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The effects of furnished cages on the behaviour of laying hens in the post-stress adaptation period

Research Article

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ABSTRACT

The aim of the study is to investigate whether housing laying hens in furnished cages in post-stress adaptation period causes any changes in behaviors or not. Due to the affects of animal welfare on production performance, the relationship between behavioral changes and egg production has also been studied. In the present study, 22 weeks old, 32 laying hens were used. The hens were subjected to transport. The transport procedure, including loading and unloading took 8 hours. Just after the transportation, birds were randomly divided into two groups as furnished and conventional cages; each consists of two subgroups with 8 hens. It was ensured that the hens in subgroups were unfamiliar with each other to induce social stress. On the top of each cage, a camera was fixed and continuous recording was done for 24 hours for 6 days. The behavior of animals was scored by time sampling method. Eating, drinking, resting, preening, wing flapping, tail-wagging, stretching, ground-scratching, gentle pecking, stereotyped and aggressive pecking behaviors were scored. In addition, the locations of the hens were also determined in furnished cages. Frequency of eating, drinking and ground-scratching behaviours significantly increased, but tail-wagging behaviour tended to increase in hens housed in furnished cages. On the other hand, resting, stretching and aggressive pecking behaviours significantly decreased in hens housed in furnished cages. In addition, the use of perch and nest rate in furnished cages significantly increased from the second day. In the conclusion, cage furnishing improves some comfort behaviour such as ground-scratching and tail wagging and decreasing aggressive pecking in laying hens. Therefore, it would be beneficial to keep stress-exposed hens in furnished cages in the post-stress adaptation period.

Keywords: laying hens, cage furnishing, animal welfare, behaviour, stress

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Introduction

Protection of poultry from stressors is crucial to improve production performance and animal welfare. Although many precautions are taken to reduce the stressors, it is not possible tocompletely eliminate stress factors in intensive poultry production. Laying hens are affected by various stressors such as fear, heat, cold, poor ventilation, physiologic or nutritional stress (Harvey et al., 1983; Mirfendereski et al., 2015; Scanes, 2016). Moreover, they are exposed to stressors more often due to their longer lifespan. For example, laying hens experience more episodes of transport than other types of chickens by first transferring from the hatchery to the growing pens, afterwards to the laying pens (Mitchell & Kettlewell 2004). When unfamiliar chicken put together in the same cage social stress occur due to the establishment of a new pecking order. Therefore,

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social stress seems to be inevitable for laying hens as they will be re-grouped after above mentioned transfers. (Cheng and Fahey, 2009). The detrimental effects of stress on laying hens range from reduced growth rate (Lara and Rostagno, 2013), poor production performance (Daghir, 2008), impaired immune response, decreased resistance to diseases, (Mashaly et al., 2004) and increased mortality (Hunter et al., 1999). Different approaches are considered to minimize the effects of stress in poultry industry (Rosales, 1994; Jones, 1996; Gamba et al., 2015). Cage furnishing is one of the methods used for this purpose (Altan et al., 2013). Furnished cages, also called enriched or colony cages, are cages for egg laying hens, which reduce welfare concerns and allow hens to express natural behaviors, which were observed in wild ancestors. Unlike conventional or battery cages, furnished cages are equipped with perch, nest, dust bath or litter and provide more cage height. Ban of conventional cages in laying hens industry accelerated the development of alternative housing systems such as furnished or enriched cages (Rodenburg et al, 2005). Consumers' preference for the products of poultry housed in housing systems that improve animal welfare has further increased the interest in furnished cages (Chang et al., 2010; Lu, 2013).

It was reported that, furnished cages increased both bone development and motor activity (Rönchen et al., 2010), improved feather and claw condition , and immune response (Nazar and Marin, 2011, Shimmura et al., 2010) in laying hens. In addition, it has been reported that furnished cages improve hens' welfare, by decreasing fear, aggression and pecking behavior (Gvaryahu et al., 1994).

Because environmental changes have a significant effect on behavioral responses, one of the best ways to detect the effects of furnishing cages on hens is to monitor changes in behavior (Li et al., 2016). Indeed, many behaviors have been using as an indicator of stress and welfare. However, behavior exhibited by hens may be affected by housing conditions and also by genetic, epigenetic factors and previous experiences (Janczak et al., 2007). For this reason, it is necessary to monitor the changes that occur in the behavior when evaluating the positive or negative effects of the furnished cages (Dawkins, 1999).

It has also been previously reported that furnished cages mitigate some of the effects of stress (Pohle and Cheng, 2009). However, the effects of furnished cages after stress exposure have not been studied yet. The changes in behaviors are appropriate parameters for both researchers and farmers to evaluate the animal's condition; therefore, the subject should be examined in more detail.

The aim of the study is to investigate whether housing laying hens in furnished cages in post-stress adaptation period causes any changes in behaviors or not. Due to the affects of animal welfare on production performance, the relationship between behavioral changes and egg production has also been studied.

Materials and Methods

Animals and experimental procedures

Bird: The experimental procedures were conducted in accordance with the rules of the Local Ethic Committee of Istanbul University. In the present study, 22 weeks old, 32 Lohmann Selected Leghorn (LSL) laying hens were used. Hens were purchased from a commercial poultry company after the hatching and reared in standard battery cages.

Stress procedures: The hens were exposed to transport and social stress in this experiment. The hens were transferred from the rearing pens to our institute's facilities by a poultry transport truck. The transport procedure, including loading and unloading took 8 h. Status of the birds was checked at regular intervals during transportation. Food and water were not supplied to the birds during transportation. Just after the transportation, birds were randomly divided into two groups as furnished and conventional cages; each consists of two subgroups with 8 hens. It was ensured that the hens in subgroups were unfamiliar with each other to induce social stress.

Cages and housing condition: The hens were housed in wire cages (wide 100× deep 100× high 150 cm) during post transportation period. The main cage area was 1250 cm2 per hen. Both of two type cages were equipped with feeder and drinker. For the furnishing, cages were equipped with a nest box, and a perch, the floor was filled with wood shavings. The nest boxes were built of water-resistant plywood (width = 30 cm, length = 45 cm, height = 17 cm). An appropriate gate was provided at the rear side of the nest box. A round wooden stick was installed 20 cm above the floor of the cage and served as a perch. In addition, polypropylene

string bunches of bright colors were hanged in the cages for pecking. The floor of the standard cages was made of wire mesh (3 × 3 cm). The pen was lit up with daylight, artificial lighting was not used. The hens received about 12 hours of daylight. The mean temperature a day in the pen was 22.8 ± 1.5°C during the observation period. Standard commercial diet and tap water were provided throughout the experiment. Protein, mineral and energy content of diets were calculated considering the requirements of the birds, according to Lohmann Management Guide for Laying Hens. A standard vaccination program was carried out.

Video recording and observation: On the top of each cage, a camera was fixed and continuous recording was done for 24 hours for 6 days. Images from each camera were recorded by the recording device as 35 minutes of digital audio tape (DAT) file. The behavior of animals was scored by selecting two videos for morning (between 07: 00-08:30), noon (between 12:00-13:30), and evening (between 18: 00-19:30) for each cage. Totally 144 video files were selected for 6 days. Two different observations were carried out to score behaviors. First, video recording was stopped at intervals of 200 seconds to determine the number of birds eating, drinking, sitting or lying and preening. In addition, the locations of the chickens (on the top of the nest, perch, and floor and in the nest) were also determined in furnished cages. Then, the video stream, that was stopped, was reanimated for about 5 seconds of every sample point, and immobile or moving hens were also detected. Thus, data was obtained at 10 sample points for each video file. At the second observation, the video was observed continuously from beginning to the end. Wing flapping, tail shaking, stretching, ground scratching, gentle pecking, stereotyped and aggressive behaviors were scored.

Definitions of observed behaviors: Eating: Standing in front of the feeder or ingesting feed. Drinking: Standing in front of the drinker or drinking water. Standing: Standing up without any activity. Moving: Walking or running between two points faster than normally observed. Resting (Lying or sitting): Sat or lay down on the floor, without any other activity. Preening: Grooming of the feathers with the beak. Ground-scratching: Scratching the floor with the feet. Aggressive pecking: Pecking each other in an aggressive manner (with the recipient bird moving away). Stereotyped behavior: Repeated walking in front of the wire (as if trying to escape) or continuous pecking of the cage. Stretching: Stretching the leg and wing of the same side of the body. Wing-flapping: Flapping both wings at the same time. Tail-wagging: shaking the tail. Feather ruffling: Ruffling all the feathers and shaking the body. Gentle pecking: Gentle pecking of another hen's feather, resulting without a reaction from the recipient hen (It seems to grooming each other).

Egg production: The number of eggs, including cracked, were recorded daily. Collected eggs were weighed. Hen-day egg production ratio (EPR %), and egg mass were calculated. Following formula was used for calculation of EPR and egg mass respectively. EPR= (Total number of eggs produced on a day / Total number of hens present on that day) x 100. Average Egg Mass = Percent egg production ratio x average egg weight.

Statistical analysis: In this study, independent samples t-test and ANOVA (parametric tests) were used to compare the means between groups, while the Mann-Whitney U and Kruskal-Wallis tests were nonparametric alternatives to independent samples t-test and ANOVA, respectively. Parametric tests are based on the assumption that the samples come from populations that are normally distributed. Also, parametric statistical tests assume that there is homogeneity of variance. Therefore, Levene's test was used to assess variance homogeneity, which is a precondition for parametric tests such as the t-test and ANOVA. If the significance from this test is less than 0.05, then variances are significantly different and parametric tests cannot be used. If the normality and homogeneity of variance assumptions are not met, either Mann-Whitney U test or Kruskal Wallis test are used depends on number of groups. For this study, Levene's test was not significant for "perch". Thus, the assumption of homogeneity of variance was met. On the other hand, Levene's test was significant for "inside of the nest" and "top of the nest". Thus, the assumption of homogeneity of variance was violated. Shapiro-Wilks test was used to test the assumption of normality for the K levels of the independent variable. The assumption of normality was met for the perch, but violated for top of the nest and inside of the nest. According to these results, ANOVA

test was used for the perch, Kruskal Wallis test was used for "top of the nest and inside of the nest" to determine whether there were significant differences between the means of six days for each variable. Tukey HSD post hoc test was used in order to find out between which days the difference for perch variable was present.

Results

Frequency of eating and drinking behaviors increased in hens housed in furnished cages compared to the hens housed in conventional cages (P = 0.001, Z = -7.521, and P = 0.013, Z = -2.488, respectively, Table 1). It was also found that the ground-scratching, which is considered as a component of feeding behavior, was higher in the furnished cages (P= 0.029, Z = -2.178). No significant difference was detected between furnished and conventional cages in gentle pecking (P = 0.213, Z = -1247). Resting activity was found to be higher in the conventional cages (P = 0.001, Z = -3.514,). On the other hand, there was no significant difference in terms of standing and moving behaviours between furnished and conventional cages (Table 2).

The effect of furnished cages on comfort behavior is presented in Table 3. There were no significant differences between furnished and conventional cages in frequency of preening, feather ruffling, and wing-flapping behaviours. On the other hand, there was a tendency in tail-wagging in hens housed furnished cages (P = 0.079, Z = -1.755). Tail-wagging slightly increased in hens housed in furnished cages.

Stretching behaviours (leg or wing stretching) in the furnished cage birds was less than that of the conventional cages (P = 0.038, Z = -2.080). Data of aggressive pecking and stereotyping are presented in Table 4. Aggressive pecking was higher in hens housed conventional cages, compared to furnished cages (P= 0.019, Z= -2.346,). No significant difference was detected in stereotyping behaviours (P = 0.702, Z = -0.383).

Table 1: The effects of cage furnishing on feeding and exploring behaviours in laying hens

	Conventional cage (n = 16)	Furnished cage (n = 16)	*P* value	Z (Mann Whitney U)
Eating	1.68 ± 2.131 x 0.076*	2.44 ± 2.131 x 0.071	0.001	-7.521
Drinking	0.41 ± 2.131 x 0.041	0.53 ± 2.131 x 0.044	0.013	-2.488
Ground-scratching	3.03 ± 2.131 x 0.674	7.64 ± 2.131 x 1.439	0.029	-2.178
Gentle pecking	7.89 ± 2.131 x 1.208	9.33 ± 2.131 x 1.154	0.213	-1.247

*Using the t table, $t_{0.05/2;15} = 2.131$. For example the 95 % confidence interval is 1.68 ± 2.131 x 0.076 for eating in conventional cage

In the presented study, the use of nest and perch ratio was also investigated in furnished cages (Table 5) According to this, the ratio of perch use was increased starting with the second day (P = 0.001, F = 18.719). On the other hand, it was decreased for those which were standing on the floor (P = 0.001, F = 99.606). However, in the sixth day, perch use ratio decreased compared to fourth day in spite the ratio of hens standing on the ground was slightly increased (Table 5). The same picture (except the sixth day) was noted in ratio of hens using the top of the nest as a perch (P = 0.001, Chi-square = 79.622). The ratio of nest use was significantlyhigher after the first two days (P = 0.001, Chi-square = 74.949). Parameters related to egg production were presented in Table 6. Egg production ratio was higher

Table 2: The effects of cage furnishing on resting, standing and moving behaviours in laying hens

	Conventional cage (n = 16)	Furnished cage (n = 16)	P value	Z (Mann Whitney U)
Resting	4.24 ± 2.131 x 0.160*	3.35 ± 2.131 x 0.127	0.001	-3.514
Standing	6.05 ± 2.131 x 0.135	6.17 ± 2.131 x 0.137	0.651	-0.452
Moving	1.33 ± 2.131 x 0.077	1.41 ± 2.131 x 0.075	0.216	-1.237

*Using the t table, $t_{0.05/2;15} = 2.131$. For example the 95 % confidence interval is 4.24 ± 2.131 x 0.160 for resting in conventional cage

Table 3: The effects of cage furnishing on comfort behaviours in laying hens

	Conventional cage (n = 16)	Furnished cage (n = 16)	P value	Z (Mann Whitney U)
Preening	2.25 ± 2.131 x 0.090*	2.06 ±2.131 x 0.080	0.177	-1.351
Feather ruffling	12.97 ± 2.131 x 1.468	9.39 ± 2.131 x 0.859	0.141	-1.473
Wing-flapping	9.11 ± 2.131 x 0.900	8.56 ± 2.131 x 1.096	0.615	-0.503
Tail-wagging	3.42 ± 2.131 x 0.409	5.33± 2.131 x 0.790	0.079	-1.755
Stretching	4.92 ± 2.131 x 0.567	3.25 ± 2.131 x 0.405	0.038	-2.080

* Using the t table, $t_{0.05/2;15} = 2.131$. For example the 95 % confidence interval is 2.25 ± 2.131 x 0.090 for preening in conventional cage

for those housed in conventional cages (P = 0.023, Z = 2.280). On the other hand, there were no significant differences between furnished cages and conventional cages in egg weight and egg mass.

location, and gentle pecking each other are exploratory behaviours seen in the feral or Jungle fowl. Actually, those are elements of consecutive nutritional behaviors. It was observed that hens housed in cages were

Table 4: The effects of cage furnishing on aggressive and stereotyped behaviours in laying hens

	Conventional cage (n = 16)	Furnished cage (n = 16)	P value	Z (Mann Whitney U)
Aggressive pecking	13.08 ± 2.131 x 1.574*	7.78 ± 2.131 x 0.946	0.019	-2.346
Stereotyped behavior	4.03 ± 2.131 x 0.712	6.14 ± 2.131 x 1.429	0.702	-0.383

* Using the t table, $t_{0.05/2;15} = 2.131$,. For example the 95% confidence interval is 13.08 ± 2.131 x 1.574 for aggressive pecking in conventional cage

Discussion

In the presented study, it was detected whether the housing of stressed laying hens in furnished cages after the stress episode, caused any significant behavioral changes. Scored behaviours were evaluated into four categories. These were feeding and exploring behaviours, activity behaviors, comfort behaviours and aggressive and stereotyping behaviours.

Drinking and feeding are the basic physiologic and behavioral patterns seen in all of the species. In this study, the frequency of eating and drinking behaviors increased in hens housed in furnished cages. Similarly Pohle and Chang (2009) also reported that the birds housed in furnished cages spent significantly more time for feeding than the birds housed in battery cages. However, there are some reports which also indicated that furnished cages do not affect eating behavior (Li et al., 2016). It is impossible to exactly explain why eating behaviors increased in furnished cages ın this study. But, increased ground-scratching behavior, a component of foraging behavior observed in those cages, may have led to stimulate feeding behaviors. Ground-scratching, and pecking at the scratched attacking to other individuals in the same cage in the case of no opportunity to express one or more of these behaviours (Duncan, 1998). For this reason, high frequency of eating, drinking, and ground-scratching behaviours in hens housed in furnished cages compared to those of housed in conventional cages were evaluated as a positive result. Because inadequate expression of these behaviors causes to frustration and as a result aggressive behaviors. As a result, it may be concluded that our findings on feeding behaviours showed that the housing of hens after stress exposure in furnished cages improves animal welfare.

In the current study, resting behaviours including both resting and laying in hens housed in conventional cages were highly expressed compared to those of housed in furnished cages. Different from our statements, Li et al., (2016) observed no significant difference between hens housed in furnished cages and conventional cages. On the other hand Pohle and Cheng, (2009), Meng et al, (2017) stated higher sitting activity in hens housed in conventional cages. Both of these two reports were in accordance with our results. In addition, Meng et al, (2017) reported lower lying activity for hens

Table 5: Nest and perch usage ratios in the post-stress adaptation period in laying hens

Days	Perch	Floor	Inside of the nest	Top of the nest
1	3.10 ± 2.131 x 0.26[a]	36.75 ± 2.131 x 0.43[a]	2.25 ± 2.131 x 0.33[a]	5.90 ± 2.131 x 0.30[a]
2	6.95 ± 2.131 x 0.46[b]	30.05 ± 2.131 x 0.54[a]	1.75 ± 2.131 x 0.27[a]	9.35 ± 2.131 x 0.68[b]
3	7.20 ± 2.131 x 0.37[b]	24.30 ± 2.131 x 0.44b	5.75 ± 2.131 x 0.24[b]	10.75 ± 2.131 x 10.31[b]
4	6.30 ± 2.131 x 0.42[b]	21.35 ± 2.131 x 0.58[b]	6.80 ± 2.131 x 0.47[b]	13.65 ± 2.131 x 0.55[b]
5	7.30 ± 2.131 x 0.38[b]	24.35 ± 2.131 x 0.78[b]	6.45 ± 2.131 x 0.22[b]	10.15 ± 2.131 x 0.65[b]
6	5.15 ± 2.131 x 0.34[c]	29.80 ± 2.131 x 0.50[a]	6.65 ± 2.131 x 0.32[b]	6.00 ± 2.131 x 0.27[a]
DF	5;114	5;114	DF 5	5
P value	0.001	0.001	P value 0.001	0.001
F (ANOVA)	18.719	99.606	Chi-square (Kruskal Wallis) 74.949	79.662

a, b, c Values within a column with different superscripts differ significantly at P < 0.001. DF = degrees of freedom

housed in large furnished cages compared to those of housed in small-furnished ones. The difference between the results of the researches was probably due to cage design. Actually, resting activity was expected to be lower since birds housed in furnished cages may express many natural behaviours that cannot be expressed by birds housed in barren cage (Mench, 1998, Shimmura et al., 2009).

types of stretching which is one of the comfort behaviours were defined and named as "bilateral and unilateral". Yet it is suspicious whether bilateral one is observed in domestic hens (Duncan. 1980). In the presented study unilateral stretching behaviour in which the wing and leg on the same side are pushed out and down behind the bird was scored. The cage must be wide enough in order to express some behaviours such

Table 6: The effects of cage furnishing on egg production in laying hens

	Conventional cage (n = 16)	Furnished cage (n = 16)	*P* value	Z (Mann Whitney U)
EPR (%)	83.14 ± 2.131 x 0.93*	80.19 ± 2.131 x 0.927	0.023	-2.280
Egg weight (gr)	57.74 ± 2.131 x 0.62	58.08 ± 2.131 x 0.71	0.990	0.012
Egg mass (gr/hen/day)	46.58 ± 2.131 x 0.78	48.04 ± 2.131 x 0.77	0.225	-1.214

* Using the t table, t0.05/2;15 = 2.131. For example the 95 % confidence interval is 13.08 ± 2.131 x 0.93 for EPR in conventional cage

Preening, feather ruffling, wing-flapping, tail-wagging, and stretching were all evaluated as comfort behaviours in the current study. Only frequency of tail-wagging slightly increased in hens housed furnished cages (P= 0,079, Table 3). No significant differences were found in other comfort behaviours. Similarly, Shimmura, et al., (2007) reported that, furnished cages do not have any influence in comfort behaviours. Unlike us Li et al., (2016) reported that furnished cages increased comfort behaviours. It is probably not enough that only the cages are furnished, the size of the cages is also influential. Because there are variations in the results of mentioned studies, it is concluded that the subject needs to be studied in a more detailed way. In Jungle fowl, two

as stretching and wing-flapping. A square of 653-1118 cm2 for stretching behaviour and more (860-1980 cm2) for wing-flapping were reported to be necessary (Broom and Freser, 2015). Although, the main cage area was 1250 cm2 per hen in this study, perches and nests that were used in furnished cages reduce the space. Therefore, leg and wing stretching activity may not be exhibited sufficiently. No significant differences were detected in stereotypic behaviours. On the other hand, aggressive pecking was higher in conventional cages compared to furnished cages. Previous studies have reported that birds housed in furnished cages express less aggression, and feather pecking (Gvaryahu et al., 1994). This report fit in the current study. Yet it must be

taken into consideration that aggressive behaviours in furnished cages were related to cage size (Shimmura, et al., 2009), cage design (Li et al., 2016) or cage density (Appleby et al., 2002). The decrease of aggressive behaviours in birds housed in furnished cages was supposed to be due to two factors. First, in the furnished cages there are more equipment such as nest or perch, where chicks can protect themselves from aggressive pecking (Rodenburg et al., 2005). In addition, wood shaving existence in the floor of furnished cages provides them foraging opportunity. Because, it is generally agreed that feather pecking or agressive pecking develops as a redirection of normal foraging and feeding behaviours, a lack of foraging or pecking material leads to pecking activity being redirected to the other hens (Huber-Eicher and Wechsler, 1998). No matter what was the underlying cause, the increase of aggressive pecking in hens housed in furnished cages during post stress adaptation period was accepted as an important finding.

As observed also in our study, both adapting to new cages and the formation of pecking order in hens exposed to transport and social stress are important. Because aggressive pecking is known to increase related to stress (El-Lethey et al., 2000). In the study represented here, the ratio of perch and nest use in hens housed in furnished cages showed a significant increase starting with the second day. It was also observed that they used the top of the nest as perch and this behaviour also increased after the second day. The data is important since it suggests that hens adapt to furnished cages in a short period. Perching is a behaviour adapted by wild ancestors especially against nocturnal predators. Nesting is also one of the natural behaviours preferred by hens when possible. Actually, laying open locations may increase cloacal cannibalism if other hens are able to see the cloaca during oviposition (Newberry, 2004). Hens housed in cages also express similar natural behaviours when possible (Valkonen, 2010).

An efficient use of these materials during adaptation period after transport might be effective or useful in diminishing the effects of stress. Because the above mentioned behaviours decrease when animals are in stress.

In the study represented here egg production ratio was noted to diminish though no significant difference of neither egg weight nor egg mass existed between groups. Valkonen (2010) reported, similar to our study, egg production decreased in hens housed in furnished cages. At the same time he expressed that this situation was only detected in an experiment where FCR also decreased. In our study this parameter couldn't be evaluated since FCR ratio was not recorded. On the other hand, it was also reported that furnished cages influenced neither egg production nor egg weight of the hens housed in the both types of cages (Abrahamsson and. Tauson. 1997; Valkonen et al., 2008; Shimmura et al., 2010). In the study reported here egg production decrease may be accepted as a negative effect. Yet the egg weight and mass, which were not altered, were accepted to be a mitigation factor.

The correct interpretation of the behaviors expressed by poultry, including their frequency, duration, and sequence, may be used to estimate their welfare. In the present study, cage furnishing improves some comfort behaviour such as ground-scratching and tail wagging and decreasing aggressive pecking. Therefore, it would be beneficial to keep stress exposed hens in furnished cages in the post-stress adaptation period.

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