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Comparison of performance parameters, stress, and immunity levels of native and commercial layers reared in different cage densities in Turkey

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Abstract: In this study, production performance, stress, and immunity levels of native Turkish and imported commercial laying hens were investigated in two different cage densities. In the trial, a total of 06 groups were formed by using two different cage densities (312.50 and 468.75 cm²/hen) on each of 03 different laying hen hybrids of Isa Brown (IB), Atak-S (A-S) and Novogen White (NW). The trial was carried out with 09 replicates in each group having 10 birds per replicate making 540 birds in total. Water and feed were provided as *ad libitum*. It was found that the native hybrid showed lower performance in comparison to the foreign hybrids in terms of production performance such as egg production (EP), feed consumption (FC) and feed conversion ratio (FCR) ($p < 0.01$), while its growth variables such as livability (L) and body weight (BW) were higher. In terms of stress and immunity levels, the native hybrid showed similar values to those of the brown foreign hybrid, while the stress levels were lower and immunity levels were higher in the white laying hens ($p < 0.001$). It was observed that the yield characteristics and stress levels were affected negatively in higher cage density ($p < 0.01$), while immunity levels were not affected ($p > 0.05$). It was concluded that the yield characteristics of the native hybrid were relatively lower in comparison to those of the foreign hybrids, while its stress and immunity levels were similar, and cage density decreased yield, increased stress and did not significantly affect immunity levels.

Key words: Cage density, immunity, laying hens, performance, stress

1. Introduction

Intensive production in poultry raises concerns about animal welfare and food safety in humans [1]. Animal health and animal welfare are important preconditions in provision of safe food [2]. Some environmental stimulants may compromise animal health and immunity [3]. Housing systems, cages, stock density, lighting, and ventilation are all stress factors for poultry [4–7].

The continual progress and the intensive production practice of the egg sector have triggered investments in animal improvement and genetics, which, in return, has increased the importance of creating new gene resources and using high-producing commercial layer hybrids to achieve sustainable success [8]. The testing of lines, developed by breeding companies, under farm conditions is crucial to determining the genotype-by-environment interaction. Thus, random sampling tests are conducted with a view to contribute to the genetic material preferences and performance analyses of egg producers [9]. In this context, it is essential for the Turkish egg sector to test hybrids developed by national improvement programmes to determine the region with the best economic potential for the raising of a given hybrid and to prevent the wastage of resources through the selection of the hybrid best fit for the environment [10]. The number of laying hen hybrids, currently available at global level, is around 15–20, and ten of these are imported into Turkey for egg production. Today, while most of the laying hen hybrids raised in Turkey are imported, approximately 2.5%–3% are comprised of the native hybrids [10–12].

Not only the selection of genetically superior hybrids but also the housing and production system preferences have an impact on the laying hen sector. With the development of modern intensive stock farming, stocking density has become one of the most important environmental and management factors [13]. The exact stocking density to be used varies based on different genotypes and different production conditions. There are studies on the effects of stocking density on the performance and the welfare of animals, but their results are inconsistent due to different genotypes and production conditions [13]. In previous studies, the performance of

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the Atak-s hybrid was reported to be lower [14] or similar [15] compared to commercial hybrids. However, studies comparing Atak-s hybrid with both white and brown layers at different cage densities are limited. Similarly, studies comparing the welfare and immunity of layers are quite limited. Given that the performance traits of laying hen hybrids alter each year as a result of animal improvement programmes, the use of hybrids after their testing for the intended region of breeding is highly significant. Due to the lack of independent testing stations in Turkey, egg producers should cooperate with research institutes and pay attention to selecting genetic material most fit for their region and market.

This study was aimed at determining the performance traits, stress-level-related heterophil to lymphocyte ratios (H/L) and immunity-level-related SRBC antibody titres of the native Atak-S (A-S) and imported Isa Brown (IB) and Novogen White (NW) hybrids, which were housed at two different cage densities (312.50 cm^2/hen and 468.75 cm^2/hen) throughout the laying period (20–72 weeks). The study results are foreseen to contribute to the selection of high-quality hybrid material and to provide scientific input on the use of native hybrids in Turkey based on the assessment of the impact of cage density on production yield, stress, and immunity.

2. Materials and methods

This study was carried out at the laying hen houses of the Poultry Unit of Atatürk University, Food and Livestock Research and Application Centre.

The study design was approved by the Local Ethics Board for Experimental Animals of Atatürk University on the basis of their Decision Number 156, which was taken at their seventh session dated 04.11.2016 and notified in their official letter dated 36643897-000-E.1600261813.

The animal material of the study was comprised of imported Novogen White (NW) and Isa Brown (IB) hybrids and native brown egg-laying Atak-S (A-S) hybrids, which had hatched on the same day and were floor-reared at the same farm. After being vaccinated at 16 weeks of age, the hens were transferred to the research centre. The vaccination programme, which was implemented during the growth period, is presented in Table 1. Out of 720 weighed hybrids, 540 that had a body weight close to the average value were placed in numbered laying cages. The uniformity percentages of the selected IB, A-S and NW hybrids were 97.50%, 96.66%, and 97.50%, respectively.

Three different hybrids (A-S, NW and IB) and 2 different cage stocking densities (8 hens/cage and 12 hens/cage) were used in the trial. A total of 540 laying hens, including 180 animals of each hybrid, were used, and each group of hybrids was divided into subgroups of 8 and 12 animals with 9 replicates (Table 2). The normal cage density (NCD) was set as 468.75 cm^2/hen, whilst the high cage density (HCD) was set as 312.50 cm^2/hen. The animals were randomly assigned to the cages.

The measurements of all cages were the same: 60 cm depth, 62.5 cm width, 46 cm rear height, 51 cm front height, 62.5 cm feed trough length, 7°-sloped floor. There were 2 nipple drinkers in each cage. Ventilation was provided by

Table 1. The vaccination programme implemented during the growth period of laying hens.

Age	Vaccine	Disease	Route
1st day	Salmonella	Typhoid	Drinking water
10th day	Nobilis Ma5+ clone 30	Ib+Nd	Spray
15th day	Nd/ıb sohol	Ib+Nd	Drinking water
20th day	Gumboro	Ibd	Drinking water
26th day	Gumboro	Ibd	Drinking water
35th day	H120	Ib	Spray
40th day	Art	Shs	Spray
50th day	Coripravac®	Coryza	IM injection
60th day	Lasota	Nd	Drinking water
70th day	Nobilis® Ib4/91	Ib	Spray
85th day	Art	Shs	Spray
95th day	Lasota	Nd	Spray
112th day	4-way mixed	Vac,Nd,Ib,Eds76	IM injection

Ib: Infectious Bronschitis, Nd: Newcastle Disease, Ibd: Infectious Bursal Disease, Shs: Swollen Head Syndrome, Eds: Egg Drop Syndrome.

Table 2. Cage density layout for each hybrid.

Hybrids	Cage density (hen/cage)	Area per hen (cm^2)	Replicate	Total
Isa Brown	8	468.75	9	72
	12	312.50	9	108
Atak-S	8	468.75	9	72
	12	312.50	9	108
Novogen White	8	468.75	9	72
	12	312.50	9	108
Total				540

natural air movement through the windows on the side walls, an air shaft on the ceiling, and an electrical negative pressure fan that was 140 cm ×140 cm in size. By means of ventilation and heating system sensors, the in-house temperature was maintained within a range of 16–24 °C. The house was lighted with white fluorescent lamps. The lighting schedule applied during the growth period was as follows: 23 h light: 1 h dark during the first 3 days, 18 h light: 6 h dark between days 3–7, 14 h light: 10 h dark between days 7–10, and 13 h light: 11 h dark from day 11 to 19 weeks of age. As of the 19th week, the duration of the daily light period was extended for 30 min each week. Once the daily light period reached a level of 17 h on week 27, the photoperiod was fixed and not altered until the end of the laying period.

The feed, of which the nutrient content is presented in Table 3, was supplied from the same feed mill. During the growth period and until being transferred into cages, the pullets were floor-reared and provided with starter and grower rations. Once housed in the cages, the animals were given a starter feed (2750 kcal/kg metabolizable energy (ME) - 17.50% crude protein (CP)) between 16 and 20 weeks, 2750 kcal/kg ME - 16.26% CP between 21 and 45 weeks, 2720 ME kcal/kg - 15.83% CP between 46 and 65 weeks, and 2720 ME kcal/kg - 15.65% CP until the end of the trial, in granulated form and *ad libitum*.

2.1. Performance traits

Egg production (EP) and liveability (L) values were recorded daily, whilst feed consumption (FC), egg weight (EW), and feed conversion ratio (FCR) (per kg egg mass) values were monitored on a weekly basis. Calculations were made using the formulae indicated below.

Egg Production (Egg Yield) = (Total number of eggs laid per day / Number of hens) ×100

Daily Feed Consumption: (Weekly feed consumption / Number of hens in cage) / 7

Feed Conversion Rate: [Feed consumption / (Egg yield ×Average egg weight)]

Broken-Cracked Egg Percentage (%) = (Number of broken-cracked eggs / Total number of eggs) ×100

Average body weight values were determined by weighing the caged animals in groups at 17 weeks of age and at 4 week-intervals between 23 and 71 weeks of age on a precision balance accurate to 5 g. The average body weight per animal in a cage was calculated by dividing the total body weight of the caged group by the number of animals housed in the cage.

Liveability was determined by recording the number of daily mortalities, and the liveability of each group was calculated separately on a daily basis. To avoid the alteration of the cage density, dead animals were replaced by new animals of the same age and from the backup flock raised in the same house, at the same stocking density. The new animals introduced into the cages were wing banded for identification.

Liveability (Survival Rate) %= Number of animals alive / Total number of animals

2.2. Stress level determination

The stress level of the animals was determined by means of the heterophil-to-lymphocyte ratio (H/L). Accordingly, at weeks 35 and 65, a hen was randomly selected from each hybrid and cage density subgroup (in total 54 hens were selected, including 1 hen per cage), and blood samples were taken from the wing vein of the selected hens. The blood samples were used for the preparation of smears, which were air-dried and stained with the May–Grünwald–Giemsa method [16]. A drop of immersion oil was placed on a thin part of the smear, and light microscopic examination was performed at x100 magnification, in different microscopic fields, using an immersion objective. Leukocytes were counted to a total of 100 cells per slide, and the types of leukocytes observed were recorded such that their percentile shares were calculated. The total leukocyte count refers to the total number of heterophils (H), lymphocytes (L), monocytes (M), basophils (B), and eosinophils (E). The H/L ratio was

Table 3. The composition of the feeds provided to the hens during the laying period.

Ingredients %	**17–20 age (weeks)**	**21–45 age (weeks)**	**46–65 age (weeks)**	**66–72 age (weeks)**
Wheat	19.06	16.70	15.43	15
Corn	47.5	49.45	49.35	52.08
Soyabean meal	18.2	16.75	17.3	14.92
Sunflower seed meal	8	4.93	4.93	4.93
Limestone	3.01	8.05	8.9	9.25
Dicalcium phosphate	3.2	1.8	1.49	1.35
Vegetable oil	0	1.35	1.6	1.59
DL-Methionine	0.07	0.05	0.1	0
L-Lysine	0.06	0.02	0	0.02
Enzyme	0.27	0.27	0.27	0.27
Sodium bicarbonate	0.18	0.18	0.18	0.16
Salt	0.2	0.20	0.20	0.19
Vitamin mineral premixes	0.25	0.25	0.25	0.25
Analyzed Value				
M. Energy (Kcal/kg)	2750	2750	2720	2720
Crude protein	17.50	16.26	15.83	15.65
Calcium	2.00	3.57	3.74	3.83
Phosphorus	0.65	0.52	0.47	0.41
Phosphorus (Diges.)	0.45	0.37	0.33	0.29
Sodium	0.16	0.15	0.15	0.15
Chloride	0.16	0.15	0.15	0.15
Lysine	0.85	0.76	0.74	0.70
Lysine (Diges.)	0.70	0.62	0.61	0.57
Methionine	0.36	0.38	0.35	0.33
Methionine (Diges.)	0.29	0.31	0.29	0.27
Meth./Cysteine	0.68	0.70	0.64	0.61
Meth./Cysteine (Diges.)	0.56	0.57	0.53	0.50
Tryptophan	0.20	0.19	0.17	0.17
Tryptophan (Diges.)	-	0.15	0.14	0.14
Threonine	0.60	0.56	0.52	0.52
Threonine (Diges.)	-	0.45	0.42	0.42
Linoleic Acid	1.00	1.74	1.39	1.13

calculated by dividing the number of heterophils by the number of lymphocytes.

2.3. Immunity level determination

The immunity levels of the animals were determined by measuring the level of antibodies produced against sheep red blood cells (SRBC). Sheep erythrocytes were obtained from sheep blood, which was collected into anticoagulated tubes at weeks 35 and 65. After being transported to the laboratory at 4 °C, the blood was centrifuged at 1000 rpm for 10 min, and the resulting upper plasma layer was discarded. The lower layer of erythrocytes was added 0.9% physiological saline (1st wash), and the resulting erythrocyte suspension was centrifuged for the second time. Following the discard of the upper layer, the lower erythrocyte layer was once again added 0.9% physiological saline (2nd wash) and centrifuged. This process was repeated twice more. Washed sheep erythrocytes were diluted with 0.9% physiological saline at a rate of 0.25%.

One randomly selected hen per hybrid subgroup and cage density subgroup (in total 54 hens, including one hen from each cage) was injected intraperitoneally with 0.5 mL of a 0.25% sheep erythrocyte suspension diluted with 0.9% physiological saline. Antibody titres were measured by performing the micro-haemagglutination test on sera extracted from the blood samples taken from the animals a week after the SRBC challenge.

2.4. Statistical analysis

The statistical and descriptive analyses of the study data were performed using the IBM SPSS v. 20 software package.

The General Linear Model (GLM) detailed below in statistical notation was used for BW data recorded at 17 weeks of age and once weekly at 4 week-intervals between 23 and 71 weeks of age, for EP and FC data, recorded once in a week at 4 week-intervals between 20 and 72 weeks of age and, for EW and FCR data, recorded once in a week at 4 week-intervals between 24 and 72 weeks of age.

$Y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$

Y_{ijk} = The value of any of the performance parameters,

μ = Population average,

a_i = Effect of the hybrid (IB, A-S, NW),

b_j = Effect of the cage density (468.75 cm^2/hen and 312.50 cm^2/hen),

ab_{ij} = Interaction between hybrid (i) and stocking density (j),

e_{ijk} = Experimental error with an average of 0 and variance of σ^2_e ($N\sim(0, \sigma^2_e)$).

Among all nonparametric tests, the chi-square (X^2) test was applied to the liveability data collected throughout the study period.

The variance analysis of repeated measurements was performed on data pertaining to the blood cell counts (H, L, M, B, E) and H/L ratio, which were used to determine the stress level, and on the SRBC antibody titres, which were used to determine the immunity level of the hens. The model used for this purpose is presented below in statistical notation:

$Y_{ijkl} = \mu + a_i + b_j + c_k + ab_{ij} + ac_{ik} + bc_{jk} + abc_{ijk} + e_{ijkl}$

Y_{ijkl} = The value of any of the parameters,

a_i = Effect of the hybrid (IB, A-S, NW),

b_j = Effect of the cage density (468.75 cm^2/hen and 312.50 cm^2/hen),

c_k = Effect of age (35 weeks, 65 weeks),

ab_{ij} = Interaction between hybrid (i) and cage density (j),

ac_{ik} = Interaction between hybrid (i) and age (k),

bc_{jk} = Interaction between cage density (j) and age (k),

abc_{ijk} = Interaction between hybrid (i), cage density (j) and age (k),

e_{ijkl} = Experimental error with an average of 0 and variance of σ^2_e ($N\sim(0, \sigma^2_e)$).

3. Results

3.1. Performance traits

While significant differences were observed in all performance parameters for genotype ($p < 0.05$), all performance parameters excluding egg weight showed statistically significant differences for cage density ($p < 0.05$) (Table 4).

The highest egg performance was determined for NW, followed by IB and A-S hybrid, respectively ($p < 0.001$). NCD egg production was 79.88%, and HCD egg productivity was 67.01% ($p < 0.001$). In the present study, the hybrid-by-cage density interaction was found to be significant ($p < 0.05$), and of the hybrids housed at NCD, IB displayed the highest egg production level, whilst of the hens housed at HCD, the hybrid NW laid the highest number of eggs. In both cage density subgroups, A-S was the hybrid with the lowest egg yield. When housed at HCD, the egg yield of the hybrids IB, A-S, and NW decreased by 19.50%, 19.23%, and 9.69%, respectively (Table 4).

The highest egg weight was determined for IB and was followed by A-S and NW ($p < 0.001$). The egg weights of the hens housed at NCD and HCD were determined as 62.43 g and 62.79 g, respectively ($p > 0.05$). The average egg weight of all study groups ranged from 61.51g to 64.35 g, and the correlation between diet and egg weight was found to be statistically insignificant ($p > 0.05$) (Table 4).

Daily feed consumption and FCR values was highest in the hybrid A-S, followed by IB and NW ($p < 0.001$). It was observed that increased cage density increased FCR values and decreased feed consumption ($p < 0.001$). The separate assessment of each hybrid housed at different cage densities revealed that the A-S hybrid displayed the highest FCRs for both stocking densities, whilst of the animals fed on a NCD, the lowest FCR value was displayed by the IB hybrid, and of the animals fed on a HCD, the lowest FCR was displayed by the NW hybrid ($p < 0.001$). In terms of the hybrid × cage density correlation, the lowest FC value was detected in the NW hybrids housed at HCD, whilst the highest FC value was detected in the A-S hybrids housed at NCD. For both cage densities, the highest amount of feed consumption was detected in the A-S hybrid, whilst the lowest feed consumption was detected in the NW hybrid (Table 4).

The study demonstrated that body weight values were significantly affected by hybrid and cage density ($p < 0.001$). Throughout the laying period, the average body weight was highest in the A-S hybrid (1790.57 g), followed by the IB hybrid (1767.75 g) and the NW hybrid (1499.03 g). The average body weights calculated for the animals housed at NCD and HCD during the laying period were calculated as 1728.54 g and 1643.03 g, respectively (Table 5).

Mortality rates are 22.2% for NW, 11.6% for IB, and 7.7% for A-S ($p < 0.001$) (Table 6). Mortality was observed

Table 4. The effect of hybrid and cage density on EP, EW, FC, and FCR.

		EP			**EW**			**FC**			**FCR**		
		Mean	SE	p value	Mean	SE	p value	Mean	SE	p value	Mean	SE	p value
Hybrid	IB	75.13[a]	1.123	<0.0001	63.62[a]	0.301	<0.0001	118.70[b]	0.652	<0.0001	2.61[b]	0.055	<0.0001
	A-S	68.46[b]			62.46[b]			122.29[a]			3.02[a]		
	NW	76.74[a]			61.75[b]			116.80[c]			2.43[c]		
Cage Density	NCD	79.88	0.917	<0.0001	62.43	0.246	0.298	121.08	0.533	<0.0001	2.38	0.045	<0.0001
	HCD	67.01			62.79			117.45			2.99		
Hybrid x Cage Density	IB NCD	83.25	1.588	0.026	62.90	0.425	0.077	119.60	0.923	0.095	2.23	0.078	<0.0001
	IB HCD	67.01			64.35			117.81			2.99		
	A-S NCD	75.75			62.39			123.93			2.59		
	A-S HCD	61.18			62.52			120.65			3.45		
	NW NCD	80.65			62.00			119.72			2.33		
	NW HCD	72.83			61.51			113.88			2.53		

[a-c]: Different letters within one column are significantly different ($p < 0.001$).
EP: Egg production, EW: Egg weight, FC: Feed consumption, FCR: Feed conversation rate, IB:Isa Brown, A-S: Atak-S, NW: Novogen White, NCD: Normal cage density, HCD: High cage density.

to be higher in HCD housed animals (16.6%) and lower in NCD housed animals (9.7%) ($p < 0.05$) (Table 6).

3.2. Stress level determination

Heterophil, lymphocyte, and monocyte counts were determined to be different ($p < 0.001$). Cage density was found to have a significant effect on heterophil and lymphocyte counts ($p < 0.01$). Age was ascertained to have very significant effects on lymphocyte, eosinophil, and monocyte counts ($p < 0.01$) and a significant effect on basophil counts ($p < 0.05$).The H/L ratio was lowest in the NW hybrid and highest in the A-S hybrid ($p < 0.001$). The increase in cage density increased the H / L ratio ($p < 0.001$) (Table 7).

3.3. Immunity level determination

The SRBC antibody titres, p values and variance analysis results of each group are shown in Table 8. The mean SRBC antibody level was lowest in the A-S hybrid and highest in the NW hybrid ($p < 0.001$). The SRBC antibody level was determined not to be affected by cage density ($p > 0.05$). It was determined that the SRBC antibody level increased with advanced age ($p < 0.001$). The hybrid-by-cage density interaction was determined to have a significant effect on the SRBC antibody levels ($p < 0.05$), and antibody levels were determined to have increased in the A-S and NW hybrids and to have decreased in the IB hybrid, when housed at HCD.

4. Discussion

The present study was an investigation of the egg production, stress, and immunity levels of a native Turkish hybrid (A-S) and two imported hybrids (IB, NW) of laying hens housed at different cage densities. When compared to the imported hybrids, the A-S hybrid showed lower performance in terms of EP, FC and FCR but a better performance in terms of BW and L (Table 4, Table 5). Similar to the results of the present study, Türker et al. [15] reported a better performance for BW and a lower performance for FC and FCR in the native A-S hybrid. In their study on the comparison of two native hybrids (Atak and Atak-S) with two imported hybrids (Nick Brown and Lohmann Brown), Fathel and Elibol [14] reported similar findings as in the present study where the A-S hybrid reported to have lower EP and FCR than other breeds. Previous studies on production traits have suggested that genotype has significant effects on EP [17], EW [18], FC [19], FCR [17], BW [20], and L [21]. The fact that it descents from white Leghorn with ax-crested, which are in the class of light breeds based on body size, may explain why the live weight of the NW [10] hybrid is lower than other hybrids, and why it consumes less feed. In the present study, liveability was highest in the A-S hybrid and lowest in the NW hybrid. The NW hybrid having a body size and cloaca smaller than the other hybrids increased the number of cloacal prolapse cases encountered in NW hens. The more active, nervous and aggressive nature of the white hybrids was observed to have led to a higher rate of cage-mate inflicted wounding in these animals. Moreover, it is considered that the white plumage of the NW hybrid increased the visibility of the haemorrhages caused by cloacal prolapse or any other

Table 5. Two-way analysis of variance results for body weights measured at different ages.

HYBRID	CAGE DENSİTY		AGE (WEEKS)		
		17	23	71	Mean
IB	NCD	1313.61 ± 6.26	1691.53 ± 14.72	1910.14 ± 23.21	1803.38 ± 14.85
	HCD	1316.53 ± 6.26	1706.02 ± 14.72	1757.45 ± 23.21	1732.12 ± 14.85
	IB-Mean	1315.07 ± 4.43[b]	1698.77 ± 10.41[a]	1833.80 ± 16.41[b]	1767.75 ± 10.50[a]
A-S	NCD	1347.15 ± 6.26	1701.81 ± 14.72	1956.25 ± 23.21	1848.24 ± 14.85
	HCD	1360.37 ± 6.26	1678.52 ± 14.72	1810.56 ± 23.21	1732.90 ± 14.85
	A-S-Mean	1353.76 ± 4.43[a]	1690.16 ± 10.41[a]	1883.40 ± 16.41[a]	1790.57 ± 10.50[a]
NW	NCD	1100.14 ± 6.26	1409.93 ± 14.72	1640.28 ± 23.21	1533.98 ± 14.85
	HCD	1085.69 ± 6.26	1394.07 ± 14.72	1501.30 ± 23.21	1464.08 ± 14.85
	NW-Mean	1092.92 ± 4.43[c]	1402.00 ± 10.41[b]	1570.79 ± 16.41[c]	1499.03 ± 10.50[b]
Total	NCD	1253.63 ± 3.61	1601.09 ± 8.50	1835.56 ± 13.40	1728.54 ± 8.57
	HCD	1254.20 ± 3.61	1592.87 ± 8.50	1689.77 ± 13.40	1643.03 ± 8.57
	Total	1253.92 ± 2.56	1596.98 ± 6.01	1762.66 ± 9.48	1685.78 ± 6.06
P value	Hybrid	<0.0001	<0.0001	<0.0001	<0.0001
	Cage Density	0.913	0.497	<0.0001	<0.0001
	Hybrid x Cage Density	0.093	0.404	0.957	0.230

[a-c]: Different letters within one column are significantly different ($p < 0.001$).
IB:Isa Brown, A-S: Atak-S, NW: Novogen White, NCD: Normal cage density, HCD: High cage density

Table 6. The effect of hybrid and cage density on the mortality rate.

Group	Number of dead animals	Total number of animals	p value
Isa Brown	21 (%11.6)	180	<0.0001
Atak-S	14 (%7.7)	180	
Novogen White	40 (%22.2)	180	
NCD	21 (%9.7)	216	0.022
HCD	54 (%16.6)	324	

NCD: Normal cage density, HCD: High cage density.

reason, and, thereby, increased the sensitivity and reaction of the hens to red colour, which eventually increased the rate of mortality due to pecking. This situation may be supported by studies reporting that white-feathered birds show more emotional and physical reactivity than colored birds. [22,23]. The high reactivity of Leghorn-descended hens may be explained by a behavioral response to indirect selection and the different physiological needs of the organism [22,24].

In the present study, it was ascertained that higher cage density was associated with decreased EP, FC, and BW and increased FCR and L. Higher cage density was observed to have negatively affected all of the parameters investigated, excluding EW. In agreement with the present study, Akbari Moghaddam Kakhki et al. [25] reported that higher cage density (413 cm^2/hen vs 310 cm^2/hen) altered EP, FC and L values in both genotypes (white and brown laying hens). However, different from the results of the present study, these researchers suggested that cage density did not cause any statistically significant alteration in the BW and FCR values. Similarly, Anderson and Jenkins [21] compared two different genotypes housed at cage densities of 482 cm^2/hen and 361 cm^2/hen and reported that increased cage density significantly negatively affected EP, L and EW in

Table 7. Means and standard errors for blood cell counts and the H/L ratio at different ages (35 wks, 65 wks) and the effects of hybrid, cage density, age, and interactions on the H/L ratio and blood cell counts of laying hens (p value).

HYBRİD	CAGE DENSİTY	AGE (WEEK)	HETEROPHIL	LYMPHOCYTE	EOSINOPHIL	MONOCYTE	BASOPHIL	H/L RATIO
Isa Brown	NCD	35	34.33 ± 2.14	52.89 ± 1.98	4.78 ± 0.84	3.78 ± 1.11	4.44 ± 0.90	0.67 ± 0.08
		65	33.22 ± 2.14	47.33 ± 1.98	3.56 ± 0.84	8.00 ± 1.11	7.89 ± 0.90	0.71 ± 0.08
		Mean	33.78 ± 1.51	50.11 ± 1.40	4.17 ± 0.60	5.89 ± 0.78	6.17 ± 0.63	0.69 ± 0.06
	HCD	35	37.22 ± 2.14	43.56 ± 1.98	4.89 ± 0.84	8.22 ± 1.11	6.11 ± 0.90	0.87 ± 0.08
		65	43.11 ± 2.14	42.11 ± 1.98	2.11 ± 0.84	7.33 ± 1.11	5.33 ± 0.90	1.06 ± 0.08
		Mean	40.17 ± 1.51	42.83 ± 1.40	3.50 ± 0.60	7.78 ± 0.78	5.72 ± 0.63	0.96 ± 0.06
	IB		36.97 ± 1.07[a]	46.47 ± 0.99[b]	3.83 ± 0.42[ab]	6.83 ± 0.55[b]	5.94 ± 0.45	0.83 ± 0.04[a]
Atak-S	NCD	35	33.44 ± 2.14	47.67 ± 1.98	6.22 ± 0.84	6.56 ± 1.11	6.11 ± 0.90	0.73 ± 0.08
		65	32.44 ± 2.14	48.89 ± 1.98	3.78 ± 0.84	8.67 ± 1.11	6.22 ± 0.90	0.68 ± 0.08
		Mean	32.94 ± 1.51	48.28 ± 1.40	5.00 ± 0.60	7.61 ± 0.78	6.17 ± 0.63	0.71 ± 0.06
	HCD	35	39.44 ± 2.14	45.11 ± 1.98	5.89 ± 0.84	5.33 ± 1.11	4.22 ± 0.90	0.89 ± 0.08
		65	43.11 ± 2.14	40.22 ± 1.98	2.44 ± 0.84	8.22 ± 1.11	6.00 ± 0.90	1.12 ± 0.08
		Mean	41.28 ± 1.51	42.67 ± 1.40	4.17 ± 0.60	6.78 ± 0.78	5.11 ± 0.63	1.01 ± 0.06
	A-S		37.11 ± 1.07[a]	45.47 ± 0.99[b]	4.58 ± 0.42[a]	7.19 ± 0.55[b]	5.64 ± 0.45	0.86 ± 0.04[a]
Novogen White	NCD	35	29.22 ± 2.14	54.22 ± 1.98	3.11 ± 0.84	7.44 ± 1.11	6.00 ± 0.90	0.60 ± 0.08
		65	31.56 ± 2.14	49.33 ± 1.98	3.33 ± 0.84	9.56 ± 1.11	6.22 ± 0.90	0.65 ± 0.08
		Mean	30.39 ± 1.51	51.78 ± 1.40	3.22 ± 0.60	8.50 ± 0.78	6.11 ± 0.63	0.62 ± 0.06
	HCD	35	31.33 ± 2.14	51.78 ± 1.98	3.44 ± 0.84	7.56 ± 1.11	5.89 ± 0.90	0.60 ± 0.08
		65	31.22 ± 2.14	46.00 ± 1.98	3.00 ± 0.84	11.78 ± 1.11	8.00 ± 0.90	0.69 ± 0.08
		Mean	31.28 ± 1.51	48.89 ± 1.40	3.22 ± 0.60	9.67 ± 0.78	6.94 ± 0.63	0.65 ± 0.06
	NW		30.83 ± 1.07[b]	50.33 ± 0.99[a]	3.22 ± 0.42[b]	9.08 ± 0.55[a]	6.53 ± 0.45	0.64 ± 0.04[b]
			P value					
Hybrid			<0.0001	0.002	0.011	0.078	0.366	<0.0001
Density			<0.0001	<0.0001	0.249	0.306	0.669	<0.0001
Age			0.195	0.003	<0.0001	0.001	0.029	0.057
Hybrid x Density			0.043	0.292	0.202	0.761	0.319	0.038
Hybrid x Age			0.903	0.462	0.632	0.058	0.954	0.914
Density x Age			0.216	0.675	0.563	0.272	0.830	0.100
Hybrid x Density x Age			0.271	0.196	0.054	0.931	0.027	0.581

a.b: Different letters within one column are significantly different ($p < 0.001$).
IB:Isa Brown, A-S: Atak-S, NW: Novogen White, NCD: Normal cage density, HCD: High cage density
H: Heterophil, L: Lymphocyte, M: Monocyte, E: Eosinophil, B: Basophil, H/L: Heterophil / Lymphocyte

both genotypes. In accordance with present study, there were also reports indicating that EP [26,27], EW [27], FC [27,28] and FCR [28] were significantly negatively affected by increasing cage density. In the present study, it was ascertained that high cage density during the laying period resulted in the production of 48 less eggs and the consumption of 4 g less feed and caused 81 g of body weight loss per hen. This decrease observed in the production traits of the animals housed at high cage density was attributed to the energy derived from feed being used for the management of stress caused by the overcrowded cages, instead of being used for egg production. Furthermore, the housing of a greater number of animals per unit area reduced the length of the feed trough available (linear feeder space) per hen. Furthermore, the decrease in the production traits could also be attributed to high cage density forcing animals to compete for feeding space and decreasing the time they spend at the feeder, when

Table 8. Means and standard errors for SRBC (the level of antibodies produced against sheep red blood cells) antibody levels at different ages (35 wks, 65 wks) and the effects of hybrid, cage density, age and interactions on SRBC antibody levels.

HYBRİD	**CAGE DENSITY**	**AGE (Weeks)**	**SRBC (log_2)**
Isa Brown	NCD	35	1.78 ± 0.34
		65	3.67 ± 0.34
		Mean	2.72 ± 0.24
	HCD	35	1.56 ± 0.34
		65	2.78 ± 0.34
		Mean	2.17 ± 0.24
	Isa Brown Mean		2.44 ± 0.17[b]
Atak-S	NCD	35	1.22 ± 0.34
		65	2.67 ± 0.34
		Mean	1.94 ± 0.24
	HCD	35	2.11 ± 0.34
		65	3.56 ± 0.34
		Mean	2.83 ± 0.24
	Atak-S Mean		2.39 ± 0.17[b]
Novogen White	NCD	35	2.89 ± 0.34
		65	3.67 ± 0.34
		Mean	3.28 ± 0.24
	HCD	35	3.11 ± 0.34
		65	3.78 ± 0.34
		Mean	3.44 ± 0.24
	Novogen White Mean		3.36 ± 0.17[a]

	p value
Hybrid	<0.0001
Density	0.397
Age	<0.0001
Hybrid x Density	0.013
Hybrid x Age	0.174
Density x Age	0.509
Hybrid x Density x Age	0.758

a.b: Different letters within one column are significantly different ($p < 0.001$).
NCD: Normal cage density, HCD: High cage density.
SRBC: The level of antibodies produced against sheep red blood cells.

compared to the feeding time at normal stocking density [29]. Although the space per animal is the same in all hybrids while forming a cage density group, the fact that the live weight of white laying hybrids is noticeably lower in comparison to other hybrids increases the area where they can move in the cage [24,30]. The NW hybrid being least affected by high cage density can be attributed to its greater mobility, owing to its smaller size and lower body weight, when compared to the other hybrids, and thus, it being less exposed to stress inflicted by overcrowding. High cage density was observed to reduce feed consumption by 3.33% and egg production by 16.11%, which explains the difference in FCR. Furthermore, the increased number of animals per unit area was considered to have strengthened the population hierarchy, resulting in the access of the weak to feed and water being avoided by the strong, the

weak being chased and driven to the corners of the cage or beneath the feeder, and the mortality rate has increased due to weak animals being pecked and squashed. Furthermore, high cage density might have increased the severity of cage layer fatigue (cage paralysis) by restricting the mobility of the animals, and thereby, might have weakened their immunity.

Reports indicate that leukocyte components are reliable indicators of stress level in poultry [31] and point out to the H/L ratio as the major indicator of chronic stress [3,32]. In the present study, the H/L ratios of the IB, A-S and NW hybrids were determined to be 0.83, 0.86 and 0.64, respectively. While Clark et al. [33] reported a heterophil percentage of 26% and a lymphocyte percentage of 66% in avian blood, Gross and Siegel [16] suggested that H/L ratios of 0.2, 0.5 and 0.8 indicated the presence of mild, moderate and heavy stress. According to these literature data, it was determined that, in the present study, brown laying hens suffered from heavy stress, while white laying hens were under medium high stress. Similarly, Peixoto et al. [34] also stated that brown laying hens showed more anxious and fearful behaviors in comparison to whites. The difference observed between genotypes may have originated from intensive genetic selection and the physiological-biochemical and cellular changes taking place in the animal body [22,35]. For example, a few quantitative characteristic loci related to fear reaction in white leghorns were found related. [34]. For this reason, genetic selection towards reducing body weight may have also affected fear in white laying hens [34]. Furthermore, physiological, biochemical and cellular changes that occur in the body may differ with the adaptation capability of the animal to external influences. This could be interpreted as possible differences between genotypes for stress tolerance and stress sensitivity. In accordance with the present study, Kozak et al. [24] also reported that genotypes in laying hens may display various behavioral needs to sustain the homeostasis of the organism. Moreover, this difference observed between the genotypes could be explained by white laying hens having a smaller body size and lower body weight, which enables them to move faster and easier within the cage, and thus, exposes them to less stress [30]. Similar to the present study, in their study on two brown and two white laying hen breeds, Bozkurt et al. [31] reported that the brown breeds had higher stress levels.

In the present study, the H/L ratios determined for the animals housed at NCD and HCD were 0.67 and 0.87, respectively. According to the limits described by Gross and Siegel [16], these values indicated a medium-high stress level for the animals housed at NCD and a high stress level for the animals housed at HCD. Environmental factors, such as cages, production systems and stocking density, being stress factors for poultry [4-7,13] supports the results of the present study. It is reported that animals are less active at high densities [7]. It is considered that, in the animals housed at HCD, increased stress elevated the blood corticosterone level, which in return increased the number of heterophils and decreased the number of lymphocytes. It has been reported that the numbers of intracellular lymphocytes and IgA-secreting cells decrease in laying hens under stress [36]. This is attributed to the adherence of glucocorticoid hormones to endothelial cells and the circulatory lymphocytes at a higher level, which eventually reduces the lymphocyte count [37]. Astaneh et al. [38] reported H/L ratios of 0.52 and 0.71 for chickens housed at stocking densities of 12 and 18 hens per cage and indicated to have detected differences between the groups. Studies reporting that the H/L ratio changes at different housing densities [26,28,39] and those stating that the H/L ratio increases in animals based on the increased stress factor [40,41] were examined. As opposed to the study, in some studies [42], it was stated that density does not affect the H/L ratio.

In the present study, the H/L ratios at 35 weeks and 65 weeks of age were determined to be 0.73 and 0.82, respectively, and these values were observed to be similar. In agreement with the present study, Onbaşılar et al. [43] reported H/L ratios of 1.09, 1.10 and 1.19 at 32 wks, 48 wks and 61 wks of age, respectively, and indicated that the values determined at different ages were similar.

The hybrid-by-cage density interaction was determined to significantly affect the H/L ratio. While high cage density was observed to have significantly increased the stress level in the brown laying hens, such a drastic increase did not occur in the NW hybrid.

In the present study, it was determined that while statistically significant alterations occurred in the monocyte, eosinophil and basophil counts with age, the hybrids differed only for the monocyte count. This could be interpreted as monocytes, which take part in allergic reactions together with eosinophils, basophils and in the immune system together with lymphocytes, being altered in number with the cellular immune response, depending on the homeostatic structure and age of the animal [44].

Understanding genetics and association of the performance and immunity characteristics of hybrids provide important information for genotype selection in commercial farming [45,46]. It was reported that the capacity of poultry to form a response against antigenic events may be measured by complex antigens that do not form infections such as SRBC [6,47]. In the present study, the SRBC antibody titres of the IB, A-S and NW hybrids were determined to be 2.44, 2.39 and 3.36 $\log_2$, respectively. It was stated that a functional immune system is required for a good health, but stress factors may have potentially negative effects on the immune system [40,41,47]. The

differences between the antibody titres of the hybrids could be related to the stress levels they were exposed to. NW hybrid used in the present study having been exposed to a lower level of stress could also explain the difference observed in the immunity levels. In a previous study conducted by Ahmed and Alamer [48], antibody titres of native and commercial laying hens were measured on days 3, 7 and 10 post-SRBC challenge, and were found to be 2.32, 3.84 and 3,47 $\log_2$, respectively, in the native hens and 0.85, 3.50 and 4.41 $\log_2$, respectively, in the commercial hens. In agreement with the results of the present study, Nath et al. [49] reported different SRBC antibody titres in different genotypes.

It was reported that housing conditions are effective on behavioral and physiological development in laying chickens, and they are also associated with adequacy of immunity [50]. It is expected for the relationship between farming conditions such as housing density and immunity to reach a higher standardization level in relation to the genetic background of animals [3]. In the present study, the anti-SBRC antibody titres of the animals housed at NCD and HCD were determined to be 2.65 and 2.81 $\log_2$, respectively. Likewise, the difference between the SRBC antibody titres of chickens housed at cage densities of 646 cm^2/hen and 323 cm^2/hen were reported to be statistically insignificant [51]. The results of three other scientific research [42,43] are also in support of the present study. Contrary to the results of the present study, it was stated that, in modern farming, stocking density has negative effects on the health and welfare of the chicken [52]. Palizdar et al. [53] determined statistically significant differences in SRBC antibody titres for stocking density. Such differences between study results could be related to differences in the use of erythrocyte suspensions, the dose and administration route of the antigen, the stocking densities tested, and the size of the trial groups established [7].

In the present study, the SRBC antibody titres during the peak laying period (week 35) and the late laying period (week 65) were determined to be 2.11 and 3.36 $\log_2$, respectively. It was ascertained that the immunity level of animals was higher at advanced age ($p < 0.001$). It is considered that the antigen administered at week 65 may have served as a repeat dose and increased the antibody titre. Contrary to the results of the present study, Onbaşılar et al. [43] reported SRBC antibody titres of 5.2, 5.7 and 5.7 $\log_2$ at 32, 48, and 61 weeks of age, respectively, and indicated the differences as statistically insignificant.

5. Conclusion

In conclusion, despite displaying lower egg production, feed consumption and FCR values, the native hybrid was determined to offer the advantages of a high body weight and a high liveability rate. Based on the results of the present study, it is suggested that the IB hybrid could be raised at NCD to achieve better production results. Furthermore, for better stress tolerance and a higher immunity level, NW could be preferred in hybrid selection. The native A-S hybrid could be preferred as a brown laying hen hybrid.

To reduce foreign dependency and improve the production traits of native breeds, there is a need for increased research on the improvement and management of native layer hybrids.

Among all the hybrids used in the present study, NW was determined to be the one least affected by cage density. While high cage density (i.e. housing of an increased number of animals per unit area) was economically advantageous, and animals housed at NCD and HCD do not significantly differ for immunity level, high stocking density adversely affects the performance and stress level of animals. Thus, utmost attention should be paid to hybrid selection and management decisions, in view of the adverse effects of HCD on the welfare and egg production of brown hybrids, which have a greater body weight.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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