages. Among the cages, behavioural diversification is higher in furnished cages, and behaviour is most restricted in the conventional cages. As confirmed in Chapter 2, supplying a larger space encourages comfort behaviour (Black and Hughes, 1974; Freeman, 1983; Nicol, 1987;

Dawkins and Hardie, 1989; Hurnik and Lewis, 1991) and movement (Appleby et al., 2002; Appleby, 2004; Shimmura et al., 2007a, 2007b), and supplying more litter space and nest box leads to increased litter exploring (Blokhuis et al., 2007; Shimmura et al., 2008a) and pre-laying (Appleby, 2004). It is, therefore, undoubted that these behaviours were more common in the non-cage systems and furnished cages, especially the former with a more enriched environment. On the other hand, behaviour was remarkably restricted in conventional cages, with little space and no resources.

In terms of the freedom from fear and distress, free-range offers high welfare, while the conventional cage and aviary have variable risks. The result that the fear response and physical stress were lower in non-cages systems than in conventional cages, which agrees with previous studies (e.g. Jones and Faure, 1981), was shown in Chapter 2. Also, a large litter space and outdoor area promote litter scratching, which restrained claw overgrowth (Shimmura et al., 2007b), although it sometimes caused footpad inflammation, as mentioned above. The short claw is one of the advantages of the non-cage systems with a large litter area because overgrowth leading to claw breakage is frequently observed in conventional cages (Hills, 1975; Tauson, 1986). On the other hand, large group size is one of the disadvantages of the non-cage systems. A number of studies have demonstrated that feather pecking and aggressive pecking were increased with increments of group size (Appleby and Hughes, 1995; Abrahamsson et al., 1995, 1996; Abrahamsson and Tauson, 1997; Appleby et al., 2002; Wall and Tauson, 2002; Vits et al., 2005; Weitzenbürger et al., 2005; Shimmura et al., 2007a, 2007b, 2008b, 2009a). In reality, these behaviours were observed more commonly in housing systems with large group sizes (Chapter 2). Although such positive correlation between group size and injurious pecking is found in groups of tens of hens, little aggressive interaction was observed when the group size is still lager. For example, the incidence of agonistic interaction was low and similar in groups of 100, 200, and 400 hens (Hughes et al., 1997). Generally, thousands or tens of thousands of hens are housed in a non-cage system on commercial farms, and that the feather pecking and aggressive pecking are low in commercial conditions is generally accepted opinion (e.g. Blokhuis et al., 2007). However, this situation also depends largely whether the beak trimmed or not, and the risk of feather pecking is extremely high in non-beak trimmed flocks in non-cage systems (Blokhuis et al., 2007). Considering that beak trimming is prohibited in most EU countries, non-cage systems would incur a higher risk of feather pecking and aggressive pecking. On this point, the risk for these aggressive behaviours is lower in cage systems with small group size. Among the non-cage systems, less feather pecking was performed in free-ranges than in aviaries (Chapter 2), which agrees with the previous results on commercial conditions (Blokhuis et al., 2007). It has been reported that the risk for feather pecking is lower when an outdoor grazing area is provided because the motivation to peck is redirected to grass or because the distances between individual hens is greater (Mohboub et al., 2004; Shimmura et al., 2008c). Therefore, free-range housing has low risks of fear response, physical stress, claw length, and injurious pecking, resulting in a high rating for the freedom from fear and distress. The low and high ratings of these indicators are mingled in conventional cages and aviary, resulting in variable risks in both systems. Among furnished cages with most of the characteristics of conventional cages, while physical stress is reduced in a small furnished cage, a large furnished cage incurs the disadvantage of increased feather pecking and aggressive pecking (Chapter 1), resulting in poor welfare in large furnished cages.

For the freedom from discomfort, the conventional cage has lower risk of poor welfare compared with the other systems. If the henhouse is windowless and the temperature is controlled, the risk of thermal discomfort is low in all housing systems. However, in these conditions, dust is increased in housing systems with dust baths, such as furnished cages and non-cage systems (Blokhuis et al., 2007). Similarly, the droppings of hens are not perfectly separated in these housing systems, resulting in a higher ammonia concentration compared with conventional cage with a wholly wire floor (Blokhuis et al., 2007). Thus, for the freedom from discomfort, the conventional cage has a lower risk of poor welfare compared with the other systems.

In terms of productivity, most developed furnished cages provide similar production, feed conversion results, and mortality compared to conventional cages (Abrahamsson et al., 1995; Appleby and Hughes, 1995; Abrahamsson and Tauson, 1997; Appleby et al., 2002; Shimmura et al., 2007b). The non-cage systems have the advantages of higher behavioural diversification and increased movement, but conversely they can lead to energy loss. Therefore, feed intake is generally increased in the systems with large floor areas, as reported in previous studies (Abrahamsson and Tauson, 1995; Abrahamsson et al., 1996b, 1998; Tauson et al., 1999; Michel and Huonnic, 2003). Some studies reported decreased egg production and feed efficiency in non-cage systems (Abrahamsson and Tauson, 1995; Abrahamsson et al., 1996b, 1998; Tauson et al., 1999; Michel and Huonnic, 2003), although these tendencies were not found in Chapter 2. In addition, as mentioned above, mortality is considerably higher in free-range systems than in housing systems without an outdoor area, resulting in the high risk of poor production in free-range systems.

With regard to egg quality, very intensive use of the nest box is observed and normally close to 100% of eggs are laid in the nest box in furnished cages (Tauson, 2005; Shimmura et al., 2007b). The proportion of dirty eggs is also similar or even lower in furnished cages than in conventional cages (Tauson, 2005). On the other hand, the risk of misplaced eggs is higher in non-cage systems, especially multi-tiered aviaries and free-range systems, because hens cannot find the nest boxes. Although the proportion of misplaced eggs is decreased now by improvement of management (e.g., rearing conditions), the incidence of misplaced eggs in multi-tiered aviary systems reportedly averages 4.6% (Van Horne, 1996), resulting in an increased number of dirty eggs. The risk of misplaced and dirty eggs is of course higher in free-range systems (Blokhuis et al., 2007). In addition, as mentioned in Chapter 2, free-range systems that allow exposure to sunlight have the disadvantage of pale egg shells.

For immune response, the positive correlation between immune response and welfare level was suggested in Chapter 2. In fact, it has been reported that the immune response is decreased by various stressors: ACTH injection (Puvadolpirod and Thaxton, 2000), heat (Thaxton and Siegel, 1970; Thompson and Lippman, 1974; Gillis et al., 1979), cold (Brown and Nestor, 1973; Subba Rao and Glick, 1977), and behaviours such as aggressive interaction (e.g. Gross and Siegel, 1965, 1973). However, few studies have investigated the relationship between physiological stress and immunity in housing systems for laying hens. The results of Chapter 2 are not fully conclusive, and further investigation of the relationship is needed.

Based on the discussion above, we summarised the advantages and disadvantages of various housing systems in Figure 6-1. Here, the furnished cages were divided into small (<20 hens) and large (≥20 hens) furnished cages for the sake of convenience, and the medium furnished cages (10 hens) discussed in Chapters 4 and 5 were included in the former. The non-cage systems were divided into aviary and free-range, and the floor-rearing, single- and multi-tiered aviaries were included in the former. This figure clarified these characteristics by a "traffic light" system applied by the LayWel project (Blokhuis et al., 2007), and a red area in an indicator indicates a high risk for that indicator. For example, the red area (black area in the monochrome Figure 6-1) in the free-range as an indicator of production indicates that the free-range has high risk in production (decreased production). The advantages and disadvantages are, as a whole, consistent with the results of the animal-based assessment in Chapter 2. Namely, the non-cage systems, especially free-range systems, offered some poor welfare in terms of the freedom from pain, injury, and disease, and some high risks, such as lower egg production and pale eggs, for productivity. On the other hand, the freedom to express normal behaviour was high. The reverse situation was found in the conventional cages, and most of the indicators were rated considerably highly. Among the furnished cages, retaining the advantages of conventional cages, furnished cages were rated higher for the freedom to express normal behaviour than conventional cages, while large furnished cages were rated lower for the freedom from pain, injury, and disease, and from fear and distress, compared with small furnished cages.

Table 6-1. The advantages and disadvantages of each housing system†. Under the 'traffic light' system, black (actually red) indicates high risk, dark grey (actually yellow) medium/variable risk, light grey (actually blue) low risk, and the white area unknown (insufficient data). The black and light grey area in the welfare indicates poor and high welfare, respectively‡.

Indicator		Conventinal cage	Furnished cage		Non-cage	
			Small	Large	Aviary	Free-range
Welfare	Pain, injury and disease					
	Hunger and thirst					
	Normal behaviour					
	Fear and distress					
	Discomfort					
Productivity	Production					
	Egg quality					
Immune reponse						

†Here, the small furnished cage was defined as one for less than 20 hens, and the large furnished cage for more than 20 hens; the floor-rearing, single- and multi-tiered aviaries were included with aviary.
‡What the colors indicate in this figure are as follow:

- : High risk (poor welfare)
- : Medium/Variable risk
- : Low risk (high welfare)
- : Unknown (insufficient data)

The results described in both Chapters 2 and 3 indicate the great usefulness of furnished cages, which is also confirmed in Figure 6-1. Namely, many advantages of conventional cages remain, with furnished cages reducing the disadvantage of behavioural restriction in conventional cages (see Figure 6-1). Actually, in the evaluation by the weighted scoring system in Chapter 2, the total score for small furnished cages was comparable to those of both non-cage systems, while the score for large furnished cages was similar to those of the conventional cages. The large furnished cages have more space, which leads to more behavioural diversification, increased activity, and strong bones, as found in non-cage systems. This suggests that large furnished cages may offer a higher welfare level than small ones if one disadvantage is resolved. The disadvantage of large furnished cages is competition for a restricted number of the resources due to increased group size, and from our previous studies, it seemed that the problem occurred because a resource was placed on one side of the cage ('localised' resource). Therefore, we designed a medium-sized furnished cage with resources on both sides of cage ('separated' resources; Chapters 4 and 5). In Chapter 4, the relationship between social order and behaviour was investigated, and it was confirmed that hens from each rank used the dust bath equally in medium furnished cages with separated resources, while dominant hens had priority for using the dust bath in the furnished cages with localised resources. In Chapter 5, the cage design was evaluated thoroughly by many-sided investigations, and the results of increased activity, decreased competition for the dust bath, and similar frequency of injurious pecking and productivity similar to those of conventional cage were obtained. These results indicate clearly the great usefulness of furnished cages with separated resources, and therefore, this new furnished cage may be world-wide pioneer design that has many advantages as a cage that promotes diversified behaviour. More recently, the size of furnished cages has been increased, e.g. 16 hens (Wall et al., 2004) and 40 hens per cage (Weitzenburger et al., 2005). In the future, further study on the usefulness of separation of resources in larger furnished cages is needed.

In Chapter 3, new welfare assessment system was developed. Most existing assessments aim to certify the farm products are produced with high welfare and to assess housing systems at the farm level. These assessments are needed to assess quickly and to correlate with animal-based evaluation. Based on Bracke's model, a science-based overall assessment system for laying hens with a careful selection of measurements from an animal, environment, and management bases was devised, and the usefulness of the assessment was evaluated by comparing it with the animal-based assessment described in Chapter 2 and the environment-based Animal Needs Index (ANI; Bartussek, 2001). This model showed that freedom from injury, pain, and disease and freedom from discomfort were better secured in the cage system, while non-cage systems scored better for natural behavior and freedom from fear and distress, and this result has strong positive links with the results of the animal-based evaluation in Chapter 2 and with the above discussion. The assessment using this model was more sensitive than ANI and is applicable to cage systems. These results suggested that this model has greater usefulness. Hereafter, further studies at various farms are needed, and there is also room for improvement on the basis of more expert opinions.

In conclusion, in this thesis, the advantages and disadvantages of various housing systems were clarified from various points and a highly useful welfare assessment was developed. These results indicated the high potential value and importance of the design of furnished cages. Although competitions for resources were observed frequently in large furnished cages, this problem can be resolved by separation of resources. Thus, comparison of six housing systems for the first time led to development of a pioneer world-wide welfare assessment system and new type of furnished cage. This thesis can provide many suggestions to producers of accepted housing systems, as well as playing a key role in the dynamically developed animal welfare.

SUMMARY

Animal welfare has progressed rapidly from a concept to laws or guidelines around the world. In the EU countries, regulation of animal welfare has been enforced by law, where conventional cages will be banned from 2012, and a variety of housing systems that consider animal welfare have been developed. In such circumstances, fundamental information about the advantages and disadvantages of various housing systems is needed, and development of modified housing systems that resolve the disadvantages is also essential. In addition, to differentiate and sell the stock farm products produced using these systems, welfare assessments evaluating various housing systems at the farm level need to be developed. In this thesis, typical six housing systems (small and large conventional cages, small and large furnished cages, single-tired aviary, free-range) were built in the same place, where about 300 laying hens were managed for one and half year. The advantages and disadvantages of the six housing systems were first clarified, and on the basis of it, a newly modified housing system eliminating the disadvantages and an overall welfare assessment for evaluating various housing systems at farm level were also developed. The five experiments conducted to achieve these objectives were as follows:

Chapter 1 (Comparison of pecking behaviour in six housing systems): First, behaviours of laying hens in each system were compared in details, by which it was found that the total frequency of beak use was almost the same regardless of the housing system. This result supports strongly a hypothesis "laying hens have an immanently strong motivation to peck something" framed by our previous study. Namely, caged hens may express the motivation for beak-related behaviour by directing it at food, drinking nipples, their own feathers, and cage wires. In other words, the motivation for beak-related behaviour may be common.

Chapter 2 (Multi-factorial investigation of six housing systems): Multi-factorial investigation of six housing systems was conducted by measuring welfare level, productivity, and immune response, and the advantages and disadvantages were clarified from the viewpoint of five freedoms: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury and disease; freedom to express normal behaviour; and freedom from fear and distress. Considering the result of Chapter 1, the frequencies of pecking behaviours such as litter pecking were excluded from the measurement. The non-cage systems, especially FR, have somewhat low ratings for the freedom from pain, injury, and disease, and some disadvantages for production, such as pale eggs. On the other hand, the rating for the freedom to express normal behaviour was high, and the immune response was high in the non-cage systems. While the total welfare score and the immune response of the small furnished cage were comparable to those of both non-cage systems, the evaluations of the large furnished cage were similar to those of the conventional cages.

Chapter 3 (Development of overall welfare assessment): To increase the validity of evaluations and facilitate expansion and maintenance of assessment systems, first a database of more than 1,000 studies on the welfare of laying hens around the world was constructed. On the basis of it, a science-based welfare assessment was devised. The usefulness of our model was evaluated by comparing it with environment-based Animal Needs Index (ANI) and animal-based measurements of Chapter 2. Assessment using our model was more sensitive than ANI and can be applied to cage systems, which suggests that my model has greater usefulness.

Chapter 4 (Relationship between social order and use of resources in new-type furnished cage): The results of both Chapters 2 and 3 indicated the high potential value of furnished cages. However, in large furnished cages, competition for a restricted number of resources was frequently observed due to increased group size, while mobility and comfort behaviour are enhanced by providing a larger total cage area. Based on this result and our previous studies, a medium-sized furnished cage with resources on both sides of the cage ('separated' resources) was designed. It was confirmed that hens from each rank used the dust bath equally in medium-size furnished cages with separated resources, while dominant hens had priority for using the dust bath in the furnished cages with localised resources.

Chapter 5 (Overall evaluation of new-typed furnished cage): In Chapter 5, the furnished cages with localised resources were evaluated thoroughly by many-sided investigations. The results of increased activity, decreased competition for the dust bath, similar frequency of injurious pecking, and productivity compared to conventional cage were obtained. These results indicate clearly the great usefulness of furnished cages with separated resources.

In conclusion, in this thesis, the advantages and disadvantages of various housing systems for laying hens were clarified, and a highly useful welfare assessment system was developed. These results indicated the great potential value and importance of the design of furnished cages. Although the competition for resources was frequently observed in large furnished cages, it can be resolved by separation of resources. These results can provide many suggestions for producers of acceptable housing systems as well as playing a key role in development of animal welfare in the future.

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和文抄録

産卵鶏の福祉的飼育システムに関する研究
- 総合福祉評価法および新型　　システムの開発 -

近年、動物福祉は思想から法律への具現化を急激に始めており、OIE の世界家畜福祉基準を始めとして、世界各国で法律・ガイドラインが制定されている。EU では、2012 年からバタリーケージを廃止とする指令が法律として施行され、様々な代替システムが考案されつつある。このような状況において必要とされるのは、各システムの長短所を明瞭化することであり、その短所を解決する改良型飼育システムを開発することである。同時に、高福祉畜産物の差別化のために、各種の飼育システムを現場レベルで評価する福祉評価法を開発することも必須であると言えよう。本研究では、コンベンショナルなケージシステムから最も開放的な放牧まで、代表的な 6 つの異なる飼育システム（小型・大型バタリーケージ、小型・大型福祉ケージ、平飼い、放牧）を同一機関内に設置して、1 年半にわたり約 300 羽の産卵鶏を継続的に飼育し、各システムの長短所を明瞭化すると同時に、その知見に基づいた福祉評価法および新型飼育システムを開発することを目的とし、以下の 5 つの実験を実施した。

第 1 章（6 システムにおける Pecking behaviour の比較）：まず各システムにおける鶏の行動を詳細に比較検討し、産卵鶏が嘴を使用する合計頻度は、いずれのシステムでも一定であることを見いだした。この結果は、先行研究から立てられた仮説、鶏は何かをつつくという強い動機づけを内在的に保有しているということを強く支持するものであった。すなわち、ケージの産卵鶏は、食草・敷料床つつきを発現できないことによる Pecking behaviour の不足分を、餌・自身の羽毛・ケージワイヤーをつ

つくことで補っている、言い換えればこれらの元となる動機づけは共通していることが明らかとなった。

第2章(6システムの総合評価):福祉レベル、生産性、免疫反応の評価により、6システムを多面的に評価し、Five freedoms（飢えと渇きからの自由、苦痛・傷害および疾病からの自由、恐怖および苦悩からの自由、物理的不快からの自由、正常行動発現の自由）の観点から長短所を明瞭化した。第1章の結果を考慮して、敷料床つつきなどのPecking behaviourの発現量は、評価指標から除外した。非ケージシステム、特に放牧は、正常行動発現の自由についての評価が高くなる一方で、苦痛・傷害および疾病からの自由についての評価は低くなり、また生産面では卵殻色が薄くなる傾向にあった。小型福祉ケージの総合的な福祉レベルおよび免疫反応は、平飼い・放牧と同等に高かった一方で、大型福祉ケージは、バタリーケージと同様の低い評価であった。

第3章(福祉評価法の開発):評価の確実性の向上および評価法の推敲・維持の容易さを達成するため、世界中の産卵鶏の福祉研究1000件以上をデータベース化し、それを基に新たな福祉評価法を開発した。さらに、代表的な評価法であるAnimal Needs Index（ANI）との比較および第2章で得られた動物ベースの評価値との関係から、本モデルを評価した。本モデルおよびANI、いずれの評価法も動物ベースの評価値と強い相関関係にあったが、本モデルは、ANIと比較して福祉レベルの検出力が高く、有用性がより高いことが示唆された。

第4章(新型福祉ケージにおける社会的順位と資源利用の関係):第2・3章の結果は、いずれも福祉ケージの高い潜在価値を示していたが、大型福祉ケージにおいては、活動量が増加する一方で、グループサイズの増加により資源競争が激化することを示唆していた。これらの知見と先行研究を基に、資源競争を緩和させる資源分散型の中型福祉ケージを新たに考案した。従来型の資源集中型福祉ケージでは、上位個体が砂浴び場を優先利用する一方で、資源分散型では、いずれの順位の個体も同等に利用していた。

第5章(新型福祉ケージの総合評価):第5章ではさらに、行動・健康状態・生産性からの多面的測定により、資源分散型の中型福祉ケージを総合的に評価した。資源分散型福祉ケージは、行動の多様化・健康状態の改善という福祉ケージの利点を保持しつつも、運動量が増加するという中型ケージの利点を示していた。また、資源集中型福祉ケージと比較すると、砂浴び場への競争が緩和されており、それにより敵対行動が減少し、生産性が高く維持されていた。これらのことから、資源分散型福祉ケージの高い有用性が示された。

以上の実験から、各種飼育システムの長短所を明らかにすると同時に、有用性の高い評価法を開発した。これらの研究は、福祉ケージの高い潜在価値およびケージデザインの重要性を示していた。大型福祉ケージでは、資源競争が激化する短所が見受けられたが、それは資源を分散することで解決されうることが示された。これらの成果は、システムを採用する生産者サイドへ多くの示唆を与えるのみならず、今後の家畜福祉学の発展においても大きく貢献するものと考えられる。