

EFFECTS OF STRAIN, CAGE DENSITY AND POSITION ON IMMUNE RESPONSE TO VACCINES AND BLOOD PARAMETERS IN LAYER PULLETS

EFFECTUL RASEI, A DENSITĂȚII ȘI POZIȚIEI CUȘTII ASUPRA RĂSPUNSULUI IMUN LA VACCINURI ȘI A PARAMETRILOR SANGUINI LA PUII DIN RASELE DE GĂINI OUĂTOARE

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Two thousand 1-day-old layer chicks were used in the study from Lohman Brown, Isa Brown, Lohman White and Bowans White breeds. The chicks were placed in the at 3 cage densities (211.8, 274.5 and 370.6 cm² per bird) and on 3 positions (as top, middle and bottom tiers). All birds were kept under standard management policy and a commercial vaccination program was practiced. Total specific antibody titres to Infectious Brochitis Virus (IBV), Infectious Bursal Disease Virus (IBDV), Newcastle Disease Virus (NDV) and Egg Drop Syndrome Virus (EDSV) vaccines at the ages of 5, 10 and 20 weeks were serologically determined by ELISA. Cell-mediated immune response was also evaluated. In commercial white egg laying strains specific antibody titres to IBV, IBDV, NDV and EDSV vaccines were greater than in Brown egg layer strains. Keeping in cage created more stress in Brown egg laying chicks than those in white egg laying chicks. As cage density increased, the ratio of heterophils to lymphocytes (H/L ratio) slightly increased. Cage position had no influence on the titres of antibodies to IBV and IBDV vaccines but the position of cage in pullets where chicks were stocked, from top to bottom, NDV and EDSV antibody titre decreased and percentage of heterophils, H/L ratio and basophil rates were low. These findings suggest that cage-related stress could be decreased, resistance to diseases and finally well-being of hens may be improved if hens are kept under proper position and density within cage systems with respect to their physiological and behavioral characteristics that controlled by genes.

Keywords: cage density, cage position, pullet, strain, stress, welfare

Introduction

In Europe, despite of the strict legal regulations that banning traditional cage systems (in 2012, 999/74/EC) and allowing hen egg production in alternative

systems such as enriched cages, aviary, free-range or organic, it appears, in near future, that hens will be kept under conditions which enable them to show all behaviour in their natural behaviour repertoire and make them to be confined at various levels (15, 17).

Traditional cages in comparison to alternative systems such as housed in floored pen have advantages of less production cost and decreased disease risk (2, 18), however they also have disadvantages such as undesired welfare conditions and significant stress disorders (14, 18).

In hens “behaviour” alone for “welfare” is not a sufficient entity and environmental changes initiate a number of behavioural and physiological (changes in stress-axis) responses that ultimately negatively affect the development of immune system and may increase disease susceptibility (4). Webster Marketon and Glaser (22) reported that physiological and pathological stress in avian species affected neuro-endocrine system (glucocorticoids, catecholamins, epinephrine, norepinephrine, prolactin and growth hormones) and reduced the lymphocyte production. Several researchers focused on the effects of caged housing (5, 18), stress (3), management (6), environmental conditions and vaccinations (6, 10, 12) on immune performance.

H/L ratio was defined as stress index in hens (10). It was reported that basophil and heterophil cell numbers in poultry under stress increased (1, 11, 16).

Although several investigations concentrated in the effect of cage density and strains on humoral and cellular immunity in birds, limited number of studies into the association between the location of cage within the battery where bird housed and humoral/cellular immunity have been published. Thus, this paper discusses the possible interactions and effects of genotype, cage density and position on humoral and cellular immunity in layer pullets.

Material and Methods

Experimental population and housing conditions

The study was carried out in the Poultry Department of Afyon Feed Factory, Afyon, Turkey as a randomized complete block designs with a 4 x 3 x 3 factorial arrangement of treatments.

A total of 2000, one-day-old layer chicks were used as 500 each of following 4 genotypes: Lohman Brown (LB), Isa Brown (IB), Lohman White (LW) and Bowans White (BW). The chicks were placed in the cages with 5 nipple drinkers per 109x68x39 cm (in width, depth and height, respectively) cage at 3 cage densities, which included 70, 55 and 40 chicks per cage providing 105.9, 134.8, and 185.3 cm² space per bird, respectively, from day 1 to 4 weeks of age. Each of these cage density groups had 3 replicates for each strain, and they were randomly distributed to the cage units in the middle row of the battery (into 36 cages). Linear feeder space was 109 cm per cage or 1.56, 1.98, and 2.73 cm per bird at the 70, 55, and 40 birds per cage density, respectively. At 4 weeks, the first replicates and second replicates were randomly moved to cages at the top and the second

replicates of the bottom rows and the third replicates remained in the middle row. Then half the remaining chicks in the replicates were moved to a nearby empty cage, thereby doubling the number of replicate cages and reducing bird density to 35, 27 and 20 birds per cage (into 72 cages total). Thus, cage space was increased to 211.8, 274.5 and 370.6 cm² per bird, and feeder space was increased to 3.11, 4.04 and 5.45 cm per bird from 4 to 16 weeks. Birds were managed with standard managements ND A vaccination Schedule was implemented to emulate commercial pullet rearing programs (15, 21).

Two ml of blood samples were taken from the brachial vein into 2 tubes (into plain and heparinized tubes) at the age of 5, 10 and 20 weeks. Blood samples were immediately transferred to the laboratory at 4°C. Blood stored in plain tubes used for the collection of serum and evaluated for the presence of total specific antibody titres using a commercial ELISA kits. A smear slides were prepared from blood samples kept in heparinized tubes and stained using May-Griend-Giemsa mixed staining technique. White blood counts were measured by the method of Feldman et al (9).

Statistical analysis

A three-factor by 4 strains (S), 3 cage densities (D) and 3 cage positions (P) factorial arrangement of randomized design was used. Independent variable strain, density and cage position were analyzed using 2 statistical models⁵ for day 1 to 4 weeks (Model 1) and for 4 to 20 weeks (Model 2) and these were:

$$Y_{ijk} = \mu + S_i + D_j + SD_{ij} + e_{ijk} \quad [Model 1]$$

$$Y_{ijk} = \mu + S_i + D_j + P_k + SD_{ij} + SP_{ik} + DP_{jk} + SDP_{ijk} + e_{ijk} \quad [Model 2]$$

Where Y_{ijk} is the observation per cage; μ is the overall mean; S_i is the bird strain effect; D_j is the cage density effect; P_k is the cage position; SD_{ij} , SP_{ik} , DP_{jk} , SDP_{ijk} are subsequent interactions; and e_{ijk} is the random error. Data were subjected to statistical analysis using the general linear models procedure of SPSS⁸, and differences among the means were partitioned using Duncan's multiple range procedure (7). Significance level was set at $P < 0.05$.

Result and Discussion

As cage density increased the antibody titres to IBDV vaccine showed a linear decrease in BW pullets (*Figure 1*). The antibody titres to NDV was influenced negatively by cage density in white egg laying strains however density did not have any significant effect on humoral immunity of IB chicks. The antibody titres to NDV vaccine of LB chicks was influenced negatively at the density of 20-35 birds/cage (*Figure 2*). Titres to EDSV vaccine were higher in Brown egg laying strains (density of 27 birds/cage) than in BW chicks (density of 20-27 birds/cage). No significant density effect was noted for LW chicks (*Figure 3*). DxP interaction did not have effect on the antibody titres to IBV, IBDV and EDS vaccines of chicks, nevertheless, the highest antibody titre to NDV was seen in all three density groups that housed in the middle cages (*Figure 4*).



Figure 1. Effect of strain x cage density interaction on antibody production at 20 weeks of age against Infectious bursal disease virus (IBDV) vaccine using ELISA test

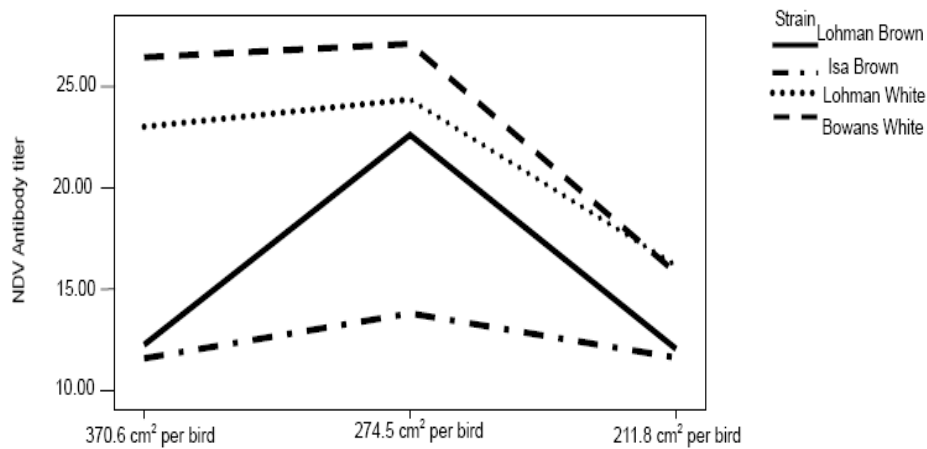


Figure 2. Effect of strain x cage density interaction on antibody production at 20 weeks of age against Newcastle disease virus (NDV) vaccine using ELISA test

Amongst the evaluated factors, interactions generated remarkable result. In a similar density, pullets of four strains showed similar or dissimilar responses. For example, housing at the density of 35 birds/cage negatively influenced the antibody titres in LW and LB pullets however it enhanced the immune response in BW pullets. In addition, the top cages were associated with more stress in white egg layer strains but it was none-stressful for Brown egg layer strains. This shows that, ignoring all these differences, to apply similar management to commercial strains which had already attained the anatomic, physiologic and behavioural diversity by genetic selection may cause the loss of their welfare aspects though indirectly.

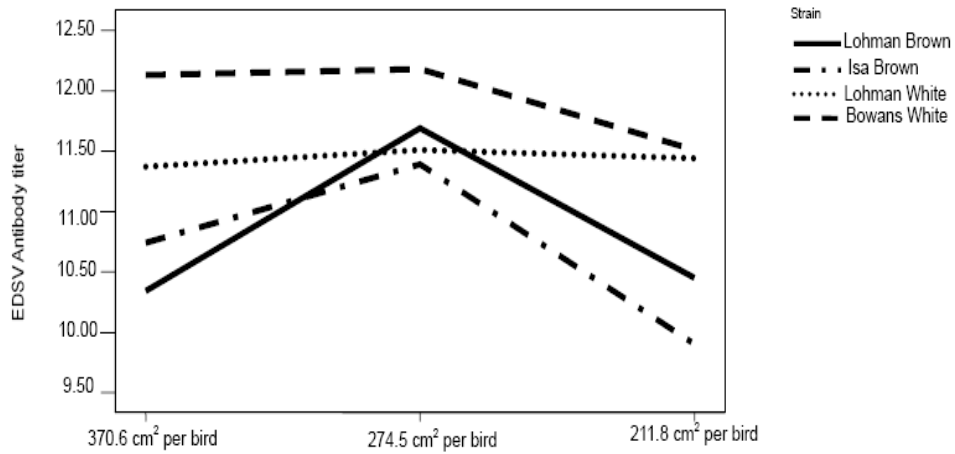


Figure 3. Effect of strain x cage density interaction on antibody production at 20 weeks of age against Egg Drop Syndrome disease virus (EDS-76 virus) vaccine using ELISA test

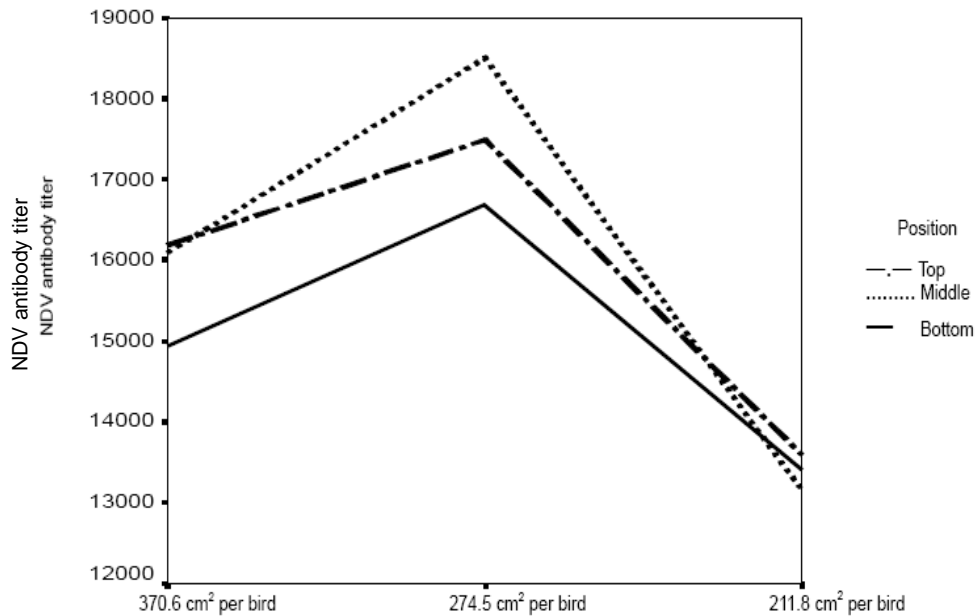


Figure 4. Effect of cage density x cage position interaction on antibody production at 20 weeks of age against Newcastle disease virus (NDV) vaccine using ELISA test

Stress hormones (catecholamines, surrenal hormones etc) are released under stress, and protein destruction increases by gluconeogenesis (6, 9), therefore the lower antibody titres in the density group of 35 birds/cage may be associated with

stress in our study. It was difficult to explain why lower antibody titres were observed in the lowest density group (20 birds/cage). However this may be associated with other factors (such as feeding policy, various environmental aspects and individual genetic differences) that affect immune system performance rather than the density related stress (12).

Housing in cage created more stress in brown egg layer pullets than in white egg layer strains, because heterophil ratio and H/L ratio were high in these birds. Previously, it was reported that the increase in H/L ratio was significantly associated with stress in avian species (11, 15, 16). Moreover, study of Al-Murrani et al. (1) showed that H/L ratio was changeable among broiler lines and concluded that the lower the H/L ratio the line was more resistant to stress.

S x P and D x P interactions an important criterion for the antibody titre to EDSV vaccine were measured ($P < 0.05$). Effects of housing at the top and bottom cages on immune response were unfavourable for LW and IB pullets however favourable for BW pullets. EDSV antibody titre of LB pullets was not influenced by the position (*Figure 5*). In the density group of 35 birds/cage, from top to bottom, EDSV antibody titre dropped whereas in the density of 20-27 birds/cage, when compared to the middle and bottom cages, the antibody titres were more negatively affected than the top cage (*Figure 6*). Important S x D x P interactions were observed in terms of the antibody titres to NDV and EDSV vaccines in pullets of 20 week-old.

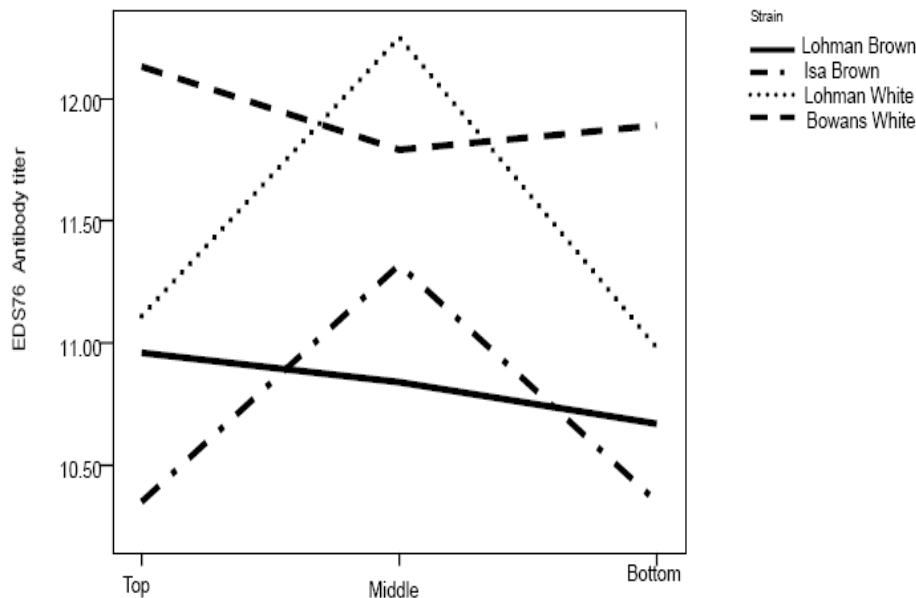


Figure 5. Effect of strain x cage position interaction on antibody production at 20 weeks of age against Egg Drop Syndrome disease virus (EDS-76 virus) vaccine using ELISA test

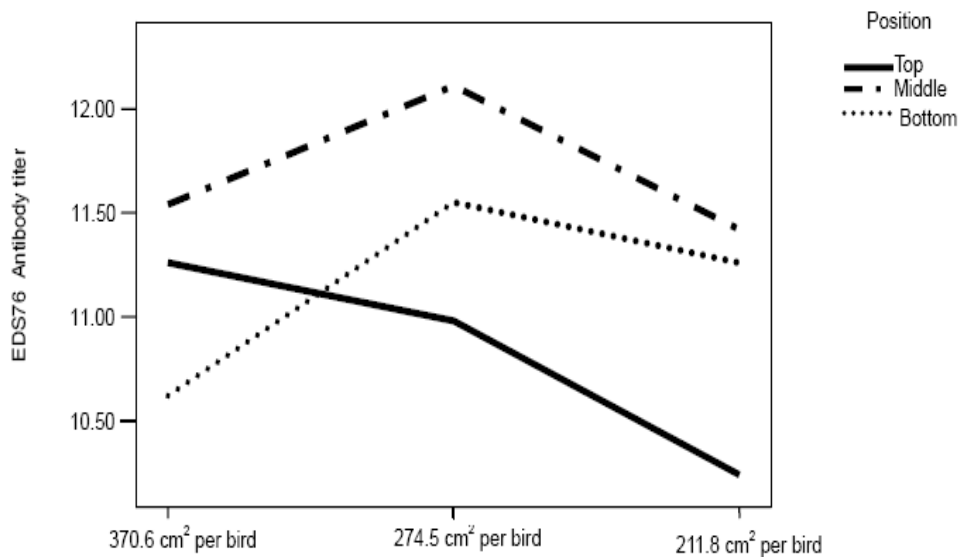


Figure 6. Effect of cage density x cage position interaction on antibody production at 20 weeks of age against Egg Drop Syndrome disease virus (EDS-76 virus) vaccine using ELISA test

Blood paramaters associated with stress

At the early life of pullets, responses of percentage lymphocytes, heterophils and H/L ratio were influenced by strain, density and position ($P < 0.01$) and later periods, because of importance of position effect, interactions between strain, density and position were also significant. H/L ratio was higher in white strains than in brown strains. Density did not significantly affect the H/L ratio alone however S x D interaction was important at the age of 20 weeks. Only LW pullets generated higher H/L ratio within the density group of 20 birds/cage.

Housing at a density of 35 birds/cage showed an increase in H/L ratio in IB, LW and BW pullets but a decrease in LB pullets (*Figure 7*). During the first 10 weeks, according to the cage position, from top to bottom, H/L ratio decreased ($P < 0.05$ and $P < 0.01$).

Significant S x P interactions (at the age of 5 and 20 weeks) showed that H/L ratio was high in white strain pullets when housed at the top cages whereas in brown strain pullets when housed at the bottom cages (*Figure 8*). The results of lymphocytes and heterophils percentages were concordant with H/L ratio. Strain and density had effect on the percentage eosinophils. The highest eosinophil ratios were noted in brown strains. Pullets housed at the density of 27 birds/cage, in comparison to other two groups, had the highest eosinophil ratio. At the age of 5 weeks, higher monocyte and basophil ratio were observed in brown strains than in white strains ($P < 0.01$), in later periods it did not differ from each other. With

respect to the cage position in the battery, from top to bottom, percentages of monocyte and basophil decreased ($P < 0.05$).

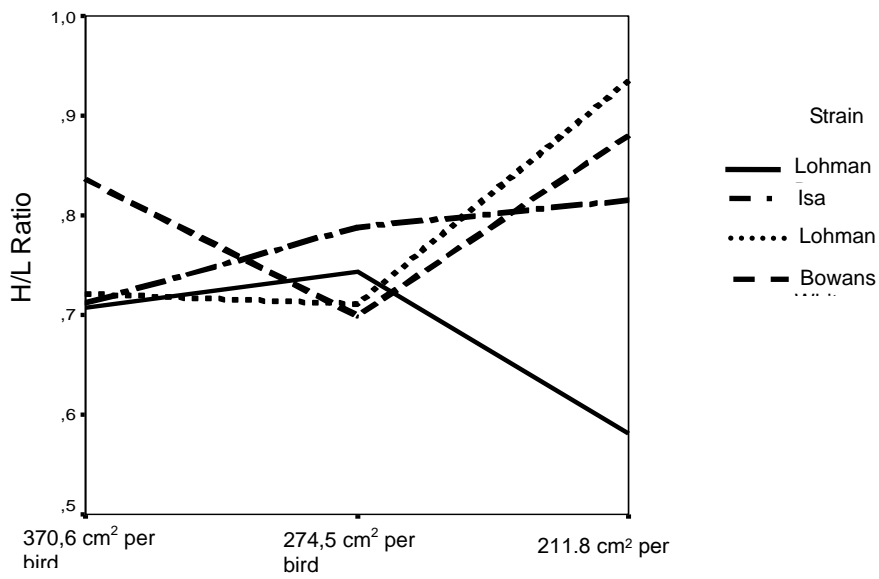


Figure 7. Effect of strain x cage density interaction on H/L ratio at 20 weeks of age

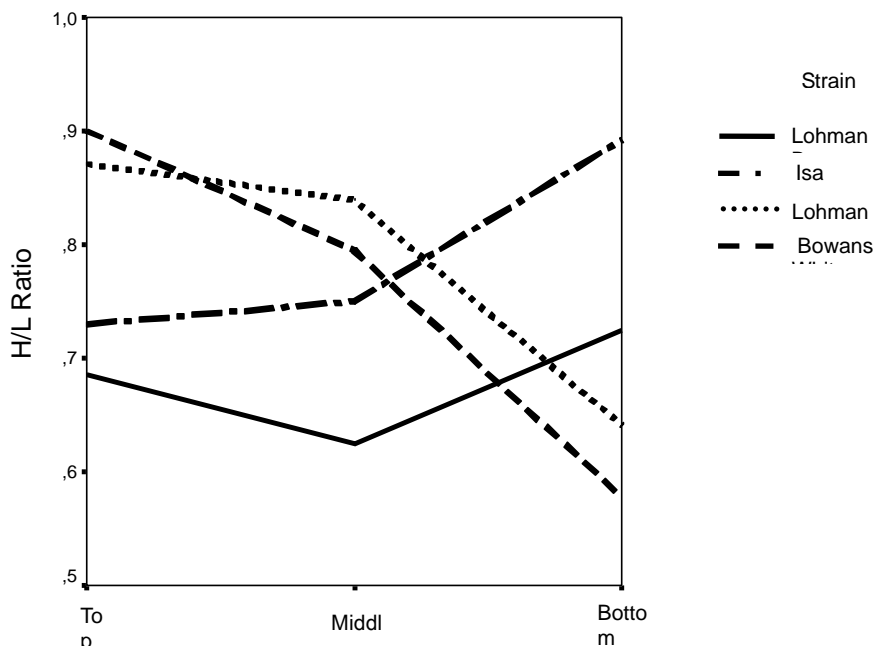


Figure 8. Effect of strain x cage position interaction on H/L ratio at 20 weeks of age

Conclusion

As birds growing, the cage position was more important stressor than the cage density and the stress had various effects on different pullets of strains. Strains showed different antibody titres to the same vaccines and stress and humoral response of each strain was closely associated to which position and what density birds were housed in the battery. The data generated here showed that cage-related stress could be decreased, resistance to diseases and well-being of hens finally may be improved if hens are kept under proper position and density within cage systems by taking in to account of their physiologic and behavioral traits that controlled by the gene.

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