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Stress parameters and immune response of layers under different cage floor and density conditions

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Abstract

The aim of the experiment was to investigate the effects of cage floor and cage density on stress parameters of laying hens. A total of 162 brown laying hens (Hyline Brown), aged 34 weeks, were used in the experiment. Compact-type battery cages, with three floors, were used. Hens were allocated as one, three or five hens in each of 18 cages to obtain three different cage density groups of 1968, 656 and 393.8 cm^2 floor area per hen, respectively. The same number of cages with different cage density were allocated to three different battery floors (first floor=top, second=middle, third=bottom) systematically. Values for body weight, mortality rate, egg weight, egg production, egg quality characteristics, egg yolk cholesterol content, the levels of blood plasma corticosterone, serum glucose, total cholesterol and triglycerides, the ratio of heterophils to lymphocytes (H–L ratio), antibody titers, claw length score, foot health score, plumage score and throat skin injuries were taken as indicators of stress. The values for egg weight ($P<0.01$) at the first floor were greater than the other floor levels. The group with five hens per cage had significantly lower mean estimates ($P<0.01$) than other groups with respects to body weight ($P<0.001$), egg production ($P<0.001$), egg weight ($P<0.001$) and plumage score ($P<0.01$), while significantly higher mean estimates for egg albumen index ($P<0.01$), Haugh unit ($P<0.01$), serum glucose ($P<0.001$), and H–L ratio ($P<0.001$). Serum cholesterol was higher in cages with one hen than that with five hens, whereas plasma corticosterone was lower. Antibody titers in cages with one hen was similar to that with three or five hens; however, those with three hens had higher titers than those with five hens. Values for egg breaking strength, yolk index, egg cholesterol content, and foot health score were not affected by cage density or floor. The results suggest that the allocation of three hens per cage had no measurable effect on health and welfare.

Keywords: Cage density; Cage floor; Laying hens; Performance; Stress parameters

1. Introduction

Battery cage systems are the most economical for the commercial layer industry, but have negative effects on hen welfare. For this reason, there has been pressure to ban battery cage production in several

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countries. Hen welfare is based on health, production, behaviour, and physiology. Stress is defined as the interaction between stress factors and protective reactions. Factors causing stress include physiological factors, such as climate, environment, nutrition, and diseases, and physical conditions, such as cage density and transport (Freeman, 1987). Under stress, rapid and temporary changes occur in the body initially; with continuous stress, these are followed by permanent and irreversible changes. At the end, a decline in yield and resistance to diseases may occur. Animals under stress become ill more easily, and excess medicine may be necessary to maintain health. As a result, drug residues increase in animal products and threaten public health directly. Stock health and welfare management are key factors in animal health and food safety. For this reason, stress conditions in animals need to be examined carefully.

Stress in poultry has been reviewed by Siegel (1980) and Hill (1983). Stress may include increases in circulating levels of corticosterone, suppression of humoral immunity, changes in the number of circulating leucocytes, with concomitant alterations in disease resistance, and a decrease in growth and egg production (Mench et al., 1986).

Many investigations have shown that, when hen density is increased, body weight (Hughes, 1975; Cunningham and Gvaryahu, 1987; Davami et al., 1987) and egg production and egg weight (Robinson, 1979; Cunningham and Ostrander, 1982; Quart and Adams, 1982) decrease, and mortality (Koelkebeck and Cain, 1984; Roush et al., 1984; Adams and Craig, 1985) increases. High levels of corticosterone have been shown to be an indicator of stress, as cage density increases (Mashaly et al., 1984; Davami et al., 1987). However, Cunningham et al. (1988) indicated that corticosterone is not a useful measurement of long-term stress or well-being of chickens. The ratio of heterophils to lymphocytes (H–L ratio) measures a physiological change, whereas the blood concentration of corticosteroid is affected by many factors (Gross and Siegel, 1983). Cage density is an another important factor in plumage condition. Some researchers (Hill and Hunt, 1978; Quart and Adams, 1982; Davami et al., 1987) reported improved plumage condition for hens housed at low cage density.

There have been some controversial results on the effects of different cage systems and cage density on the stress parameters of laying hens. Therefore, the aim of this study was to assess the response of laying hen to different management systems (cage floor and cage density), including productive performance, egg quality characteristics, blood parameters, antibody response and external appearance.

2. Materials and methods

2.1. Animals and diets

A total of 162 brown laying hens (Hyline Brown), aged 34 weeks, were used in the experiment. Compact-type battery cages with three floors, having 18 cages and 54 cages in total, were used. Each cage was $48\times41\times46$ cm^3 (width×depth×height). Hens were allocated as one, three and five hens, in each of 18 cages, to obtain three different cage density groups: 1968, 656 and 393.8 cm^2 floor area per hen, respectively. The same number of cages, with different cage density, were allocated to three floors (first floor=top, second=middle, third=bottom) systematically. To maintain cage density, any dead hen in the treatment was replaced on day of death from a reserve stock maintained at the same density. The experimental period was 22 weeks. Feed and water were provided ad libitum and the diets were presented in mash form.

2.2. Traits measured

Hens were weighed individually at 34 and 56 weeks of age. Eggs were collected daily and egg production was calculated as a hen–day basis. Laying hens were examined for mortality during the experiment. Throughout the experiment, one egg was collected from each subgroup (18 eggs for each group) at 4-week intervals to determine the egg quality characteristics. Individual eggs were weighed and their shape index, egg-shell breaking strength and shell thickness were measured. Yolk height and width, and albumen height, width and length were determined. By using these values, yolk index, albumen index and Haugh unit were calculated (Card and Nesheim, 1972). Egg and shell quality analyses were completed within 24 h of the eggs being collected. One egg from each subgroup was obtained at the end of the experiment for cholesterol measurement. Eggs

were weighed and boiled for 5 min. The yolk and albumen were separated and were weighed. Yolk cholesterol was extracted according to the AOAC (1990) method.

At 0, 2, and 4 months, one hen was randomly selected from each cage and bled from the brachial vein. Blood samples were taken in two tubes, one contained EDTA for estimating plasma corticosterone levels and the H–L ratio, and the other had no anticoagulant for estimating cholesterol, glucose and triglyceride levels. The bleeding procedure was limited to 1 min or less to minimize the influence of handling stress. All blood samples were collected at the same time in the morning and centrifuged.

Plasma was frozen (−20 °C) until analyzed for corticosterone determination. Plasma corticosterone levels were measured using the kits (Gamma-B ^{125}I Corticosterone, Code AA-13 F1) for IDS double antibody RIA technique, with a Berthold LB211 gamma counter. Blood samples were smeared on to a glass slide for the determination of the H–L ratio. After drying, the smears were stained with May–Grünwald–Giemsa stain (Gross and Siegel, 1983). The total leukocyte count includes heterophils, lymphocytes, monocytes, basophils, and eosinophils. One hundred leucocytes were counted, once on each slide, using a light microscope at ×1000 magnification. The H–L ratios were determined by dividing the number of heterophils by the number of lymphocytes.

Serum cholesterol, glucose, and triglyceride levels were determined using a Hitachi autoanalyzer (Hitachi, Tokyo; Serial Number 1238-23) and its accompanying commercial kits.

At 54 weeks of age, one hen was randomly selected from each subgroup and injected with 0.1 ml of 0.25% suspension of sheep erythrocytes (SRBC) in 0.9% saline. Circulating anti-SRBC antibody titers were determined by the microhemagglutination technique from samples taken at 5 days after the immunization. All titers were expressed as the $\log_2$ of the reciprocal of the serum dilution (Arda, 1997).

External appearance traits, including plumage condition, throat skin injuries, claw length and foot health were measured. At 56 weeks of age, hens were individually taken out of their cage and examined for feather damage using a scoring system. A score (graduated from 1=very poor plumage to 4=intact plumage) was assigned for plumage condition for each area of the body (neck, breast, back, wings, and tail). Throat skin injuries were scored from 0 to 3 points, where 0=no ateromata defects on skin and 3=large defects. Claw length was measured on a 1–4 scale, where 1=extremely long and 4=normal to short claw. Foot health and injuries to the claw fold were scored on a 1–4 scale, where 1=intact matrix and 4=severely injured matrix (Davami et al., 1987).

2.3. Statistical analyses

Statistical analyses were performed using the SPSS software package for Windows (SPSS, Chicago, IL, USA). Data were tested for distribution normality and homogeneity of variance. Antibody titers for each hen were converted to appropriate natural logarithms.

A repeated-measures ANOVA was conducted on blood parameters (serum glucose, cholesterol, triglyceride, and plasma corticosteroid, H–L ratio) and hen–day egg production to examine the time-effect with cage density and cage floor. A three-way ANOVA, with cage density and cage floor and time as main factors, was used to detect any change in egg weight or quality. A two-way ANOVA was used to determine differences between cage density and cage floors and their interactions with respect to body weight, egg cholesterol, antibody titers, claw length, foot health, and plumage score. When a significant difference was

Table 1
Effect of cage floor and cage density on body weight at the end of the experiment

	Body weight (g)
CD1	2124a
CD3	2096a
CD5	1861b
CF1	2019
CF2	2049
CF3	2013

Two-way ANOVA

	df	Mean squares
CF	2	13,670
CD	2	1,171,058*
CF×CD	4	12,489

ab: means within columns with different letters are significantly different ($P<0.05$).
CD: cage density, CF: cage floor, CF×CD: CF by CD interaction.
* $P<0.001$.

found among cage densities or among cage floors, Bonferroni's test was used. When a significant interaction between cage density, cage floor or period was detected, a one-way ANOVA was used to detect differences among different conditions (Saunders-Dawson and Trapp, 1990).

3. Results and discussion

3.1. Body weight

Differences in body weight, at the beginning of the experiment, versus cage density and cage floor were not statistically significant (data not shown). Increasing cage density, from one and three to five hens/cage, resulted in a lower body weight; however, cage floor position had no effect on body weight (Table 1). This is in agreement with other studies (Hughes, 1975; Hill and Hunt, 1978; Roush et al., 1984; Cunningham and Gvaryahu, 1987; Davami et al., 1987; İşcan et al., 1998), which also reported low body weight with high cage density. In contrast, Cook and Dembnicki (1966) and Wayman et al. (1969) found that cage density had no effect on body weight of layers, and Grover et al. (1972) reported that body weight of hens maintained on the top cage floors was higher than those on lower floors.

Table 2
Effect of cage floor, cage density, and period on hen–day egg production

	Egg production (%)	Repeated-measures ANOVA		
CD1	94.1a		*df*	Within-subjects contrast (mean squares)
CD3	89.3b	T	2	183.315*
CD5	78.5c	CF×T	4	8.236
CF1	86.5	CD×T	4	20.387
CF2	87.3	CD×CF×T	8	20.830
CF3	88.2			Between subjects (mean squares)
T1	85.4x	CF	2	15.084
T2	88.7y	CD	2	1317**
T3	87.8y	CF×CD	4	19.125

abc, xy: means within columns with different letters are significantly different ($P<0.05$).
CD: cage density, CF: cage floor, T: period, CF×CD: CF by CD interaction, CF×T: CF by T interaction, CD×T: CD by T interaction, CF×CD×T: CF by CD by T interaction.
* $P<0.01$.
** $P<0.001$.

Table 3
Effect of cage floor and cage density on egg weight

	Egg weight (g)	Three-way ANOVA		
CD1	63.4a		*df*	Mean squares
CD3	63.6a	CF	2	349.906*
CD5	62.4b	CD	2	773.051**
CF1	64.0d	T	2	5334.128**
CF2	62.9e	CF×CD	4	148.712***
CF3	62.5e	CF×T	4	25.693
T1	60.4x	CD×T	4	40.527
T2	63.7y	CF×CD×T	8	35.460
T3	65.3z			

ab, de, xyz: means within columns with different letters are significantly different ($P<0.05$).
CF: cage floor, CD: cage density, T: period, CF×CD: CF by CD interaction, CF×T: CF by T interaction, CD×T: CD by T interaction, CF×CD×T: CF by CD by T interaction.
* $P<0.01$.
** $P<0.001$.
*** $P<0.05$.

3.2. Mortality

During the experiment, three (5.56%), zero (0.00%), and two (3.70%) hens died on the first, second, and third floor, respectively. One (5.56%), one (1.85%), and three (3.33%) hens died in the groups having one, three, and five hens per cage, respectively. Mortality was not affected by the cage floor or density ($P>0.05$). Other researchers reported similar results, i.e., that cage floor (Adams and Jackson, 1970; Grover et al., 1972) and cage density (Cunningham and Ostrander, 1981, 1982; Cunningham, 1982; Davami et al., 1987; İşcan et al., 1998) had no effect on mortality. These results contrast with those of some researchers (Koelkebeck and Cain, 1984; Roush et al., 1984; Adams and Craig, 1985), who found that mortality increased with cage density. Feather pecking increased mortality rates in laying hens (Rodenburg et al., 2003). Increasing the cage density may stimulate feather pecking; however, in the present study, feather pecking was not observed.

3.3. Egg production and egg weight

Increasing density had a negative effect on egg production, as shown in Table 2. Cage floor position did not affect hen–day egg production. Egg produc-

tion was lower at the beginning of the study, then increased. Cage floor, density, and time had a significant effect on egg weight (Table 3). Egg weight increased significantly with time. Eggs in the first floor were heavier than the second or third floor. Increasing cage density from one and three to five hens per cage resulted in a lower egg weight. There was an interaction between floor and density. The heaviest eggs were detected on the first floor, having one or three hens per cage. The first floor was fresher than the others, and this may contributed positively to egg weight.

The results of egg production and egg weight were similar to those reported in some studies (Robinson, 1979; Cunningham and Ostrander, 1982; Quart and Adams, 1982; Sütö et al., 1997; Anderson et al., 2004). In contrast, other investigators (Koelkebeck et al., 1987, Brake and Peebles, 1992; Carey et al., 1995; İşcan et al., 1998) reported that cage density had no effect on egg production or egg weight. Reports by Cook and Dembnicki (1966) and Dorminey and Arscott (1971) indicated that higher cage densities causes an increase in egg weight. The differences between the present study and literature reports may be due to the genotype and age of birds, season, feeder space, and housing conditions. Davami et al. (1987) concluded that hens in lower density cages were allowed more movement within the cage, which may have resulted in a less stressful environment. Food is partitioned between body functions, including maintenance, growth, reproduction, and health. In healthy animals, 10% of food ingredients consumed are used to maintain health, while the remaining portion is divided into three equal parts for reproduction, maintenance, and health (Siegel and Gross, 2000). In stress, most of the consumed food is used to cope with unpleasant conditions (Siegel and Gross, 2000). In the present experiment, this condition may explain the reduction in the egg production, egg weight, and body weight in groups with five hens in cages.

3.4. Egg quality characteristics and egg yolk cholesterol content

Egg shape index (data not shown), egg-shell breaking strength (data not shown), albumen index, yolk index, and Haugh unit of laying hens were similar for cage floor positions, whereas egg shell thickness on the third floor was larger than that on the first floor (Table 4). Cage density had no effect on egg shape index, egg-shell breaking strength, shell thickness or yolk index. The values of albumen index and Haugh unit were higher in cages containing five hens than in the cages with one or three hen, but some changes were detected in shell thickness, yolk index, and Haugh unit of laying hens with time. None of the interactions of cage density×cage floor, cage density×period and cage floor×period were significant for these egg quality characteristics. Egg yolk cholesterol content was not affected by cage density or floor position (data not shown).

Similarly, some researchers found no differences in egg Haugh unit at different cage floors (Adams and Jackson, 1970), in egg-shell breaking strength (Wells,

Table 4
Effect of cage density, cage floor, and period on some egg quality characteristics

	Shell thickness (μm)	Albumen index (%)	Yolk index (%)	Haugh unit (%)
CD1	389	7.48a	45.2	74.6a
CD3	403	8.11a	44.5	76.8a
CD5	397	8.90b	45.1	79.5b
CF1	393d	8.37	45.0	77.9
CF2	398de	8.06	45.1	76.6
CF3	399e	8.12	44.6	76.6
T1	381x	8.20	43.9x	77.7xy
T2	391x	8.83	46.6y	80.3y
T3	416y	7.78	45.4bz	74.5x
T4	397xy	8.00	43.9xz	76.0x

	df	Three-way ANOVA (mean squares)			
CF	2	24.861*	1.395	5.881	35.608
CD	2	5.921	23.121**	6.413	290.058**
T	3	40.997**	6.322	54.344***	232.652**
CF×CD	4	9.331	5.435	7.764	89.806
CF×T	6	2.802	2.355	10.833	62.988
CD×T	6	12.169	0.687	3.546	20.639
CF×CD×T	12	4.960	2.471	4.512	41.920

ab, de, xyz: means within columns with different letters are significantly different ($P<0.05$).
CF: cage floor, CD: cage density, T: period, CF×CD: CF by CD interaction, CF×T: CF by T interaction, CD×T: CD by T interaction, CF×CD×T: CF by CD by T interaction.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

Table 5
Effect of cage floor, cage density and period on plasma corticosterone, serum glucose, cholesterol, triglyceride concentrations and H–L ratio

		Plasma corticosterone (ng/ml)	Serum glucose (mg/dl)	Serum cholesterol (mg/dl)	Serum triglyceride (mg/dl)	Heterophils/lymphocytes
CD1		1.65a	221a	133a	1280	0.62a
CD3		1.72ab	231b	122ab	1218	0.57a
CD5		1.93b	250c	114b	1089	0.95b
CF1		1.80	235	121	1245	0.70
CF2		1.74	232	125	1148	0.68
CF3		1.77	234	122	1194	0.76
T1		1.88x	211x	120	1086x	0.72
T2		1.69y	255y	118	1205xy	0.72
T3		1.73y	236z	130	1297y	0.70
	df	Repeated-measures ANOVA (within-subjects contrast, mean squares)				
T	2	0.5230*	26,124*	1996.41	602,178**	0.00484
CF×T	4	0.1720***	24.4	94.802	30,707	0.00211
CD×T	4	0.0396	386.5**	573.506	113,317	0.00393
CD×CF×T	8	0.0130	67.2	655.465	75,563	0.00268
	df	Between subject (mean squares)				
CF	2	0.0168	71.167	81.706	42,001	0.03124
CD	2	0.382***	3952*	1654**	171,326	0.783*
CF×CD	4	0.0118	37.997	183.329	41,903	0.007479

abc, xyz: means within columns with different letters are significantly different ($P<0.05$).
CD: cage density, CF: cage floor, T: period, CF×CD: CF by CD interaction, CF×T: CF by T interaction, CD×T: CD by T interaction, CF×CD×T: CF by CD by T interaction.
* $P<0.001$.
** $P<0.05$.
*** $P<0.01$.

1972), or in shell thickness (Davami et al., 1987; Sütö et al., 1997), at varying densities. On the contrary, Davami et al. (1987) and Sütö et al. (1997) reported that Haugh unit values were not affected by cage density.

3.5. Plasma corticosterone, serum glucose, cholesterol, triglyceride concentrations, and H–L ratios

The repeated-measures ANOVA revealed a decrease in plasma corticosterone and an increase in serum glucose and triglyceride concentrations at the end of the experiment compared to admission levels (Table 5). Analysis between subjects showed significant effects of cage density on plasma corticosterone, serum glucose, cholesterol concentrations, and H–L ratios, although there were no significant cage floor effect on blood parameters.

Increasing cage density, from one to five hens per cage, resulted in a significant increase of the plasma corticosterone ($P<0.01$) and serum glucose ($P<0.001$) concentrations and a decrease of the serum cholesterol ($P<0.05$) concentrations. During stress conditions, neural impulses come to the hypothalamus and are converted to neuro-humoral factors. Corticotropin-releasing factor stimulates the anterior hypophysis to secrete ACTH, which, in turn, stimulated the adrenal for corticosterone secretion (Hill, 1983; Siegel, 1985). Therefore, higher corticosterone levels in the 5 hens/cage groups could reflect higher stress conditions. The corticosterone levels found in the present study are in agreement with the findings of some researchers (Mashaly et al., 1984; Craig et al., 1986a; Koelkebeck et al., 1986; Beuving et al., 1989). However, others (Cunningham et al., 1987, 1988) reported that plasma corticosterone was not significant in chronic stress. Differences in corticosterone secretion between experiments may be caused by a number of external factors, such as light, temperature, group size, density, and methodological factors of taking blood, or internal factors, such as genetic stock, age, background, or inherent variation (Littin and Cockrem, 2001).

Increasing blood glucose levels, due to the effect of glucocorticoids (Scnukro, 1974; Simon, 1984), are described as an important indicator of stress conditions. The glucose concentrations found in the present study are in agreement with the findings of Lagadic et al. (1990). Cholesterol concentrations of hens were lower at the higher density, as was also found by Clemens et al. (1986). On the other hand, Pesti and Howarth (1983) found no changes in cholesterol levels of broilers at different population densities.

The ratio of heterophils to lymphocytes of the group having 5 hens/cage was higher ($P<0.01$) than those of groups having 1 or 3 hens/cage. This condition could be explained by the elevated concentration of corticosterone in blood circulation, which causes an increase in heterophil count and a decline in lymphocyte count (Hill, 1983; Siegel, 1985). The results obtained in the present study are in agreement with the findings of other researchers (Gross and Siegel, 1983; Beuving et al., 1989).

3.6. Antibody response

Cage density significantly affected antibody titers, as shown in Table 6. However, cage floor had no significant effect on antibody response in a two-way ANOVA.

Table 6
Effect of cage floor and cage density on antibody titers and plumage score

		Antibody titers (Log_2)	Plumage score[a]
CD1		2.22ab	16.56a
CD3		2.57a	14.85a
CD5		1.96b	12.42b
CF1		2.29	14.64
CF2		2.21	14.09
CF3		2.25	15.10
	df	Two-way ANOVA (mean squares)	
CF	2	0.0330	8.895
CD	2	1.658*	182.677*
CFXCD	4	0.0699	3.504

ab: means within columns with different letters are significantly different ($P<0.05$).
CD: cage density, CF: cage floor, CF×CD: CF by CD interaction. $P<0.001$.
[a] Plumage score: 1 (very poor plumage)–4 (intact plumage) for each area of the body (neck, breast, back, wings, and tail).
* $P<0.01$.

Increasing cage density from 3 to 5 hens/cage resulted in a significant decrease ($P<0.01$) in the antibody titers to SRBC in the present study. This result agrees with the findings of Hester et al. (1996). In contrast, Patterson and Siegel (1998) and Heckert et al. (2002) observed that cage density treatments had no significant effect on hemaglutinin titers to SRBC. Some of these differences may be due to the genetic background of the birds, different cage densities or the use of different suspensions of sheep erythrocytes.

3.7. External appearances

The plumage score of hens were found to be lower in densely populated cages, as shown in Table 6. There were no significant differences in plumage score among different cage floors. However, throat skin injuries were found only on the hens maintained on the first floor (5.6% of hens). This may be possibly explained by the difference in light intensity at different cage floor positions. The poorer plumage score of densely populated cages can be caused by abrasion against cage wire or other hens. This result agrees with the findings of some researchers (Quart and Adams, 1982; Craig et al., 1986b; Cunningham and Gvaryahu, 1987; Davami et al., 1987; Koelkebeck et al., 1987). In contrast, plumage score comparisons in the study of Cunningham et al. (1988) were not significantly different between the groups having different cage population sizes (4–6/cage). Higher density appears to cause increased levels of nervousness and feather-pecking activity. Some strains have a greater ability to adapt to high-density environments and this may explain the differences between experiments.

Interestingly, cage density and cage floor had no significant effect on the scores of claw length and foot health (data not shown).

4. Conclusion

The study indicates that significant differences in egg weights were detected among different cage floor positions. The allocation of hens at 5/cage resulted in a reduction in productive performance, antibody response to the antigen SRBC and plumage score.

The heaviest eggs were observed in groups having 1 and 3 hens/cage on the first floor. Generally, there were no differences in stress parameters between the groups having 1 hen/cage or 3 hens/cage. Therefore, it is suggested that the allocation at 3/cage is feasible, without any major measurable effects of health and welfare of hens. Further studies on the effect of cage density and cage floor position on productive, behavioral, and physiological parameters are necessary to determine the optimal environment for laying hen well-being.

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