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BEHAVIORAL AND VOCAL CHARACTERISTICS OF LAYING HENS^UNDER DIFFERENT HOUSING AND FEEDING CONDITIONS

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ABSTRACT

We analyzed the behavioral and vocal characteristics of laying hens in this study, with the aim of assessing the level of animal welfare in laying hen flocks in South Korea. Sixty- four hens (farm A:n=18, farm B:n=18, farm C:n=28) at three farms under different housing and feeding conditions were observed. We analyzed their eating, exploring, resting, preening, ^and aggressive feather pecking, as well as special vocalizations with three replications. Time spent on eating for farm C was higher than for farm A ($P<0.05$). Time spent resting was higher in birds at farm B than those at farm C ($P<0.05$). Time spent eating increased as the group size increased and width of the cage declined, whereas resting and preening tended to increase as the group size and eating time decreased. Two special vocalizations squawk and alarm calls were more frequently recorded at high stocking density. Recording vocalizations could be a useful method to support the analysis of behavior in laying hens. The group size, width of the cage, stocking density, feeding frequency and feeding time were major factors affecting the behavior and vocalization patterns in laying hens. Analysis of these key factors may be utilized as a useful method to measure the level of animal welfare in actual hen farms where various environmental factors worked compositely.

Keywords: Animal welfare, Behavior, Group size, Hy-line Brown, Laying hens, Vocalization.

INTRODUCTION

Animals feel various emotions, such as pleasure, expectancy, curiosity, anger, fear, pain, excitement, and anxiety^(Grandin and Johnson, 2005). Emotionally relevant external events are able to stimulate a complex central nervous network that regulates endocrine feedback and behavior in order to maintain or regain homeostasis. Thus, particular states of mood or emotion may be accompanied by specific behaviors (Manteuffel *et al*., 2004).

Behavioral patterns of livestock can be affected not only by genetic and physiological factors, such as species, age, sex, pregnancy, body condition score, individual characteristics, and hormones (Keyserlingk and Weary, 2007), but also by diverse environmental factors, including the presence/absence of companions, social and sexual behaviors with companions, starvation, thirst, diseases, seasons, shelters, breeding methods, kinds of feeds and bedding, pregnancy and suckling, group sizes, body condition score, handling, separation, moving, and introduction of new animals (Hemsworth *et al*., 1996; Grandin, 2001; Wilson *et al*., 2002; Hagen and Broom, 2003; Petherick, 2005; Solano *et al*., 2007; Vieira *et al*., 2008; Szyszka and Kyriazakis, 2013). Because animal behaviors reflect physical, emotional, and physiological responses to given environmental stimuli, and onemeasurable with minimal invasiveness by the observer, they can be important factors for^easily and^quickly^analyzing the level of animal welfare (Manteuffel *et al*., 2004).

Providing animal welfare that minimize the pain^and discomfort (disease, injury and stress) experienced by farm animals is becoming an important issue both domestically and internationally, due to increasing free trade among nations. Also, good animal welfare practices can provide a supply of healthy and stable livestock products^for human consumption. Studies on animal welfare and behaviors targeting livestock have viewed these issues from various perspectives (Blokhuis and Beutler, 1992; Hansen *et al*., 1993; Savory, 1995; Appleby, 1997; Rodenburg and Koene, 2004; Mench, 2008; Fraser, 2009; Temple *et al*., 2011)

Laying hens raised in henhouses with a^narrow floor area and low height cages, live generally in restricted condition with no perches, nesting boxes, dust baths, and other birds. Cheon *et al*. (2015) reported that restriction in such conditions brings about frequent displacement behavior including frequent movement inside the cage, accompanied by frustration associated gakel calls. When a conflict situation is presented by long duration of displacement behavior, there is a high possibility of the development of stereotype behavior (Mimura, 1997). Usually, laying hens make continuous vocalizations as an auditory signal. A squawk call is a vocalization of high frequency that can be made when the bird is startled and/or experiencing momentary pain and has been reported to be related to feather pecking (Konishi, 1963; Wood-Gush, 1971; Collias, 1987). An alarm call is a rather soft turmoil reaction that occurs in response to the appearance of animals or people, things, and unfamiliar sounds (Konishi, 1963; Collias, 1987). In both of these special vocalizations, external environmental stimuli cause a sudden change in the hen's emotional state. Therefore, squawk and alarm calls are recognized as important vocalizations from an animal welfare perspective.

Due to recent concern about infectious diseases of livestock in Korea, a spotlight has been put on stable livestock products and livestock raising environments and such interest has given rise to animal welfare aware livestock product consumers. But, most henhouses in Korea still adopt a cage or battery type housing, although the concept of animal welfare has recently been introduced. For the welfare of laying hens, the top priority lies in changing cage type henhouses into plane type, aviary, or multistage henhouses. However, in addition to the basic effort in changing hen housing efforts to apply various other aspectsof animal welfare management such as improving stocking density and controlling the systems of ventilation, ambient temperature and relative humidity are also necessary. Systems which conduct real-time monitoring of livestock behavior and sound non-invasive animal-based criteria which can detect the environment surrounding livestock sensitively and promptly. Behavior and sound data accumulated through monitoring can be utilized as a crucial data to assess the level of animal welfare, and monitor improvements.

Although environmental changes have been shown to affect hens' behavior and vocalization, there is little research identifying the key factors affecting animal welfare of commercial laying hen farms in Korea (Sohn *et al*., 2011; Ahammed and Ohh, 2013; Kim *et al*., 2013; Sohn *et al*., 2014; Cheon *et al*., 2015; Sohn *et al*., 2015). In order to better understand the level of animal welfare, we must investigate the actual conditions in which livestock are being reared. Therefore, this study analyzed the behavioral and vocal characteristics of laying hens reared in different housing and feeding conditions.

MATERIALS AND METHODS

Selection of experimental farms: For collecting samples of behaviors and vocalizations, we selected three farms ranging in size from 7,200 to 55,000 hens per poultry house kept under different environmental conditions. Sampling was conducted from 12 to 22 September 2012. Detailed information on the housing conditions on each farm are given in Table 1. Laying hens at the three farms were all Hy-line Brown. The photoperiod of the three farms was 16:8h, with lights on from 04:00 to 20:00h. The cage width (cm) allowed per bird was 13, 9.5, and 7.9, and stocking density (cm^2/bird) was 455, 333, and 430 in farm A, B and C, respectively. All three farms used the same kind of commercial laying-hen feed (Hyundai Feed Co. Ltd., Korea). Hens were fed six times per day at farm A, three times per day at farm B, and seven times per day at farm C. All animal-based procedures used in this study were approved by the Institutional Animal Care and Use Committee at Konkuk University (KU12097).

Table 1. Experimental conditions for laying hens at the three farms

Items	Farm A	Farm B	Farm C
Farm capacity (birds/house)	7,200	13,000	55,000
Group size(birds/cage)	2	3	7
Cage size (width × length × height, cm)	26 × 35× 42	28.5× 35 × 42	55 × 54.7 × 55
Stocking density (cm^2/bird)	455	333	430
House condition	Open	Open	Windowless
Ventilation system	Free	Free	Forced
Ambient temperature (°C)	21–27	21–27	21–25
Light:dark (h)	16:8	16:8	16:8
Breed	Hy-line Brown	Hy-line Brown	Hy-line Brown
Age (weeks)	37	27	47
Feeding frequency (count/day)	6	3	7
Feeding time (h)	04:00, 7:00, 10:00, 13:00, 15:00, 18:00	04:00, 10:00, 17:00	07:00,09:00, 11:00, 13:00, 15:00, 17:00, 19:00

Behavioral recording and observation: For behavioral recording, a total of 64 (farm A = 18, farm B = 18, farm C = 28) hens located inside the middle zone of three commercial farms were selected randomly. Closed-circuit television cameras connected to a multiplexer were installed at a height of 150–180cm in front of the cage so that the movement of the hens could be seen clearly^ (Fig 1). All behaviors were recorded continuously during the light period (16h per day). Recording samples were collected from each farm every 3 days, from 12 to 22 September 2012.

The behaviors of each of the 64 laying hens observed for 16h daily for 3 days were analyzed. Categories of behavior included eating, resting, exploring, preening, and aggressive feather pecking (for full^definitions, see^Table 2). Expressed behaviors were recorded as a unit of time or frequency.

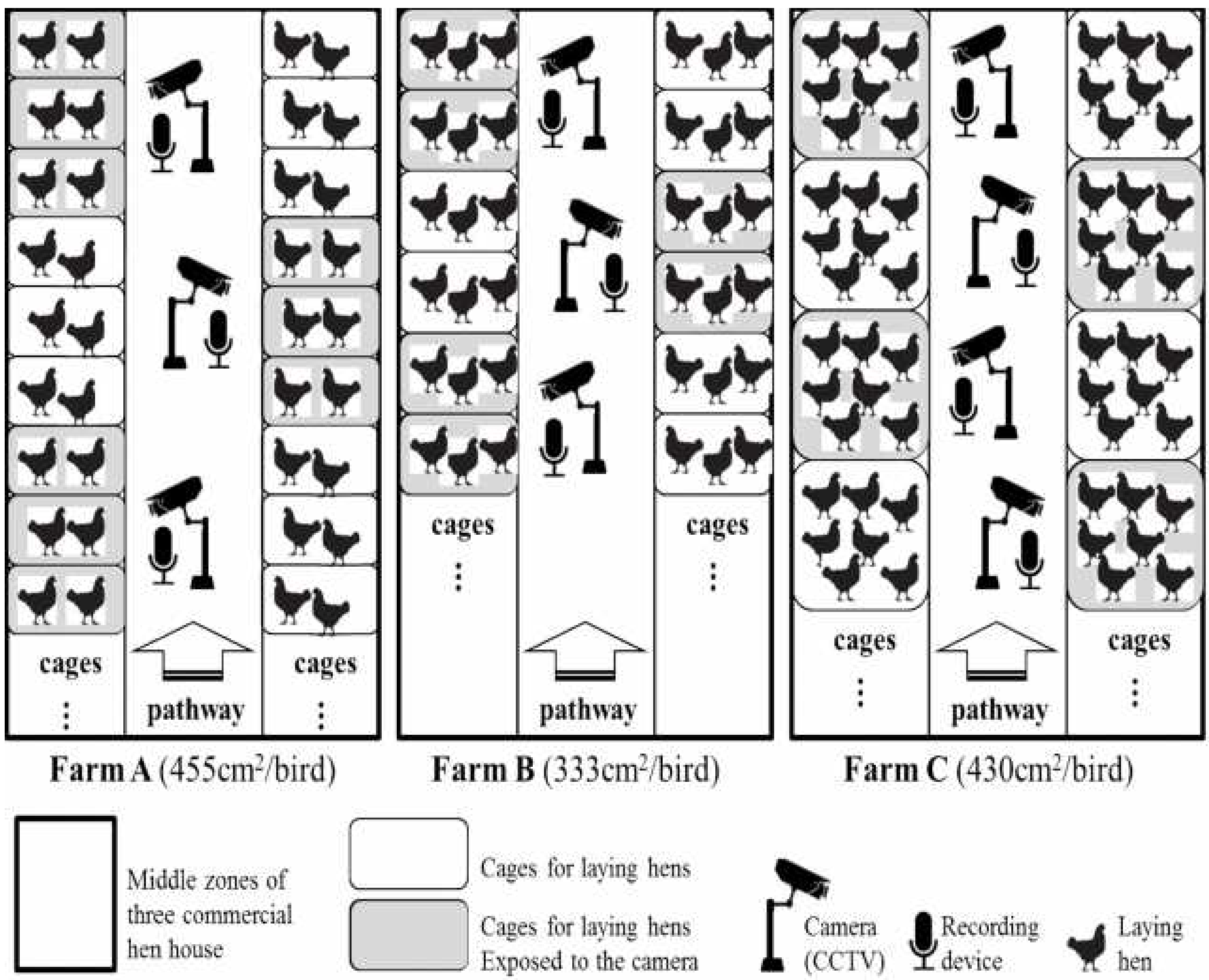


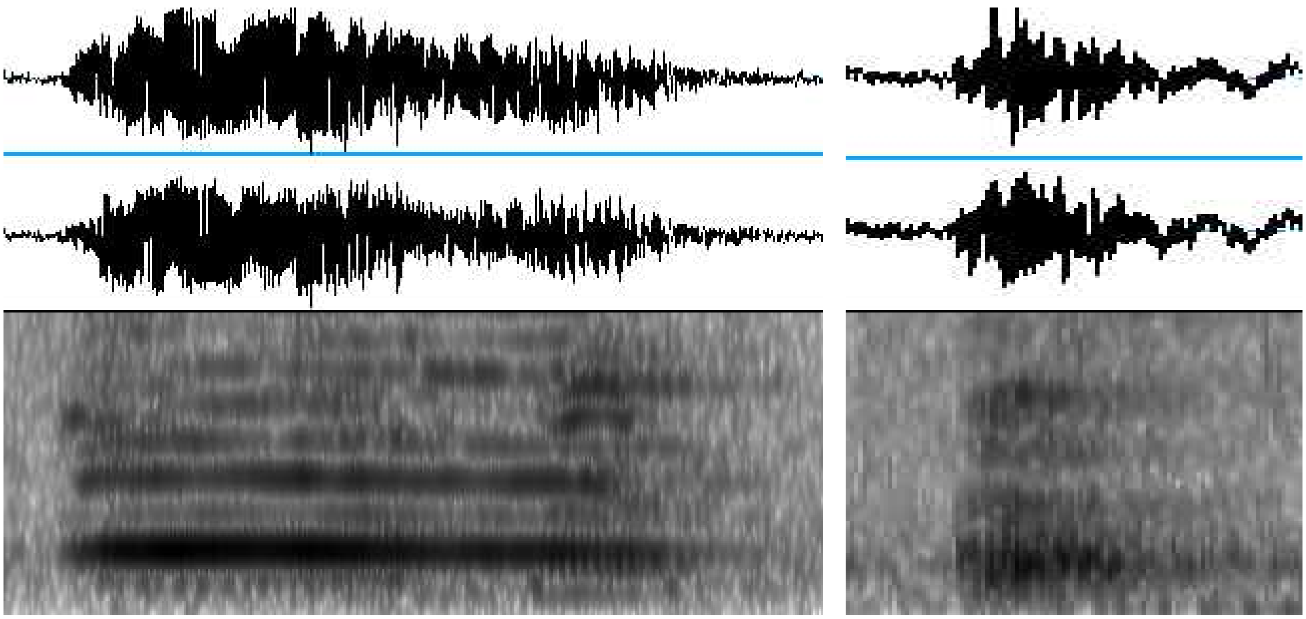
Figure 1. Diagram of behavior and vocalization recording of three commercial laying hen farms

Table 2. Descriptions of recorded behaviors

Behavioral category	Description (observation standard)
Eating	Picking up^and swallowing feed from the feeder^by bending the head down to the shoulder
Resting	Lower part of the bird from belly to hips touches the ground while sitting with both legs bent
Exploring	All standing behaviors except eating which includes^pecking, walking, drinking and observing by turning the head
Preening	Beak moves while touching another part of the bird’s own body
Aggressive feather pecking	Sharp forward movements with the head towardanother bird

Vocalization recording and analysis: Audio recordings were made over a continuous 24-h period for three days (three replications). ^For audio recording, vocalization samples in middle zone of each of the three hen houses where the noise from ventilation fan or automatic machine could be minimized were selected. At each farm, recording devices (PCM-M10, Sony, Japan)^were installed in front of each camera at the height of 1.5m in the central passage. The recorded data was saved in 24kb, more than 16bit, and as a wave file.

This study focused on the expression levels of squawk and alarm calls. In this study, we recorded the expression frequencies of squawk and alarm calls on 24h at each farm. The frequencies of squawk and alarm calls were counted by audio engineering software (sound forge v.9.0, ^Sony, Japan).In regards to squawk and alarm calls, only those with intensity of over 55dB and withclear identification of sound manifestation on audio and spectrum of program were counted. Accuracy of measurement was enhanced through such methods and errors that could be generated due to differences in the quantity of laying hens per group between the three farms was minimized by adjusting the counting range of the samples. Below Figure2 illustrates the spectrum and spectrogram sample of a squawk and alarm call.



Squawk call (0.29sec) Alarm call (0.13sec)

Figure 2. Spectrum and spectrogram samples for squawk and alarm call of laying hens

Statistical analysis: The mean time and frequency values for behaviors and vocalizations, respectively of laying hens were compared among the three farms. Factors were analyzed by using analysis of variance or the general linear model procedure in SAS (ver. 9.1, SAS Institute, Cary, NC, USA). When a statistically significant difference was recognized in the analysis, Duncan's multiple range test was used to detect statistical significance (*P<0.05*) among treatment groups. Meanwhile, the average time and frequency values for diurnal patterns of behaviors and vocalizations per individual of laying hens were also compared among the three farms.

RESULTS AND DISCUSSION

Comparison of behavioral expression time and frequency among different feeding conditions (Table 3): The average time spent on eating, resting and exploring per individual hen among different three farms is given in Table 3. Hens at farm C (430cm^2/bird) spent the longest time eating (average 551.3min) and showed significant differences when compared with farm A (455cm^2/bird; average 452.3min) (*P<0.05*). This likely occurred because farm C had the biggest group size (7 birds/cage) and hens at farm C were fed at the greatest frequency, seven times daily. Also, seven birds could not eat simultaneously because the cages at farm C were deep with a narrow width (7.9cm per bird). The impact of feeding competition is another factor that likely increased eating time at farm C. Mimura (1997) noted that cages that were short and wide improved feed efficiency because cages which were long and narrow ^ increased laying hens' movement and stress when eating. On the other hand, the stocking densities at farms A and B were 455and 333 cm^2/bird, respectively, and no differences in eating time were found.

Hens at farm A and B spent similar amounts of time resting (279.8 and 285.2min respectively), whereas this value was only 82.1 min at farm C (*P<0.05* vs. farm B). This difference appeared due toan increase in eating time with the group size of seven birds per cage and lack of cage width allowed per bird likely hindering resting at farm C. Exploring time among the three farms showed no significant differences. Exploring time tended to be affected by group size (farm A= 2, farm B= 3, and farm C= 7 heads/cage) and the cage width (farm A= 13, farm B= 9.5, and farm C= 7.9cm/bird) allowed per bird when eating.

Table 3. Time spent and expression frequency of behaviors and vocalization in laying hens at the three farms with different feeding condition

Items	Farm A	Farm B	Farm C	Average
Time (min/16h/individual)				
Eating	452.3±52.7[b]	447.3±87.5[ab]	551.3±33.2[a]	483.6±58.6
Resting	279.8±57.4[ab]	285.2±105.9[a]	82.1±34.5[b]	215.7±115.7
Exploring	227.9±46.1	227.5±78.1	326.6±39.7	264.3±71.5
Frequency (no./16h/individual)				
Preening	80.3±35.1	52.3±57.7	33.3±18.3	55.3±23.6
Aggressive feather pecking	35.6±12.3	52.1±40.8	20.1±14.1	35.9±16.0
Frequency (no./24h/individual)				
Special vocalizations	42.4±16.6	74.3±36.0	50.8±25.3	55.8±20.0

Means with different superscript letters are significantly different (*P<0.05*).

Table 3 also shows the average frequencies of preening and aggressive feather pecking per individual Hens at farm A showed the greatest frequency of preening (80.3 times per 16h), followed by those at farms B and C, but there was no significant difference among farms. The result likely stemmed from the complex relationship between resting time and group size, and wasn't affected by stocking density.

Aggressive feather pecking is a social behavior expressed in individuals, and in severe cases, it is an abnormal behavior that leads to cannibalism (Hughes and Duncan, 1972). In this study, there was no significant difference among farms, but the highest frequency of feather pecking was recorded at farm B. The stocking density of farm B was 333cm^2/bird, which is far poorer than the certified stocking density of domestic non-antibiotic-treated hens (420cm^2/bird). Other studies have also noted a connection between aggressive feather pecking and stocking density (Hughes and Duncan, 1972; Savory, 1995). Nicol *et al.* (1999) reported that flock size as well as stocking density could influence feather pecking. According to Bilcik and Keeling (2000), when fixing stocking density at 2000cm^2/bird and changing stocking size within the cage to 15, 30, 60, or 120 birds, the frequency of aggressive feather pecking increased significantly as group size increased. In the present study, however, farm C had the largest group size, but the lowest frequency of feather pecking. This finding suggests that the relationship between group size and aggressive feather pecking is weak when breeding groups are less than 15 birds and under sufficient low stocking density. Therefore, it appears that stocking density has a greater influence on this behavior than group size per cage.

Comparison of diurnal patterns of behaviors among different feeding conditions: The diurnal patterns of behaviors (mean time and frequencies) among three farms with different feeding condition were compared (Figure 3-7).^Eating time (Figure 3) at all farms increased after lights-on. Later, before and after the main egg-laying time (10:00-11:00h) and feeding time, the increase in eating behavior was repeated and eating time drastically decreased before lights-out. The eating time of farms A and B showed a clear pattern based on feeding times, while consistent intake was realized during the day in farm C. Savory (1980) reported in his review that the most important factor affecting diurnal feeding patterns of domestic fowl is reproductive state (layers or non-layers), while the strain of fowl, age of birds, particle size, diet, cage shape, and lighting also had some influence. In most commercial poultry farms, however, other factors besides fixed feeding amount and feeding time may have an effect. Savory and Maros (1993) reported that feeding frequency had a significantly effect on eating, drinking, walking, standing, and sitting behavior of broiler breeder chickens (*P<0.05*).In this experiment, the hens' eating patterns tended to be directly influenced by feeding frequency and feeding time. According to Savory and Maros (1993), the behaviors that appear to best reflect feeding motivational state were walking before feeding time and drinking, pecking floor litter, and sitting after feeding time. We also observed these behaviors before and after eating at the three farms. However, the duration of eating after feed was provided varied among the three farms, with farm A showing the shortest time. That is, eating time increased and decreased sharply before and after each feeding time at farm A, where hens were fed six times a day. This trend suggests that feed is quickly consumed at farm A, likely because the cage width per bird (13cm) was sufficient so all hens could eat simultaneously. At farm B, where hens were fed three times a day, eating time increased less sharply at each feeding time compared to that of farm A, reflecting the fact that the amount of feed given to each bird per feeding time at farm B was more than that at farm A. At farm C hens ate steadily throughout the day, which appeared to result from frequent feeding (seven times daily, ca. every 2h) but also the relatively narrow cage width per bird (7.9cm), which lead to a continuous competition for feed. The first daily feeding was at 07:00 at farm C, so the expression of eating behavior was low between 04:00 and 06:00h. Savory (1980) reported that more intensive eating occurs around sunset than at sunrise in laying hens. Similarly, we found that at farms B and C, eating time was very high around 18:00h.

Resting time (Figure 4) was inversely proportional to eating time. There were differences in each farm, but resting peaked at 09:00 and between^15:00–16:00h. In particular, resting time increased significantly between 19:00–20:00h, in the hour before the lights were turned off and when eating was almost finished. The diurnal patterns of resting at farms A and B were similar, whereas hens at farm C rested little throughout the day. This pattern at farm C likely reflected the interaction between frequent feeding and large group size.

Although there were no clear differences in exploring time (Figure 5) among the farms, this behavior was expressed mainly between 11:00–13:00h and 15:00–17:00h. In farm C, however, exploration peaked between 04:00–07:00h, which was the period between lights-on and the first feeding. However, hens at farm C may have reacted more sensitively to light because they were kept in a windowless poultry house. In addition, unlike farms A and B, feed was not given until shortly after lights-on at farm C.

We also found few differences in the diurnal pattern of preening behavior among the farms (Figure 6). At all three farms, the frequency of preening was greater in the morning than in the afternoon and evening. The maximum preening frequency occurred at 06:00 at farm A, from 08:00 to 09:00 at farm B, and at^05:00 at farm C.

Aggressive feather pecking (Figure 7) increased between 09:00 and 17:00 at farms A and B, whereas it tended to increase between 05:00 and 07:00 at farm C and remained low thereafter.This tendency is similar to exploring behavior, indicating that aggressive feather pecking is likely to be expressed while hens were exploring. Blokhuis and Beutler (1992) reported that feather pecking is associated with psychological fear in laying hens and TI (tonic immobility) is consistently presented as anentity resulting in a high degree of feather pecking behavior. According to a study by Hansen *et al*. (1993), TI duration was longer in laying hens raised in cages compared to those in amultistage henhouse. Savory (1995) has classified feather pecking into GFP (gentle feather pecking) and SFP (severe feather pecking) based on its intensity. Although GFP is a behavior associated with strong explorative traits, SFP is a behavior which causes feathers to be plucked and skin damage and it was mainly associated with a power struggle or competition within group (Rodenburg and Koene, 2004). Such SFP was observed in the current experiment associated with competition for resources. At 05:00h, SFP was observed frequently in front of the feeding rack among laying hens at farm C. Hens likely showed this competitive behavior because lights had been on for 1h but no new feed had been provided. Thus, the expression of SFP at farm C was a redirected eating behavior. The frequency of SFP increased at farm B between 08:00 and 11:00h. Because this time span is consistent with the laying time, the hens at farm B, which had the highest stocking density, were likely competing to secure a position for laying.

Occurrence frequencies of squawk and alarm calls among different feeding conditions: Table 3 presents the average frequency of squawk and alarm calls of the laying hens at the three farms. Although there was no significant difference in the frequency of both special vocalizations among the farms, the frequency was directly proportional to stocking density, with the greatest frequency at farm B (average 74.3 times). The hens at farm B also showed the most frequent SFP. These results are consistent with several studies reporting that special vocalizations, including squawk call, are correlated with the frequency of feather pecking behavior (Konishi, 1963; Wood-Gush, 1971; Collias, 1987; Bright, 2008).

Diurnal pattern of squawk and alarm call frequencies among different feeding conditions: The diurnal patterns of squawk and alarm call (mean frequencies) at the three farms are shown in **Figure 8**. Squawk and alarm calls tended to be infrequent during the egg-laying time (06:00–10:00h), but more frequent between 14:00 and 19:00h.At this time, farms with higher stocking density clearly showed a higher frequency of special vocalizations. There are several reasons why special vocalizations increased in the afternoon. First, in the morning, hens make strong and continuous laying vocalizations, while the afternoon is the time when they can concentrate more on external stimuli once laying is finished. Squawk and alarm calls at farm B showed a remarkably high frequency between 14:00 and 21:00h. Hens tend to make a cautionary sound in response to changes in the external stimuli, and respond sensitively and together as a group to the abrupt change of such sounds. As reported by Mimura (1997), the sensitive reaction of hens at farm B likely reflected the higher stocking density. Also, feed was given only three times a day at farm B (at 04:00, 10:00, and 17:00h),^so the frequency of vocalization increased in association with the long feeding gap. Within the windowless hen house at farm C, the special calls were most frequent during the lights-on period (between 04:00^and 20:00h), showing that visual stimuli affect vocalizations as well as the behaviors of the laying hens.

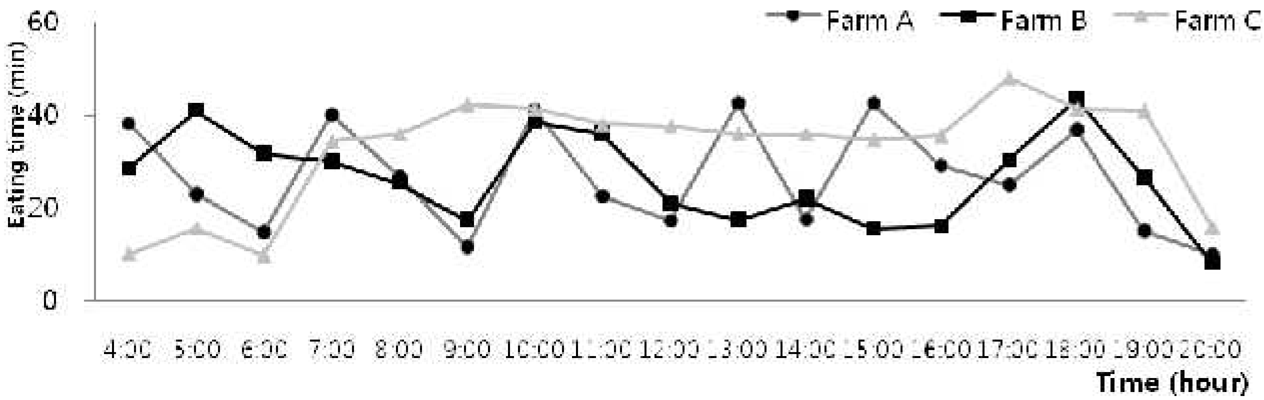


Figure 3.^Diurnal behavioral pattern of eating in laying hens during the lights-on period at three farms with different feeding conditions

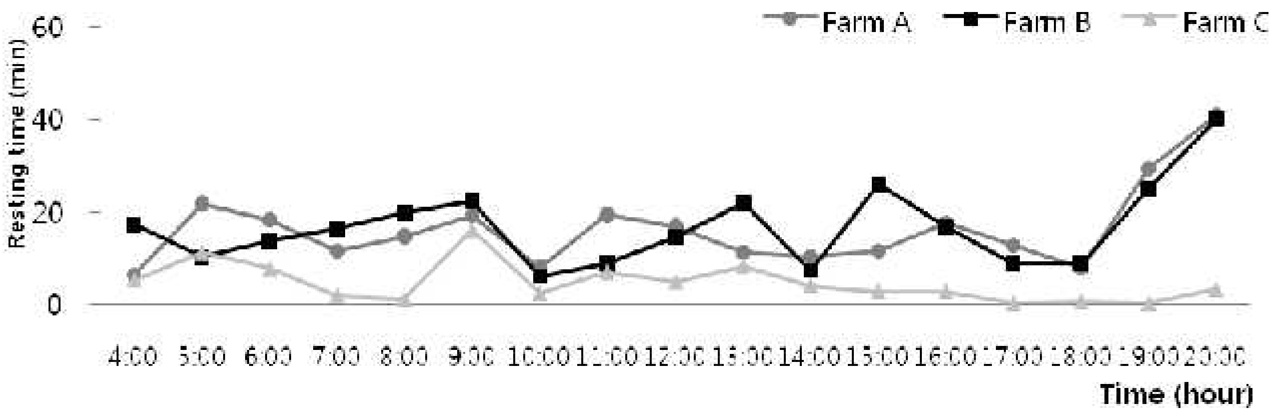


Figure 4. Diurnal behavioral pattern of resting in laying hens during the lights-on period at three farms with different feeding conditions

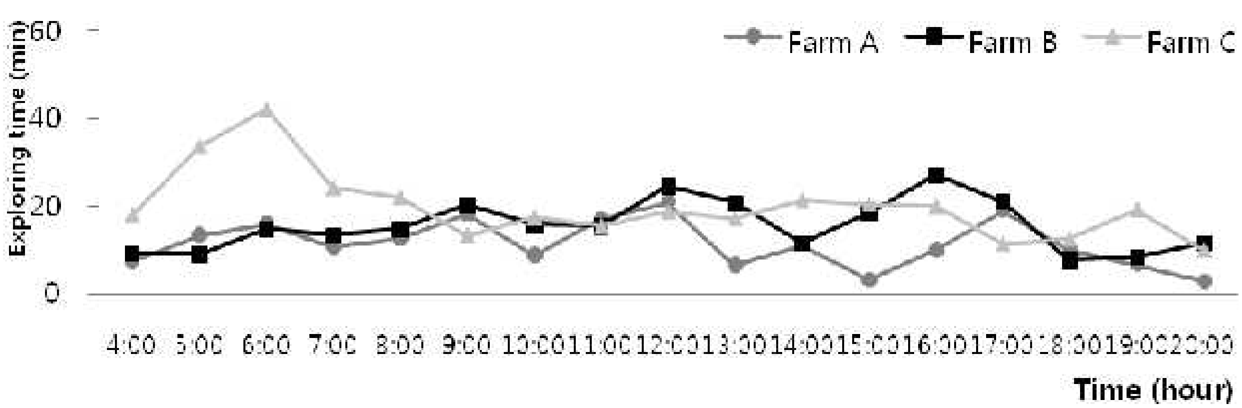


Figure 5.^Diurnal behavioral pattern of exploring in laying hens during the lights-on period at three farms with different feeding conditions

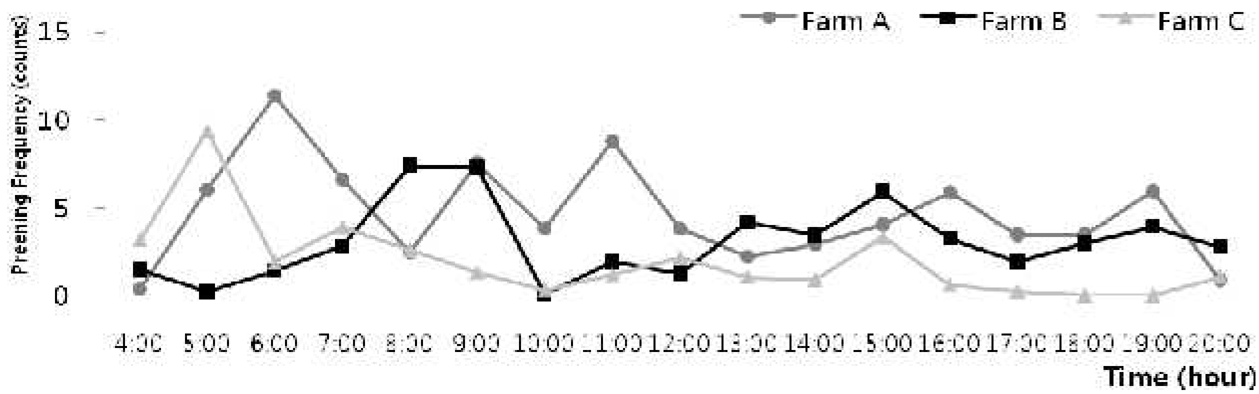


Figure 6. Diurnal behavioral pattern of preening in laying hens during the lights-on periodat three farms with different feeding conditions

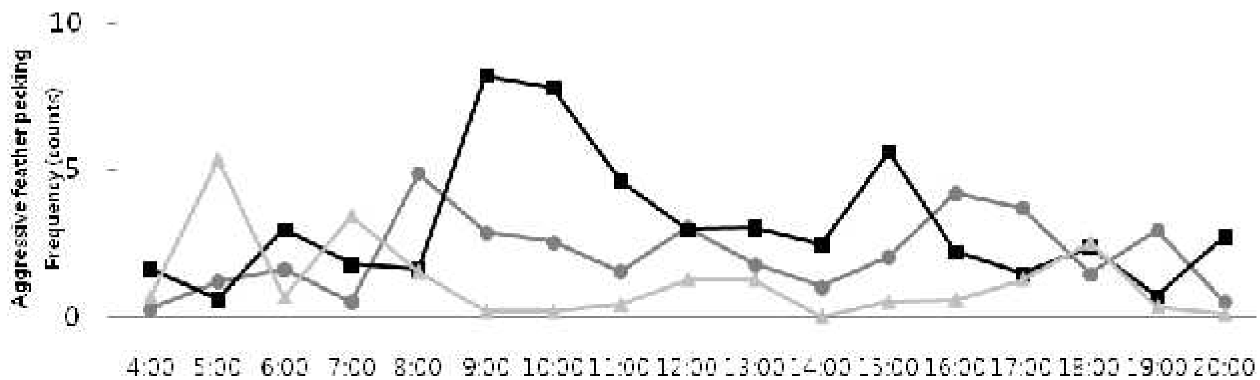


Figure 7. Diurnal behavioral pattern of aggressive feather pecking in laying hens during the lights-on period at three farms with different feeding conditions

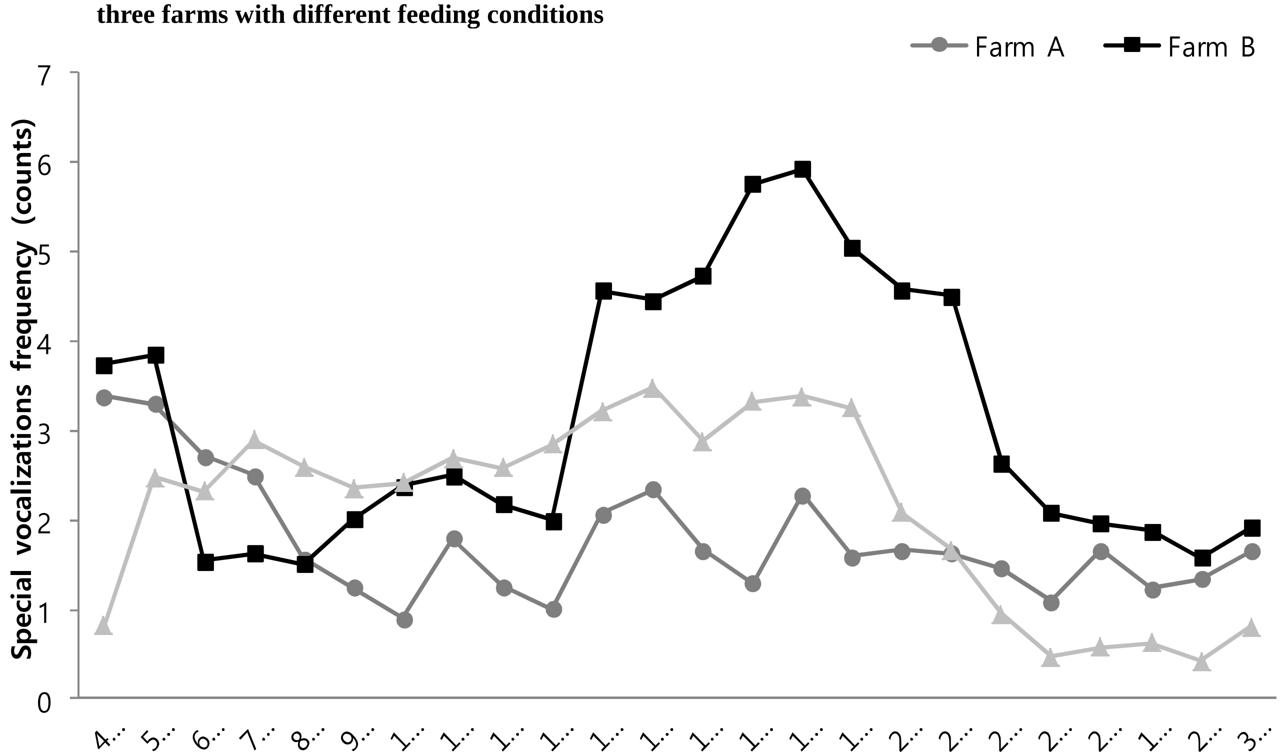


Figure 8. Diurnal pattern of squawk and alarm callsby laying hens at three farms with different feeding conditions

Conclusion: The behaviors of laying hens in cages were affected by some housing and feeding factors. Behaviors of eating and resting were especially affected by group size and the width of the cage. There was no significant difference in aggressive feather pecking in this study among farms,but the highest frequency of feather pecking was recorded at farm B (333cm^2/bird) where stocking density was highest. Moreover special calls tended to be closely related with stocking density especially in the afternoons. Recording vocalizations could be a useful method to support the analysis of behavior and welfare in laying hens.

Our findings are important because this study was conducted at actual commercial caged laying hen farms in Korea. The group size, width of cage, stocking density, feeding frequency and feeding times were the major factors influencing diurnal pattern of behavior and vocalization in laying hens. Analysis of these key factors can be utilized as a practical method to measure the level of animal welfare in commercial hen farms where various environmental factors worked compositively.

Further research illustrated below needs to^be conducted to overcome some ofthe limitations of the current study. Since 1) Stocking density and 2) group size are factors associated with direct physical damage to the^hen followed by feather pecking, there is a necessity to conduct similar behavior and sound experiments under controlled conditions. Also, factors such as 3) ambient temperature and relative humidity and 4) ammonia and carbon dioxide concentrations which were^not been incorporated into this experiment but may have very crucial influence on behavior and sound in reality shall be re-considered.

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