Research Note

Effects of stock density on the laying performance, blood parameter, corticosterone, litter quality, gas emission and bone mineral density of laying hens in floor pens

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ABSTRACT

The effects of stocking density on the performance, egg quality, leukocyte concentration, blood biochemistry, corticosterone levels, bone mineral density, and noxious gas emission for laying hens were investigated. Eight hundred 34-week-old Hy-Line Brown laying hens (Gallus gallus domesticus) were randomly assigned to one of 4 treatments, each of which was replicated 4 times. Four stocking densities, including 5, 6, 7, and 10 birds/m², were compared. A commercial-type basal diet was formulated to meet or exceed nutrient recommendations for laying hens from the National Research Council. The diet was fed to the hens ad libitum for 8 wk. Results indicated that hen-day egg production, egg mass, and feed intake were less (P < 0.01) for 10 birds/m² stock density than other stock densities. Production rate of floor and broken eggs and eggshell strength were greater (P < 0.01) for 10 birds/m² stock density than other stock densities. There were no significant differences in the level of leukocytes among densities. However, heterophils and the H/L ratio were greater (P < 0.01) for 10 birds/m² than in stock density of 6 or 7 birds/m². Serum corticosterone was greater (P < 0.01) 10 birds/m² than stock density than other stock densities. Litter moisture and gas emission (CO₂ and NH₃) were greater (P < 0.01) for 10 birds/m² than stock density than 6 and 7 birds/m² stock density. Bone mineral content was not influenced by increasing stock density. However, bone mineral density was less (P < 0.05) for 10 m² stock density than other stock densities. These results indicate that increasing the density beyond 5 birds/m² elicits some negative effects on laying performance of Hy-Line brown laying hens.

Key words: bone mineral density, corticosterone, laying performance, stock density, Hy-line Brown laying hens, animal welfare

INTRODUCTION

The welfare of laying hens raised in standard commercial cages has been placed under intense scrutiny. The traditional housing of egg-type chickens in conventional cages, long perceived as the most efficient method of housing laying hens, is now widely considered to have a negative effect on their welfare (Appleby, 1993; Appleby et al., 1993; Craig and Swanson, 1994). The limited environmental complexity and confinement in conventional cages physically restrict hens and eliminate many of their natural behaviors such as nesting, roosting, and scratching (Nicol, 1987; Baxter, 1994; Tactacan et al., 2009). The use of non-cage housing systems for laying hens increased after the 2012 EU ban on conventional cages was implemented. The European Union Council Directive 1999/74/EC (European Communities, 1999) outlined minimum standards for the welfare of laying hens after 1 January 2012, allotting a maximum stock density of 9 birds/m². However, for those organizations that began applying these standards on 3 August 1999, a stock density of 12 birds/m² was allowed where the usable area corresponded to the available ground surface (until 2012, when the new standards were applied). The Korean Council Directive 2012–68 (APQA, 2012), which lays down minimum standards for the welfare of laying hens in Korea, imposes a maximum stock density of 7 birds/m². Stocking rate has been examined as a factor in a number of epidemiological surveys of laying hen behavior (Gunnarsson et al., 1999). There have been numerous studies demonstrating the negative effect of cage or floor density on egg production, egg weight, feed intake, feather pecking, and plumage scores (Bell, 1981; Roush et al.,...
STOCK DENSITY EFFECTS ON LAYING HEN WELFARE

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Welfare Committee of the National Institute of Animal Science, Rural Development Administration, Republic of Korea.

**Birds and Experimental Design**

Eight-hundred 34-week-old Hy-Line Brown laying hens (Gallus gallus domesticus) were randomly assigned to one of 4 dietary treatments with each treatment replicated 4 times. Four stocking rates of 5 (n = 50), 6 (n = 50), 7 (n = 50), and 10 (n = 50) birds/m² in floors with deep litter of rice hulls were used for each replicate, respectively. A commercial-type basal diet was formulated to meet or exceed the nutrient recommendations for laying hens issued by the National Research Council (NRC, 1994) (Table 1). During the 8-wk experimental period, hens were provided with feed and water ad libitum and were exposed to a 16 h:8 h (light:dark) schedule. The temperature and humidity of the laying house were maintained at 20 ± 3°C and 65 to 70% respectively.

**Laying Performance**

Hen-day egg production rate, floor eggs, broken egg production rate, and egg weight were recorded daily; whereas feed intake and the feed conversion ratio were recorded weekly. Egg mass was calculated as per Hayat et al. (2009):

\[
\text{Egg mass} = \text{weekly number of eggs in a replicate} \times \text{average egg weight.}
\]

**Table 1.** Composition and nutrient content of experimental diet.

| Ingredients (g/kg) |  
|-------------------|---|
| Corn              | 411.5 |
| Wheat             | 150.0 |
| Soybean meal      | 250.0 |
| DDGS              | 50.0  |
| Canola meal       | 20.0  |
| Tallow            | 5.0   |
| Molasses          | 5.0   |
| Dicalcium phosphate | 7.0   |
| Limestone         | 97.0  |
| Sodium chloride   | 2.0   |
| Vitamin premix    | 1.5   |
| Mineral premix    | 1.0   |
| Total             | 1,000.0 |
| Energy and nutrient content |  
| MEn, MJ/kg        | 11.4 |
| Crude protein, g/kg | 142.0 |
| Calcium, g/kg     | 40.0  |
| Available P, g/kg | 3.3   |
| Lysine, g/kg      | 7.5   |
| Methionine, g/kg  | 3.0   |

1Provided per kilogram of the complete diet: vitamin A (vitamin A acetate), 12,500 IU; vitamin D₃, 2,500 IU; vitamin E (DL-α-tocopheryl acetate), 20 IU; vitamin K₃, 2 mg; vitamin B₁₂, 2 mg; vitamin B₅, 5 mg; vitamin B₆, 3 mg; vitamin B₁, 18 μg; calcium pantothenate, 8 mg; folic acid, 1 mg; biotin, 50 μg; niacin, 24 mg.

2Provided per kilogram of the complete diet: Fe (FeSO₄·7H₂O), 40 mg; Cu (CuSO₄·H₂O), 8 mg; Zn (ZnSO₄·H₂O), 60 mg; Mn (MnSO₄·H₂O) 90 mg; Mg (MgO) as 1,500 mg.

3Nutrient contents in all diet were calculated.

**Determination of Egg Quality Parameter**

Ten eggs per replicate were randomly collected at the end of each week. Eggshell strength, eggshell thickness, egg yolk color, and Haugh units (HU) were measured. Eggshell strength was measured by the Texture Systems Compression Test Cell (model T2100C, Food Technology Co., Ltd., Rockville, MD) and expressed as units of eggshell surface area (kg/cm²). Eggshell thickness is defined as the mean value of measurements at 3 different locations on the egg (air cell, equator, and sharp end) and was measured with a dial pipe gauge (model 7360, Mitutoyo Co. Ltd., Kawasaki, Japan) and calculated using the following formula (Yannakopouls and Tserveni-Gousi 1986):

\[
\text{Eggshell thickness} = (\text{sharp point thickness} + \text{equator point thickness} + \text{air cell thickness})/3.
\]

Egg yolk color was evaluated by the Roche Yolk Color Fan (Hoffman-La Roche Ltd., Basel, Switzerland; 15 = dark orange; 1 = light pale). Haugh unit values were calculated using a micrometer (model S-8400, Ames, Waltham, MA) with the following formula described by Eisen et al. (1962):

\[
\text{HU} = 100 \log(H - 1.7W^{0.37} + 7.6),
\]

where W is egg weight, and H is albumen height.
Hematological Analysis

At the end of the 8-wk feeding trial, 2 birds/replicate (i.e., 8 birds per treatment) with a body weight near the mean were selected to be were euthanized by cervical dislocation. Immediately after death, a 5 mL blood sample was collected from the jugular vein of each bird using EDTA-treated and non-EDTA treated vacuum blood collection tubes (Becton Dickinson, Franklin Lakes, NJ). The whole blood samples were kept on ice and used immediately for hematological analysis. Leukocytes (white blood cells, heterophils, lymphocytes, monocytes, eosinophils, and basophils) from the blood samples were analyzed using the Hemavet® Multi-species Hematology System (Drew Scientific Inc., Oxford, CT). The H/L ratios were determined by dividing the number of heterophils by the number of lymphocytes. Serum samples were obtained by centrifuging the samples for 20 min at 25,000 × g and 4°C and were stored at −15°C. Total cholesterol, triglyceride, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and calcium in the serum were quantified using an ADVIA 1650 Chemistry System (Bayer Diagnostic, Puteaux, France). Serum corticosterone concentration was measured using a commercial EIA kit (No. 500655, Cayman Chemical, Ann Arbor, MI) according to the instructions of the manufacturer.

Litter Moisture and Gas Emissions

Litter samples were collected at 4 wk from 4 predetermined points in each pen, and moisture content was measured as described in AOAC method 934.01 (AOAC, 1990). To determine gas emission, litter gas was sampled using a Gastec Gas Sampling Pump (Model GV-100; Gastec Corp., Japan, Gastec Detector Tube No. 3 M and 3 La for ammonia; No. 4LL and 4LK for hydrogen sulphide).

Bone Mineral Density (BMD)

At the end of experiment, 5 birds per treatment were selected and killed by cervical dislocation at the end of experiment for bone analysis. The bone mineral density and bone mineral content of the left tibia of each intact bird was measured using a dual energy x-ray absorptiometry x-ray densitometer (DEXA; GE Healthcare, Lunar Prodigy Advance PA+130472, Small Animal software, Diegem, Belgium). Scanning began at the proximal end of the bone and lasted for approximately 10 min.

Statistical Analysis

All data were analyzed by one-way analysis of variance as a completely randomized design using the PROC MIXED procedure (SAS Institute Inc., Cary, NC). Outlier data were identified by the UNIVARIATE procedure of SAS, but no outliers were found. Least squares means were calculated and the means among treatments were compared by the PDIF option with the Tukey adjustment. Significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

**Laying Performance**

Over the course of the entire experiment (34 to 41 wk) hen-day egg production, egg mass, and feed intake were lower for ($P < 0.01$) 10 birds/m² stock density than other stock densities. Floor eggs and broken egg production rate were greater for ($P < 0.01$) 10 birds/m² stock density than other stock densities (Table 2). In this study, laying performance was shown to decline in response to increased stocking density. This finding supports previous studies that have shown that decreasing egg production is attributable to a reduction in the amount of feeding area per hen (Hester and Wilson, 1986; Craig and Milliken, 1989; Lee and Moss, 1995; Suto et al., 1997) and increased stocking density (Adams and Craig, 1985). Anderson et al. (2004) found that higher stocking density in Hy-Line W36 and Dekalb XL commercial layer genotypes decreased hen-day egg production. Similarly, Onbasilar and Aksoy (2005) determined hen-day egg production to be 94.1%, 89.3%, and 78.5% at 968, 655, and 393.8 cm² per hen respectively. Wells (1972), Carey (1987), and Shanawany (1988) all reported a decrease in feed intake with increasing stock density. Furthermore, the

| Table 2. Effects of stock density on laying performance of laying hens.¹ |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                | 5               | 6               | 7               | 10              | SEM²            | P-value         |
| Hen-day egg production, %      | 78.6ᵃ           | 78.2ᵃ           | 77.9ᵃ           | 75.7ᵇ           | 0.69            | 0.01            |
| Floor eggs, %                  | 1.34ᵇ           | 1.06ᵇ           | 1.30ᵇ           | 3.86ᵃ           | 0.555           | <0.01           |
| Broken egg production rate, %  | 0.77ᵇ           | 0.56ᵇ           | 0.52ᵇ           | 3.77ᵃ           | 0.230           | <0.01           |
| Egg weight, g                  | 61.9            | 61.8            | 62.4            | 61.6            | 0.24            | 0.70            |
| Egg mass, egg weight, g/eggs   | 48.7ᵃ           | 48.3ᵃ           | 48.6ᵇ           | 46.7ᵇ           | 0.51            | 0.04            |
| Feed intake, g/bird            | 115.9ᵃ          | 116.2ᵃ          | 115.8ᵃ          | 112.4ᵇ          | 0.44            | <0.01           |
| Feed conversion ratio          | 2.39            | 2.41            | 2.39            | 2.45            | 0.03            | 0.20            |

ᵃᵇValues in row with no common superscripts letter are significantly different ($P < 0.05$).

¹Data are least squares means of 4 observations per treatment.

²Pooled error of mean.
increased occurrence of floor or broken eggs in the highest stocking density may be secondary to increased competition for nest space.

**Egg Quality Parameters**

There were no significant differences in egg quality among stocking density treatments (eggshell thickness, egg yolk color, and Haugh unit; Table 3). However, eggshell strength was less ($P < 0.05$) for 10 birds/m$^2$ than stock density than other stock densities. Eggshell strength of 4.31, 4.39, 4.41, and 4.11 kg/cm$^2$ were measured for the respective stocking densities of 5, 6, 7, and 10 birds/m$^2$.

**Hematological Analysis**

There were no significant differences in the level of leukocytes (Table 4). However, heterophils and the H/L ratio were greater ($P < 0.01$) for 10 birds/m$^2$ than stock density than 6, and 7 birds/m$^2$ stock density. Mean H/L ratio was 0.34, 0.37, 0.37, and 0.52 for the 5, 6, 7, and 10 birds/m$^2$ treatments, respectively. There were no significant differences in the serum biochemistry (Table 5). During the initial (34 to 37 wk) and final 4 wk of the experiment (38 to 41 wk), serum corticosterone was greater ($P < 0.01$) 10 birds/m$^2$ than stock density than other stock densities (Table 6). Blood parameters are good indicators of the physiological, pathological, and nutritional status of an animal, and changes in hematological parameters have the potential to be used to elucidate the impact of nutritional factors and additives supplied in the diet of any living creature. For example, leukocytes are known to increase sharply when infection occurs as they are one of the first lines of defense of the body (Ganong, 1999; Alzawqari et al., 2011; Masoudi et al., 2011). Leukocyte count has also been used as a measure of immune function in birds (Johnson and Zuk, 1998). Many factors, such as

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**Table 3. Effects of stock density on egg quality of laying hens.$^1$**

| Items                                      | Stock density (birds/m$^2$) |                  |                  | SEM$^2$ | $P$-value |
|--------------------------------------------|-----------------------------|------------------|-----------------|--------|-----------|
| Stock density (birds/m$^2$)                | 5                           | 6               | 7               | 10     |           |
| Eggshell strength, kg/cm$^2$               | 4.31$^{a,b}$                | 4.39$^a$        | 4.41$^b$        | 4.11$^b$ | 0.032     | 0.04     |
| Eggshell thickness, μm                     | 350.0                       | 363.0           | 354.0           | 365.0  | 12.29     | 0.59     |
| Egg yolk color                             | 8.6                         | 8.6             | 8.3             | 8.6    | 0.02      | 0.20     |
| Haugh unit                                 | 90.5                        | 89.2            | 89.7            | 89.8   | 0.49      | 0.21     |

$^{a,b}$Values in row with no common superscripts letter are significantly different ($P < 0.05$).

$^1$Data are least squares means of 10 observations per treatment.

$^2$Pooled error of mean.

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**Table 4. Effects of stock density on blood parameter of laying hens.$^1$**

| Items                                      | Stock density (birds/m$^2$) |                  |                  | SEM$^2$ | $P$-value |
|--------------------------------------------|-----------------------------|------------------|-----------------|--------|-----------|
| Leukocytes$^3$                             |                             |                  |                  |        |           |
| WBC, K/μL                                  | 15.90                       | 15.66            | 18.86           | 21.26  | 2.23      | 0.83     |
| HE, K/μL                                   | 3.59$^{b,c}$                | 3.01$^c$         | 4.48$^{a,b}$    | 5.48$^a$ | 0.37      | <0.01    |
| LY, K/μL                                   | 11.20                       | 8.29             | 12.74           | 11.24  | 1.05      | 0.35     |
| SI, HE:LY                                  | 0.34$^b$                    | 0.37$^b$         | 0.37$^b$        | 0.52$^a$ | 0.06      | 0.04     |
| MO, K/μL                                   | 1.51                        | 0.94             | 1.80            | 1.49   | 0.24      | 0.46     |
| EO, K/μL                                   | 0.21                        | 0.07             | 0.22            | 0.16   | 0.09      | 0.99     |
| BA, K/μL                                   | 0.04                        | 0.01             | 0.02            | 0.02   | 0.02      | 0.48     |

$^{a,b}$Values in row with no common superscripts letter are significantly different ($P < 0.05$).

$^1$Data are least squares means of 4 observations per treatment.

$^2$Pooled error of mean.

$^3$Leukocytes: WBC = white blood cells; HE = heterophils; LY = lymphocytes; MO = monocytes; EO = eosinophils; BA = basophils.

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**Table 5. Effects of stock density on blood biochemistry of laying hens.$^1$**

| Items                                      | Stock density (birds/m$^2$) |                  |                  | SEM$^2$ | $P$-value |
|--------------------------------------------|-----------------------------|------------------|-----------------|--------|-----------|
| Total cholesterol, mg/dL                   | 160.9                       | 163.9            | 161.7           | 168.6  | 20.63     | 0.82     |
| Triglyceride, mg/dL                        | 1,033.9                     | 1,382.9          | 1,254.8         | 1,550.5 | 279.42    | 0.25     |
| Glucose, mg/dL                             | 177.1                       | 173.5            | 154.0           | 159.5  | 14.97     | 0.31     |
| Calcium, mg/dL                             | 3.36                        | 3.40             | 3.89            | 3.17   | 0.401     | 0.57     |
| AST, U/L                                   | 7.28                        | 6.63             | 8.05            | 7.11   | 0.761     | 0.80     |
| ALT, U/L                                   | 222.9                       | 222.2            | 216.4           | 246.1  | 11.82     | 0.26     |

$^1$Data are least squares means of 4 observations per treatment.

$^2$Pooled error of mean.
exposure to various microbes and chemicals, can cause changes in both granulocytic white blood cells (Lucas and Jamroz, 1961). The lack of adequate data on the role of increasing stock density in altering blood parameters in poultry requires further research.

The H/L ratio has proved to be a valuable measurement in stress-related research in poultry (Post et al., 2003; Zulkifli et al., 2003). Evaluation of the higher H/L ratio indicates increased stress in poultry with increasing stock density (Martrenchar et al., 1997; Feddes et al., 2002). El-Lethey et al. (2000) also reported that the H/L ratio was influenced by housing conditions (e.g., stock density).

Blood corticosterone concentration has been widely used as a measure of environmental stress in poultry (McFarlane and Curtis, 1989; Zulkifli et al., 2003). Craig et al. (1986) reported that increased stock density increased total plasma corticosteroids in some experiments. Hocking et al. (2001) also reported that mean corticosterone concentration in broiler breeders at 6 wk of age was 0.5 ng/mL under usual stocking density (9 birds/m²). Cheng and Muri (2004) reported that laying hens showed significantly lower plasma corticosterone levels in single-bird cages (525 cm²/bird) than in the 10-bird cages (419 cm²/bird), indicating that social stressors could be a factor in higher production of corticosterone in hens. Later work showed that serum corticosterone levels increased when population density was increased due to birds being forced to compete for feeding and watering space (Petsi and Howarth, 1983; Mashly et al., 1984; Craig et al., 1986).

**Litter Moisture and Gas Emissions**

Over the course of the experiment, litter moisture, gas emission (CO₂ and NH₃) were greater (P < 0.01) for 10 birds/m² than stock density than in 5, 6, and 7 birds/m² stock density (Table 7). Additionally, as stocking density increased, the amount of caked litter in the pens also increased. Higher stock density increases nitrogen and moisture level in the litter and thus favors microbial activity. Sorensen et al. (2000) reported increased litter moisture of broilers as stocking density increased from 622 to 455 cm²/birds. As stocking density increased, the amount of caked litter in the pens also increased, and the presence of caked litter could have served as a seal, altering the production of ammonia. Also, caked litter corresponds to high litter moisture or areas where litter becomes anaerobic, which suppresses ammonia volatilization (Carr et al., 1990). The wetter the litter, the more likely it will promote the proliferation of pathogenic bacteria and moulds. Litter condition is governed by the type of material, depth, friability, and moisture as well as housing, technical equipment, and management. From a welfare point of view, stock densities that are too high may create various problems such as increased air ammonia and heat produced from the birds, which can lead to stressful conditions and cause the death of individual hens.

**Bone Mineral Density**

Bone mineral content was not influenced by increasing stock density (Table 8). However, bone mineral density was less (P < 0.05) for 10 m² stock density than other stock densities. One of the main welfare concerns in cage is the hen’s inability to exercise, which leads to development of bone weakness and at peak production can easily result in skeletal damage (Webster, 2004). In the previous research, the bone mineral density of the tibia and humerus was higher in birds housed in enriched cages (Tactacan et al., 2009). Knowles and
Table 8. Effects of stock density on bone mineral density of laying hens.

| Items       | Stock density (birds/m²) | SEM² | P-value |
|-------------|--------------------------|------|---------|
| BMC, g      | 3.76                     | 0.491| 0.50    |
| BMD, g/cm²  | 0.28a                    | 0.22b| 0.030   |

a,bValues in row with no common superscripts letter are significantly different (P < 0.05).

CONCLUSION

These data indicate that increasing the stock density from 5 to 10 birds/m² of floor space negatively influenced laying performance, leukocytes concentration, serum corticosterone, litter moisture, gas emission, and bone mineral density, but blood biochemistry was not significantly altered.

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