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Effects of Strain, Cage Density and Position on Immune Response to Vaccines and Blood Parameters in Layer Pullets

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Summary

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 cm^2 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 Bronchitis Virus (IBV), Infectious Bursal Desease 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*

Piliçlerde Genotip, Kafes Yoğunluğu ve Pozisyonunun Aşılara Karşı Oluşan İmmun Yanıta ve Kan Parametrelerine Etkileri

Özet

Çalışmada Lohman Brown (LB), Isa Brown (IB), Lohman White (LW) ve Bowans White (BW) genotiplerinden 1 günlük yaşta 2000 adet civciv kullanıldı. Civcivler 3 kafes sıklığında (sırasıyla 211.8, 274.5 ve 370.6 cm2/piliç) ve 3 kafes pozisyonunda (üst, orta ve alt kat kafesleri) barındırıldı. Piliçlere standart bakım-besleme uygulandı ve ticari bir aşı programına uygun aşılama yapıldı. Beş, 10 ve 20 haftalık yaşlarda alınan kan örneklerinde Infectious Brochitis Virus (IBV), Infectious Bursal Desease Virus (IBDV), Newcastle Disease Virus (NDV) ve Egg Drop Syndrome Virus (EDSV) aşılarına karşı oluşan toplam spesifik antikor üretimi ticari ELISA kitleri kullanılarak belirlendi. Hücresel immun yanıt da incelendi. Ticari beyaz yumurtacı piliçlerde IBV, IBDV, NDV ve EDSV aşılarına karşı spesifik antikor üretimi kahverengi yumurtacı piliçlerden daha yüksekti. Kafeste barındırılma kahverengi yumurtacı piliçlerde beyaz yumurtacı piliçlere göre daha fazla stres oluşturdu. Kafes sıklığı arttıkça, heterofil ve heterofil/lenfosit (H/L) oranları hafifçe arttı. Pozisyon etkisi IBV ve IBDV aşılarına karşı antikor üretimini etkilemedi, ancak piliçlerin barındırıldıkları kafesin pozisyonu bataryada üstten alta doğru indikçe NDV ve EDSV antikor üretimi azaldı, yüzde heterofil, H/L oranı ve bazofil oranları düştü. Bu bulgular, piliçler kafes sistemleri içerisinde genleri tarafından kontrol edilen fizyolojik ve davranımsal özelliklerine uygun sıklık ve pozisyonda barındırılırlar ise kafes kaynaklı stresin azaltılabileceğini, hastalıklara karşı dirençlerin ve dolayısı ile refahlarının da arttırılabileceğini gösterdi.

Anahtar sözcükler: *Kafes yoğunluğu, Kafes pozisyonu, Piliç, Genotip, Stres, Refah*

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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 [1,2].

Traditional cages in comparison to alternative systems such as housed in floored pen have advantages of less production cost and decreased disease risk [3,4], however, they also have disadvantages such as undesired welfare conditions and significant stress disroders [5,6].

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 [7]. Webster Marketon and Glaser [8] reported that physiological and pathological stress in avian species affected neuroendocrine system (glucocorticoids, catecholamins, epinephrine, norepinephrine, prolactin and growth hormones) and reduced the lymphocyte production. Several researchers focused on the effects of caged housing [4,9,10], stress [11] management [12,13], environmental conditions and vaccinations [14,15] on immune performance.

H/L ratio was defined as stress index in hens [16,17]. It was reported that basophil and heterophil cell numbers in poultry under stress increased [18,19].

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 desings 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 cm^2 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 cm^2 per bird, and feeder space was increased to 3.11, 4.04 and 5.45 cm per bird from 4 to 16 weeks.

Brooding temperature was kept at 33°C on day 1, 24 h/day lighting was maintained for the first 2 days, then temperature and light were reduced gradually and set point of 21°C and 13 h/day, respectively, as recommended by the Lohman, Bowans ve Isa commercial management guides [20-23]. Birds were fed with a 3-phase program of starter, grower and developer diet that consisted of 20%, 17% and 14% crude protein and 2800, 2750 and 2750 kcal ME/kg, respectively, during 1-8, 9-14 and 15-16 weeks *(Table 1)*. Feed and water were supplied ad libitum and birds were fed twice throughout the day at 09:00 and 15:00 h. Chicks were beak trimmed at the age of 10 days. The results of chicks' performance were published elsewhere [24].

Vaccination Program

A vaccination Schedule was implemented to

emulate commercial pullet rearing programs [25].

Newcastle Disease Virus (NDV), Infectious Bursal Desease Virus (IBDV), Infectious Bronchitis Virus (IBV) and Egg Drop Syndrome Virus (EDSV) vaccines used in the study were purchased from CEVA (Animal Health, South Africa Ltd./ Johannesburg) and Novartis (Novartis International AG. Basel, Switzerland) and administered according to the manufacturer's recommendations. Marek's disease vaccine was given to chicks immediately after hatching and then were kept in the study pen.

Maternal antibody levels were determined at Bornova Veterinary Control and Research Institute, Izmir, Turkey in order to plan the vaccination program *(Table 2).*

Blood sampling, determination of humoral and cell-mediated immunity

Two ml of blood samples were taken from the brachial vein into 2 tubes (into plain and heparinized tubes) at at the age of 5, 10 and 20 weeks. Blood samples were taken from 8 birds from each subgroup (8 birds from each of 9 strain subgroups and 8 birds from 12 density subgroups (4 strain x 3 density) at 5 weeks of age; 144 birds from 18 strain subgroups, 96 birds from 24 density subgroups (4 strain x 3 density) and 96 birds from 24 position subgroups (4 strain x3 density) at 10 and 20 weeks of age. After collection, 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 (BioCheck, Gouda/Holland) as described by Puthpongsiriporn and Scheideler [12]. The absorbency (Optical Density [OD] of 405 nm) was measured with a multi-well ELISA plate reader. Results were expressed on a numerical scale with a standard positive sample used to set the highest value for each group separately.

Leucocyte diffential count were obtained in blood smcar stained with MayGrunwald-Giemsa Stain, and calculated the percentage of each of the five basic leucocyte (heterophils, eosinophils, lymphocytes and monocytes) [26]

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. Factors were evaluated for major effects and their interactions. Response variables included antibody titre to IBV, NDV, IBDV and EDSV vaccines and ratios of lymphocytes, reactive lymphocytes, H/L, eosinophils, monocytes and basophils. Independent variable strain, density and cage position were analyzed using 2 statistical models [27] for day 1 to 4 weeks *(Model 1)* and for 4 to 20 weeks *(Model 2)* and these were:

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

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

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 eijk is the random error. Data were subjected to statistical analysis using the general linear models procedure of SPSS[27], and differences among the means were partitioned using Duncan's multiple range procedure [28]. Significance level was set at $P<0.05$.

RESULTS

Maternal Gumboro (IBDV) antibody titres amongst brooding flocks obtained from chicks of LB, LW, IB and BW strains were found to be 6132, 7495, 8863 and 5874 respectively.

The ELISA cut-off values were determined for IBDV as S/P>0.20 (mean negative control OD=0.132, mean possitive control OD=0.572), for NDV as S/P>0.20 (mean negative control OD=0.132, mean possitive control OD=0.572), for IBV as S/P>0.30 (mean negative control OD=0.158, mean possitive control OD=0.610), and for EDSV as S/P>0.35 (mean negative control OD=0.121, mean possitive control OD=0.510).

Specific Antibody Titres

Specific antibody titres of chicks to IBV (5, 10 and 20 weeks of age, P<0.01), IBDV (5 and 20 weeks of age, P<0.01 and P<0.05), NDV (5, 10 and 20 weeks of age, P<0.01, P<0.05 and P<0.05) and EDSV vaccines (at 140th day, P<0.01) were

significantly affected by the strain *(Table 3)*.

At the 5th and 10th weeks, although serum IBV antibody titres were the highest in LB and IB chicks this trend, in advancing stage, was changed in favour of white egg laying strains. As antibody titre in Brown egg laying strains markedly decreased, it rapidly increased in white egg laying strains (P<0.05). In 7, 16 and 23 day-old chicks, specific humoral response formed during the first 5 weeks against IBDV vaccine was higher in LB and IB chicks than those in other chicks (P<0.05). The highest antibody titre was observed at the age of 20 weeks in IB chicks followed, in order, by LW, BW and LB strains. In general IBDV antibody titre in BW chicks during growing period increased in a linear fashion however there was a decrease in other three strains on day 70.

Strain effect on the level of antibody titres to the first 3 NDV vaccines was irregular in chicks. After the 4th and 5th vaccination antibody titres to NDV increased rapidly in white egg layer strains but rather decelerated in Brown egg layer strains (P<0.05).

Density had influence on the immune response directed to IBV in early stage and also affected antibody titres to IBDV, NDV and EDSV vaccines after 10 weeks of age. Chicks grown in medium density group (274.5 cm^2/bird) gave the highest immune response to these vaccines whereas chicks grown in the most dense cages (211.8 cm^2/bird) generated the lowest immune response (P<0.05). Interestingly, in density group 3 (370.6 cm^2/bird) where the welfare anticipated to be the best, the antibody titre was low. Despite of no positional effect at the ages of 5 and 10 weeks when compared to chicks grown in middle and top cages at the 20th week immun response of bottom cage chicks to NDV and EDSV vaccines was lower (P<0.01 and P <0.05, respectively) *(Table 3)*.

S x D interaction influenced the antibody titre to IBDV (P<0.05) and NDV vaccines (P<0.01) at the age of 20 weeks. Density of both 20 bird/cage and 35 birds/cage system resulted in a decrease in the antibody titres to IBDV in LW and LB chicks but increased in IB chicks.

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)*.

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 *(Table 3)*.

Blood paramaters associated with stress

Data associated with blood parameters are summurised in *Tables 4* and *5*. 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 (Table 4). 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 pecentages were concordant with H/L ratio. Strain and denstiy 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 *(Table 5)*. 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 ($P<0.05$).

As shown in S x P interactions (important criteria for percentage basophil, monocyte and basophil ratio) increased in pullets housed at a density of 27 birds/cage at the bottom cages but not at the top and middle cages. Percentage of monocytes in all strains were influenced by housing at the middle cage. IB housed at the top and BW at the bottom cages possessed higher monocyte ratio than other strains ($P<0.01$).

DISCUSSION

Commercial white egg layer strains had higher titre of specific antibody to IBV, IBDV, NDV and EDSV vaccines than those of brown egg layer strains in this study. Lymphocyte ratio was also higher in white egg layer strains. In mammals and avian species antibodies are produced by lymphocyte cells within the immune system. Therefore changes in lymphocyte ratio further influences circulating blood antibody levels. These results indicated that genotype had effect on immunity in egg layer pullets in our study as stated by several researchers that the amount and period of antibody production by a B-lymphocyte is governed by the genes [11,29,30]. In addition, after the age of 5 weeks, differences between each strain regarding H/L ratio and basophil ratio considered as an indicator of stress eventually disappeared. This may suggest that all pullets established an adaptation skill to overcome cage-related stress. Thus, this period could differently affect welfare and antibody responses of strains having different behavioural and physiological characteristics [12,31,32]. Commercial lines obtained as a result of selection for different characteristics may hold genetic diversity that effect immune response [9,33]. 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. Proviously, it was reported that the increase in H/L ratio was significanlty associated with stress in avian species [16,19,34].

Moreover, study of Al-Murrani et al.[17] 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. There was a large variation between different strains in terms of immune response in hens [35]. Variations in H/L ratio between strains in our study may be attributable to the anatomic structure of birds because brown strains possessed greater body weight which may be, negatively, further associated for the use of space within confined cage area. The average body weights of LB, LD, LW and BW pullets were 1492, 1438, 1202 ve 1115 g, respectively [24]. This was also supported by the previous work of Siversides and Budgell [36].

Antibody titre was closely influenced by the cage density. Antibody titres were lower in medium density group (274.5 cm^2/bird) however it was higher in comparison to the other two density groups. As cage density increased there was a slight drop in lymphocyte ratio and an increase in heterophil, H/L and basophil ratios but a decrease in eosinophil ratio which suggest that stress could be affected parallel to the cage density [16]. Stress hormones (catecholamines, surrenal hormones etc) are released under stress, and protein destruction increases by gluconeogenesis [10,37], therefore the lower antibody titres in the density group of 35 birds/cage may be associated with stress in our study. Borges et al.[38] and Donker et al.[39] reported that stress increased as temperature raised in birds. Similarly, in the current study, the higher the density in the cage the higher temperature (possibly created by metabolic process of several birds) was recorded and this could further cause the chronic heat stress since lower heterophil, basophil and H/L ratio were observed in these particular groups. These findings support the study of Glaser et al.40 who showed that chronic stress could have effect on IgG stability or number of cells releasing IgG after the vaccination. It was difficult to explain why lower antibody titres was 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 [13]. One of these factors could be body development in which 16-week-old pullets had more heterogen body weight than other density groups [24]. In these groups, development disorders of body could negatively affect the immune responses in the pullet [14]. Nevertheless, Humphrey [41] showed in an experimental study that the potential resistance of birds in a good welfare condition could be influenced by other environmental factors. In the study R^2 values (R^2: 0.06-0.54) of statistical model used for the evaluation of antibody responses showed a wide variation, thus other factors rather than strain, density and cage position could influence the variations in antibody titres amongst pullets. Effect of these factors may be inconsistent according to the vaccine type and vaccination periods [15,41]. In terms of stres, blood parameters, lower variation in R^2 values of models indicated that stress in pullets used in this study may be significantly associated with strain, density and cage position [27].

The cage position did not show sero-response to IBV and IBDV vaccines however the antibody titre to NDV and EDSV decreased and percentage heterophils, H/L ratio and basophil ratio were low from top to bottom cage in the battery. This indicates that pullets at the top cage could face to more stress than at the lower cages. However this finding disagrees with the conclusion of Fraisse and Cockrem [42] who showed that there was no significant differences in the corticosterone concentration between tiers. In our study, birds were used during the growth period (developmental stage of their immune system) and factors such as the size of groups and controlled environment may have effect. Interestingly, albeit advantageous in terms of stress, the rate of lymphocytes producing antibodies was high but titres were low in pullets housed at the bottom cages. Again this is difficult to explain, however microenvironment present at the bottom cage effecting directly to B-lymphocytes could pressure antibody synthesis [5,30,40]. It gives rise to a tought of the other factors such as lymphokines produced by T and B lymphocytes or monokines produced by monocytes and macrophages [43].

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.

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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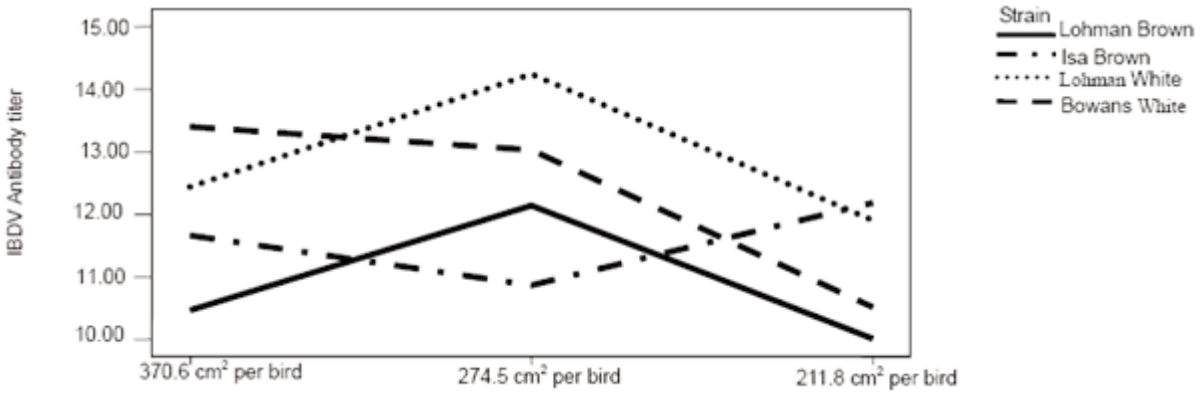

Fig 1. Effect of strain x cage density interaction on antibody production at 20 weeks of age against Infectous bursal disease virus (IBDV) vaccine using ELISA test

Şekil 1. 20 haftalık yaşta İnfeksiyöz Bursal Disease virus (IBDV) aşısına karşı oluşan ve ELISA test ile belirlenen antikor üretimine genotip x kafes yoğunluğu interaksiyonunun etkisi

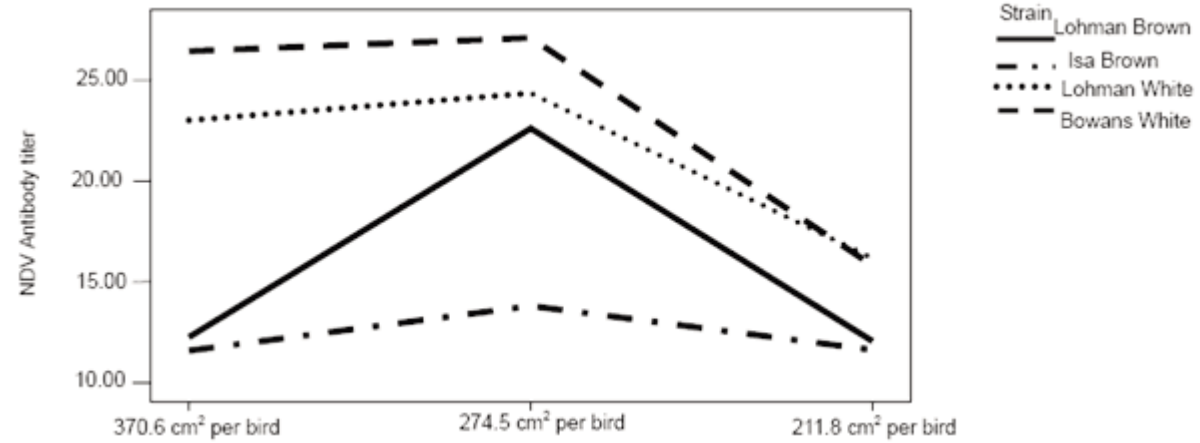

Fig 2. Effect of strain x cage density interaction on antibody production at 20 weeks of age against Newcastle disease virus (NDV) vaccine using ELISA test

Şekil 2. 20 haftalık yaşta Newcastle disease virus(NDV) aşısına karşı oluşan ve ELISA test ile belirlenen antikor üretimine genotipxkafes yoğunluğu interaksiyo-nunun etkisi

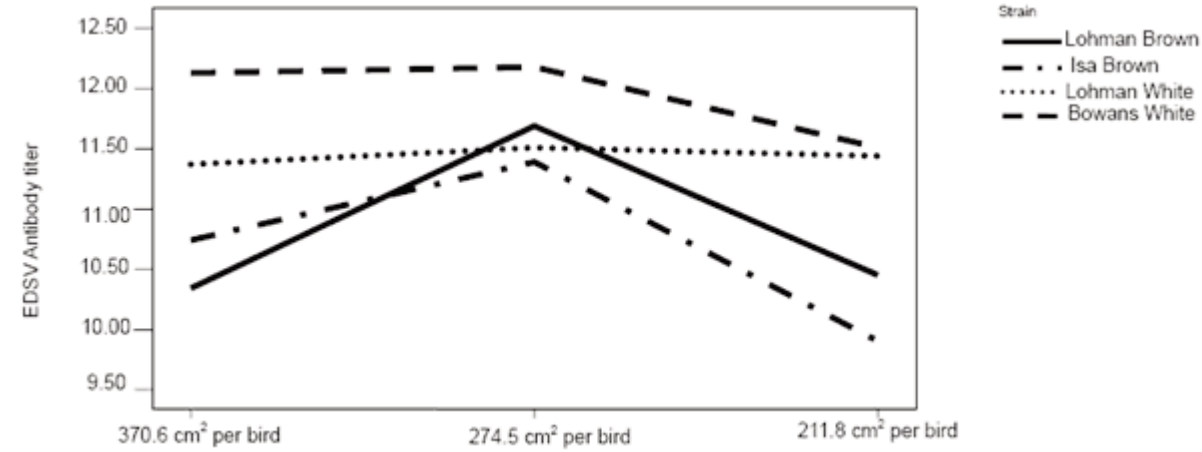

Fig 3. Effect of strain x cage density interaction on antibody production at 20 weeks of age against Egg Drop Syndrome disease virus (EDS-76 virus) vaccine using ELISA test

Şekil 3. 20 haftalık yaşta Egg Drop Syndrome disease virus (EDS-76 virus) aşısına karşı oluşan ve ELISA test ile belirlenen antikor üretimine genotip x kafes yoğunluğu interaksiyonunun etkisi

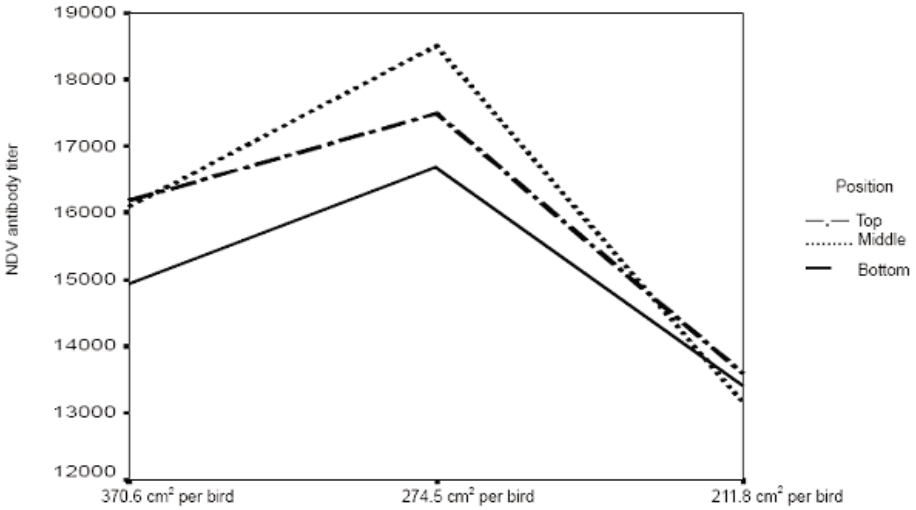

Fig 4. Effect of cage density x cage position interaction on antibody production at 20 weeks of age against Newcastle disease virus (NDV) vaccine using ELISA test

Şekil 4. 20 haftalık yaşta Newcastle Disease virus (NDV) aşısına karşı oluşan ve ELISA test ile belirlenen antikor üretimine kafes yoğunluğu x kafes pozisyonu interaksiyonunun etkisi

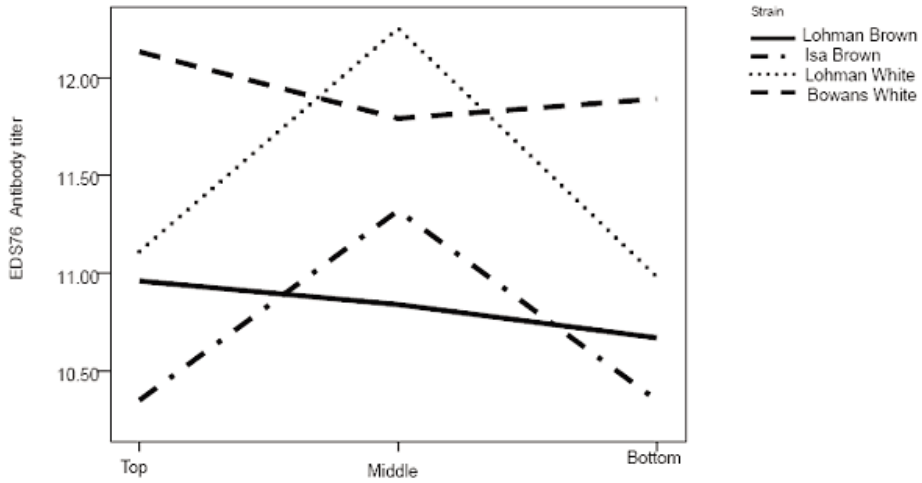

Fig 5. Effect of strain x cage position interaction on antibody production at 20 weeks of age against Egg Drop Syndrome disease virus (EDS-76 virus) vaccine using ELISA test

Şekil 5. 20 haftalık yaşta Egg Drop Syndrome disease virus (EDS-76 virus) aşısına karşı oluşan ve ELISA test ile belirlenen antikor üretimine genotip x kafes pozisyonu interaksiyonunun etkisi

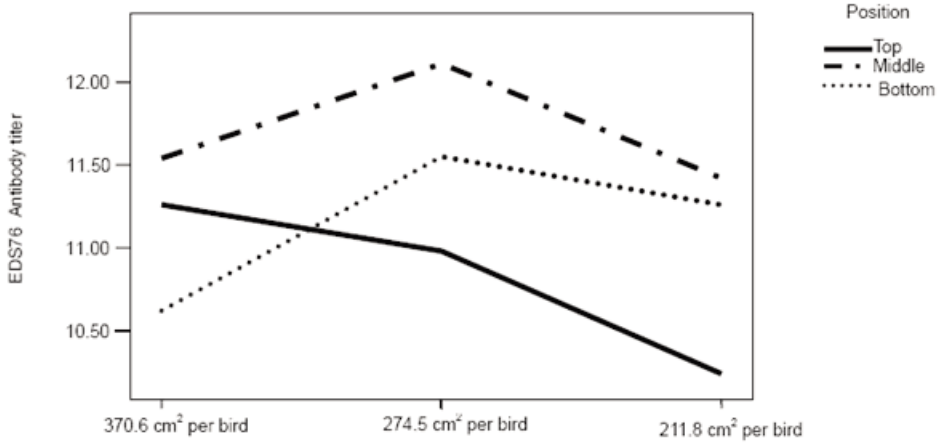

Fig 6. Effect of cage density x cage position interaction on antibody production at 20 weeks of age against Egg Drop Syndrome disease virus (EDS-76 virus) vaccine using ELISA test

Şekil 6. 20 haftalık yaşta Egg Drop Syndrome disease virus (EDS-76 virus) aşısına karşı oluşan ve ELISA test ile belirlenen antikor üretimine kafes yoğunluğu x kafes pozisyonu interaksiyonunun etkisi

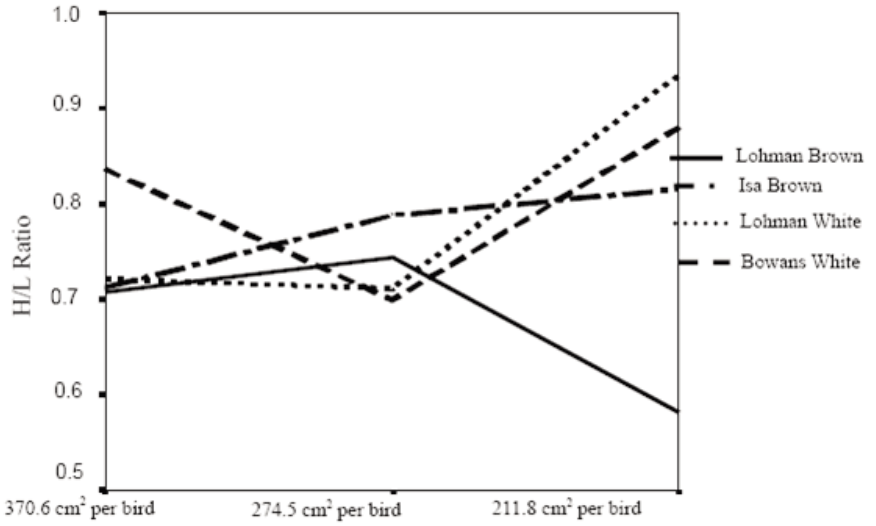

Fig 7. Effect of strain x cage density interaction on H/L ratio at 20 weeks of age
Şekil 7. 20 haftalık yaşta H/L oranına genotip x kafes yuğunluğu interaksiyonunun etkisi

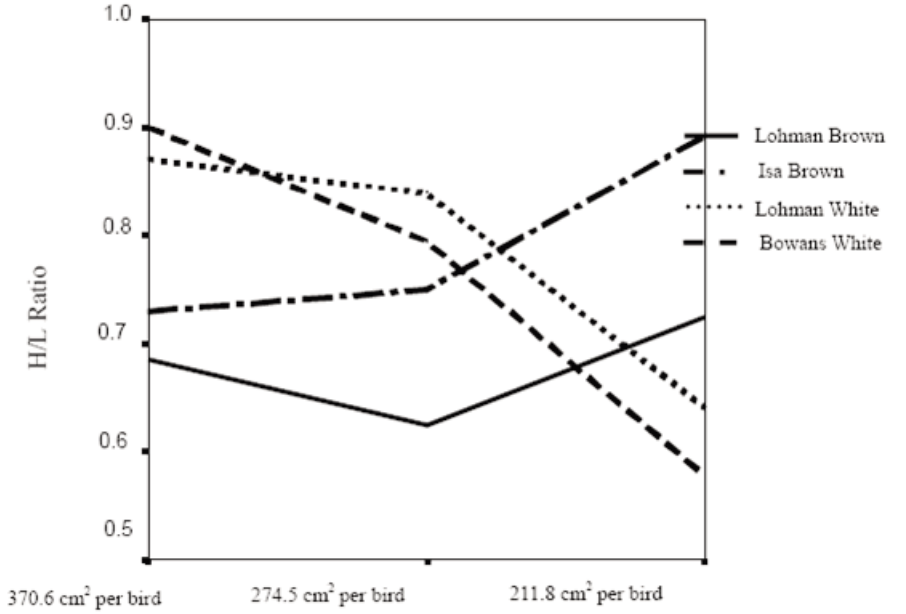

Fig 8. Effect of strain x cage position interaction on H/L ratio at 20 weeks of age
Şekil 8. 20 haftalık yaşta H/L oranına genotip x kafes pozisyonu interaksiyonunun etkisi

Tablo 1. *Deneysel dietlerin kompozisyonu ve içerikleri*
Table 1. *Ingredients and nutrient composition of experimental diets*

Ingredients	Diets		
	Starter (%)	Grower (%)	Developer (%)
Corn	30	20	20.7
Wheat	27	34	40
Barley	0	15	15
Sunflower meal	13.6	17.5	18.5
Corn bran	6.5	2.75	0
Soybean meal	20.8	5.4	0
Cotton seed meal	0	2.5	2.5
Limestone	0.50	1.2	1.64
DCP	0.7	0.7	0.7
Salt (NaCl)	0.35	0.35	0.30
Vitamin premix[1]	0.25	0	0
Vitamin premix[2]	0	0.25	0.25
Mineral premix[3]	0.1	0.1	0.1
DL-Methionine	0.07	0	0.02
Lysine	0.02	0.14	0.18
Natuphos[4]	0.06	0.06	0.06
Natugren blend[5]	0.05	0.05	0.05
Calculated analysis			
Dry matter %	88.16	88.32	88.36
Crude Protein, %	20.0	17.0	14.0
Crude sellulose %	5.0	6.2	6.0
Crude ash %	5.7	6.0	6.0
Metabolisable energy (ME) ,kcal/kg	2800	2750	2750
Calcium, %	0.75	1.1	1.4
Total phosphorus, %	0.72	0.71	0.71
Sodium, %	0.16	0.16	0.16
Chloride, %	0.28	0.30	0.28
Potassium, %	0.85	0.74	0.68
Methionine%	0.4	0.28	0.28
Methionine + cystine, %	0.7	0.57	0.55
Lysine, %	0.86	0.70	0.60
Triptophan, %	0.23	0.19	0.17

[1] **Rovimix 121-L,** Provided per 2.5 kg of diet: Vitamin A 12.000.00 IU; Vitamin D_3 2.500.000 IU; Vitamine E 20.000 mg; Vitamine K_3 4.000 mg; Vitamin B_1 3.000 mg; Vitamin B_2 6.000 mg; Vitamin B_6 5.000 mg; Vitamin B_{12} 20 mg; Niacin, 25.000 mg, Ca-D-Pantotenate, 6.000 mg; Folic acid, 750 mg; Choline clorit, 250.000 mg

[2] **Rovimix 122-E,** Provided per kg of diet: Vitamin A: 10.000.000 IU, Vitamin D_3 1.000.000 IU,vitamin E: 25.000 mg, vitamin K: 3.000 mg, Vitamin B_1: 2.000 mg, Vitamin B_2: 25.000 mg, Niacin :20.000 mg, Calcium D-pantothenate 8.000 mg, Vitamin B_6: 4.000 mg, Vitamin B_{12}: 15 mg, Folic acid: 800mg, Choline Chlorid: 300.000 mg

[3] **Remineral S provided per 2.5 kilogram of diet:** Mn, 40.000 mg; Fe, 60.000 mg; Zn, 5.000 mg; Cu, 500 mg; Co, 2.000 mg; Se, 150 mg; Ca, 223.905 mg

[4] **Natugrain Blend** Provided per 1000 gr: Endo-xylanase: 11.000.000 U, Beta –Glucanase: 240.000 U.

[5] **Natuphos** Provided per 1000 gr: Fitaz 500.000 U

Table 2. *Vaccination schedule used in this study*
Tablo 2. *Çalışmada kullanılan aşılama programı*

Age (week)	Administration	Disease and Vaccine	Vaccine Name
1	Subcutaneous injection	Attenuated live vaccine: MDV	AVINEW [1]
7	Subcutaneous injection	Inactivated vaccine: NDV + BDV Live Vaccine:NDV + IBV	CECAV ND IBD K[2] CEVAC BRON H120[2]
16	Drinking water	Live Vaccine: IBDV	CEVAC IBD L [2]
23	Drinking water	Live Vaccine: BDV	CEVAC IBD [2]
26	Drinking water	Live Vaccine: NDV	CEVAC VITAPEST L[2]
40	Drinking water	Live Vaccine: IBV	CEVAC BRON H120[2]
60	Drinking water	Live Vaccine: NDV	New Castle Lasota [3]
92	Drinking water	Live Vaccine: NDV	H120/Clone [2]
110	Intra Muscular Wing-web	Inactivated Vaccine: NDV + IBV + EDS Live Vaccine: Pox+	CEVAC ND IB EDS K[2] CEVAC FP L[2] CEVAC TREMOL AE[2]

MDV: Marek's disease **ND:** NewCastle Virus, **IBDV:** Infectious Bursal Deases, **IBV:** Infectious Brochitis Virus, **EDS:** Egg Drop Sendrome 76 , **AE:** Avian Encephelomiyelitis. [1]- Merial Select, Inc., Gainesville, GA. [2]- CEVA Animal Health, South Africa Ltd., Johannesburg
[3]-Novartis International AG. Basel, Switzerland.

Table 3. *Antibody titres to IBV, IBDV, NDV and EDSV vaccines of layer pullets strains at different ages, cage densities and positions*
Tablo 3. *Farklı yaşta, kafes yoğunluğu ve pozisyonunda barındırılan yumurtacı piliç genotiplerinin IBV, IBDV, NDV and EDSV aşılarına karşı antikor titreleri*

Treatment			Infectious Bronchitis (IB)			Infectious Bursal Disease (IBD)			Newcastle (ND)			Egg Drop Syndrome (EDS-76)
			Weeks									
Strain	Density[1]	Position[2]	5	10	20	5	10	20	5	10	20	
			(%)									
Lohman Brown			3.62 [a]	8.23 [a]	9.46 [a]	6.18	10.83 [b]	10.87 [b]	6.77 [b]	12.59 [a]	15.64 [b]	10.83 [c]
Isa Brown			4.62 [a]	7.91 [a]	9.48 [a]	7.39	12.64 [a]	11.57 [a]	6.42 [b]	10.59 [b]	12.33 [c]	10.68 [cb]
Lohman White			2.06 [b]	4.78 [b]	8.89 [b]	7.67	11.78 [ab]	12.86 [ab]	6.67 [b]	13.44 [a]	21.18 [a]	11.44 [ab]
Bowans White			2.05 [b]	5.37 [b]	8.86 [b]	9.14	11.21 [b]	12.32 [b]	7.50 [a]	11.47 [ab]	23.11 [a]	11.94 [a]
	1 (35)		3.01 [b]	6.24	9.53	6.67	11.93	11.15 [b]	6.72	12.53	13.91 [c]	10.83 [b]
	2 (27)		3.59 [a]	6.74	8.85	9.06	11.41	12.57 [a]	6.68	11.34	21.97 [a]	11.67 [a]
	3 (20)		2.66 [b]	6.74	9.14	7.05	11.52	11.93 [b]	7.12	12.18	18.32 [b]	11.14 [b]
		1(Top)		6.78	9.08		11.75	11.92		11.94	18.37 [a]	11.14 [ab]
		2(Milde)		6.52	9.11		11.57	12.06		11.82	18.71 [a]	11.55 [a]
		3(Bottom)		6.42	9.33		11.53	11.74		12.29	17.12 [b]	10.97 [b]
SEM of model			0.11	0.21	0.15		0.52	0.22	0.14	0.34	0.29	0.08
Main effect												
Strain			**	**	*	NS	NS	**	*	*	**	**
Density			*	NS	NS	NS	NS	*	NS	NS	**	**
Position			NS	NS	NS	NS	NS	NS	NS	NS	**	*
SXD			NS	NS	NS	NS	NS	*	NS	NS	**	NS
SxP			NS	NS	NS	NS	NS	NS	NS	NS	*	*
DxP			NS	NS	NS	NS	NS	NS	NS	NS	NS	*
SDxP			NS	NS	NS	NS	NS	NS	NS	NS	*	*
R^2			0.26	0.30	0.08	0.08	0.06	0.11	0.03	0.19	0.54	0.24

a-c Means in a column and treatment variable with no common superscript differ significantly (P<0.05). ***** P<0.05. ****** P<0.01, **NS** : Nonsignificant.
[1]- 35, 27 and 20 birds Per cage [2]- The top, middle and bottom rows of the battary. n=72 birds from each strain, 96 birds from each density groups at 5 weeks of age
n=144 birds from each strain, 192 birds from each density groups and 192 birds from each position groups at 10 and 20 weeks of age

Table 4. *Percentages of lymphocyte, heterophile and H/L of layer pullets strains at different ages, cage densities and positions*
Tablo 4. *Farklı yaşta, kafes yoğunluğu ve pozisyonunda barındırılan yumurtacı piliç genotiplerinin lenfosit, heterofil ve H/L oranları*

Treatment			Lymphocyte			Heterophyle			H/R Ratio		
			Weeks								
Strain	Density[1]	Position[2]	5	10	20	5	10	20	5	10	20
			(%)								
Lohman Brown			51.2 [b]	54.8	52.5 [a]	43.2 [a]	38.5	40.8	0.89 [a]	0.76	0.80
Isa Brown			51.1 [b]	52.5	52.5 [a]	43.2 [a]	39.4	39.5	0.91 [a]	0.81	0.77
Lohman White			57.8 [a]	54.6	57.3 [b]	35.1 [b]	37.2	37.4	0.64 [b]	0.72	0.67
Bowans White			58.9 [a]	53.7	54.2 [b]	34.0 [b]	38.8	41.1	0.61 [b]	0.75	0.77
	1: (35)		53.9 [b]	52.2	53.2	39.7 [a]	40.1	40.7	0.78	0.81	0.79
	2: (27)		52.6 [b]	54.6	54.9	40.8 [a]	37.8	38.6	0.83	0.73	0.73
	3: (20)		57.7 [a]	55.3	54.8	36.1 [b]	37.1	39.7	0.68	0.72	0.74
		1(Top)	52.6 [b]	49.2 [c]	52.8 [b]	41.0 [a]	42.8 [a]	41.0	0.80 [a]	0.90 [a]	0.79
		2(Milde)	54.6 [b]	55.7 [b]	54.9 [a]	39.8 [a]	36.6 [b]	39.6	0.84 [a]	0.69 [b]	0.74
		3(Bottom)	57.68 [a]	57.5 [a]	55.5 [a]	35.3 [b]	35.2 [b]	37.7	0.64 [b]	0.66 [c]	0.71
SEM of model			0.84	0.84	0.66	0.87	0.84	0.66	0.76	0.46	0.79
Main effect											
Strain			**	NS	*	**	NS	NS	**	NS	NS
Density			*	NS	NS	*	NS	NS	NS	NS	NS
Position			*	*	*	*	**	NS	*	**	NS
SXD			NS	NS	NS	NS	NS	NS	NS	NS	*
SxP			**	NS	NS	**	NS	*	NS	NS	*
DxP			*	NS	NS	*	NS	NS	**	NS	NS
SDxP			*	*	NS	*	*	NS	NS	**	NS
R^2			0.52	0.51	0.38	0.55	0.48	0.35	0.49	0.47	0.35

a-c Means in a column and treatment variable with no common superscript differ significantly (P < 0.05)
***** P < 0.05.** P<0.01, **NS** : Nonsignificant. [1]- 35, 27 and 20 birds Per cage [2]- The top, middle and bottom rows of the battary
n = 36 birds from each strain, 48 birds from each density groups at 5 weeks of age
n = 72 birds from each strain, 96 birds from each density groups and 96 birds from each position groups at 10 and 20 weeks of age

Table 5. *Percentages of eosinophile, monosite, basophile and reactive lymphocyte of layer pullets strains at different ages, cage densities and positions*
Tablo 5. *Farklı yaşta, kafes yoğunluğu ve pozisyonunda barındırılan yumurtacı piliç genotiplerinin eozinofil, monusit, bazofil ve reaktif lenfosit oranları*

Treatment			Eozinophyle			Monocyte			Basophye			Reactive Lymphocyte		
			Weeks											
Strain	Density[1]	Position[2]	5	10	20	5	10	20	5	10	20	5	10	20
			(%)											
Lohman Brown			2.22 [a]	3.27	2.11 [b]	4.70	0.72	0.68	0.65 [b]	0.72	0.68	0.93	0.72	0.82
Isa Brown			2.04 [b]	3.26	1.98 [ac]	5.13	0.76	0.78	0.61 [b]	0.76	0.78	1.10	0.75	0.83
Lohman White			2.29 [b]	2.96	2.67 [b]	3.14	0.76	0.80	0.90 [a]	0.76	0.80	0.83	1.17	0.91
Bowans White			2.93 [b]	3.10	3.48 [a]	3.34	0.82	0.78	0.92 [a]	0.82	0.78	0.77	1.10	1.31
	1: (35)		2.26 [b]	3.08 [b]	2.60	4.06	0.81	0.79	0.78	0.81	0.79	0.95	0.69	0.84
	2: (27)		2.78 [a]	3.71 [a]	2.58	4.23	0.74	0.74	0.84	0.74	0.74	0.86	1.16	0.91
	3: (20)		0.93 [b]	2.85 [b]	2.33	4.07	0.73	0.74	0.68	0.73	0.74	0.93	1.06	1.15
		1(Top)	2.32	3.33	2.56	3.92	0.90 [a]	0.80	0.81 [a]	0.90 [a]	0.80	0.82	0.94	1.09
		2(Milde)	2.36	3.43	2.33	3.64	0.69 [b]	0.75	0.84 [a]	0.69 [b]	0.75	1.09	0.89	0.67
		3(Bottom)	2.35	2.67	2.69	4.78	0.67 [c]	0.71	0.64 [b]	0.67 [c]	0.71	0.86	0.95	1.11
SEM of model			0.13	0.16	0.11	0.17	0.03	0.02	0.03	0.03	0.02	0.10	0.11	0.10
Main effect														
Strain			*	NS	**	*	NS	*	**	NS	NS	NS	NS	NS
Density			*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Position			NS	NS	NS	NS	NS	NS	*	**	NS	NS	NS	NS
SXD			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SxP			NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS
DxP			NS	**	NS	NS	NS	NS	**	NS	NS	NS	NS	NS
SDxP			NS	NS	NS	*	NS	NS	NS	**	NS	NS	NS	NS
R^2			0.37	0.44	0.36	0.50	0.29	0.29	0.49	0.47	0.357	0.209	0.162	0.188

a-c Means in a column an d treatment variable with no common superscript differ significantly (P<0.05)
***** P < 0.05.** P<0.01, **NS**: Nonsignificant. [1]:-35, 27 and 20 birds per cage [2]-The top, middle and bottom rows of the battary
n = 36 birds from each strain, 48 birds from each density groups at 5 weeks of age
n = 72 birds from each strain, 96 birds from each density groups and 96 birds from each position groups at 10 and 20 weeks of age