



Effect of Stocking Density on the Performance and Immunity of 1- to 14-d- Old Broiler Chicks

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ABSTRACT

The current experiment was conducted to evaluate the effect of stocking density (SD) on the performance and immunity of 1- to 14-d-old broilers. A total of 1836 one-day-old Cobb 500 broilers were housed at four different SD (30, 60, 90 and 120 chicks/m²). Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were estimated on d 2, 5, 8, 11 and 14. Relative lymphoid organ weights, maternal antibody titers against IBV, IBD and NDV, and stress indicators were estimated on d 3, 6, 9 and 12. The results indicated that age significantly ($p < 0.001$) affected the performance and immunity of broiler chicks. Stocking density significantly ($p < 0.001$) affected the performance and physiological stress indicators of broiler chicks, but not maternal immunity, relative lymphoid organ weights, or blood glucose levels. A significant interaction between age and density was determined for BWG, FI and FCR, and maternal antibody titers against IBD and NDV. The results also indicated that the effects of SD were age-dependent: as SD increased, worse performance, lighter lymphoid organs, and stronger stress responses were observed as broilers aged. It is concluded that the higher the SD during the first two weeks of life, the worse is the performance as broilers age.

INTRODUCTION

To the best of our knowledge, there are no documented publications on stocking density (SD) of broiler chicks grown until 14 days of age. Most of the published studies aimed at evaluating the effects of SD at the end of grow-out cycle (e.g., 20 birds/m² during the entire cycle). The typical SD applied in commercial settings is brooding chicks in one third or one half of the house, and then releasing them to the entire house area, but no accurate record is available. The first two weeks of the production cycle were targeted in this study because of the immune system of the newly-hatched chicks is immature and has limited capacity to endogenously synthesize antibodies. Consequently, the early brooding period is crucial for proper development and protection of chicks from invading pathogens (Grindstaff *et al.*, 2003; Hamal *et al.*, 2006; Panda & Reddy, 2007; Davison *et al.*, 2008).

Short grow-out cycles (30 days) are required for broiler marketing in Saudi Arabia (Al-ghamdi, 2005). In Saudi Arabia, broilers are commonly grown in conventional houses (typically, 14 m wide and 110 m long) from hatch to slaughter. The brooding period lasts 10 to 14 days post-hatch (Bruzual *et al.*, 2000), and represents 33-47% of the production cycle of broilers targeted for slaughter at 30 days of age in Saudi Arabia. Therefore, any mismanagement during this period may result in suboptimal performance (Smith & Service, 1996; Lin *et al.*, 2006; Demeke, 2007; Afolayan & Afolayan, 2008), and cannot be compensated for during the remaining rearing period.



In order to achieve their full genetic growth potential, broilers must be reared under optimal environmental conditions, and any deviation may impair their performance, cause immunosuppression, and change their physiological responses, increasing their susceptibility to diseases (Dohms & Metz, 1991; Johnson *et al.*, 1992; Lin *et al.*, 2006; Demeke, 2007; Afolayan & Afolayan, 2008).

Stress in broilers can be caused by different environmental factors (Dohms & Metz, 1991), and stocking density is considered as an important stress factor in modern broiler production. Broilers are housed at different stocking densities, depending on local regulations, production system, and target body weight, aiming at minimizing fixed costs and maximizing profitability (Puron *et al.*, 1995; Muniz *et al.*, 2006; Buijs *et al.*, 2009; Skomorucha *et al.*, 2009). However, it is well documented that high stocking densities adversely affects broiler performance, health, livability, and immunity (Puron *et al.*, 1995; Pettit-Riley & Estevez, 2001; Heckert *et al.*, 2002; Thaxton *et al.*, 2006; Estevez, 2007; Pandurang *et al.*, 2011), mostly as a result of reduced access to feed and water (Jones *et al.*, 2005; Thaxton *et al.*, 2006). In addition, air flow at bird level is reduced, hindering the dissipation of body heat (Ravindran *et al.*, 2006; Pandurang *et al.*, 2011).

Immune stress is the loss of immune homeostasis induced by external forces, such as SD (Cravener *et al.*, 1992; Huff, 2001; Hassan *et al.*, 2009; Yang *et al.*, 2011). When broilers are exposed either to natural or induced stressors, the weight of both primary and secondary lymphoid organs decreases (Heckert *et al.*, 2002), and the profile of circulating leukocytes changes: lymphocyte numbers decrease and heterophil numbers increases, resulting in high H:L ratio. High H:L ratio is a reliable indication of high glucocorticoid levels (Gross & Siegel, 1983; Patterson & Siegel, 1998).

The most commonly assessed immune parameters in poultry are the weight of lymphoid organs, where avian immune cells differentiate and which also reflects the body's ability to provide lymphoid cells during an immune response (Pope, 1991; Heckert *et al.*, 2002; Thaxton *et al.*, 2006; Yang *et al.*, 2011). Maternal antibody titers and antibody response against a foreign antigen are used to assess the immune status or immunocompetence of poultry (Gross & Siegel, 1980; Montgomery *et al.*, 1991; Scott *et al.*, 1994; Hamal *et al.*, 2006). Measurements of heterophil: lymphocyte ratios and serum glucose and cholesterol concentrations indicate the level of stress in poultry (Cravener *et al.*, 1992; Al-Murrani *et al.*, 1997; Altan *et al.*, 2000; Thaxton *et al.*, 2006; Turkyilmaz, 2008; Davis *et al.*, 2008; Sekeroglu *et al.*, 2011; Houshmand *et al.*, 2012).

The objectives of the present study, therefore, were to assess the performance and the functional development of the immune system of broilers reared at different stocking densities during the first two weeks of life.

MATERIALS AND METHODS

Birds, husbandry and treatments

In total, 1836 one-d-old Cobb 500 chicks were obtained from a local hatchery. Upon arrival at the experimental facilities, chicks were weighed and uniformly distributed, according to weight, into 12 floor pens with fresh wood-shavings litter in a conventional broiler house similar to commercial settings.

The experimental treatments consisted of four SD (30, 60, 90, or 120 birds/m²) and with three replicates each. Each pen (2.34 m²) was equipped with water line (11 nipples per line) and tube plastic feeders. Feeder spaces of 6.7, 3.3, 2.2, and 1.7 cm per bird were provided in the SD treatments of 30, 60, 90, and 120 birds/m², respectively. The numbers of birds per water nipple were 2.7, 5.5, 8.2 and 10.9 birds per nipple for the SDs of 30, 60, 90 and 120 birds/m², respectively. Birds were supplied a commercial crumbled starter feed (Table 1), and had *ad libitum* access to feed and water. Critical control points such as temperature, lighting, and ventilation were controlled according to the genetic strain guidelines (Cobb 2012) for chicks from 0 to 14 days of age, except for SD per target weight.

Measurements

The live performance parameters body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and mortality were determined on d 2, 5, 8, 11, and 14. Sixteen chicks were randomly selected for blood sampling on day of arrival, and then three chicks per pen were sampled on d 3, 6, 9, and 12. Blood samples were collected into clean, non-heparinized Vacutainer® (BD, Germany) tubes. One drop of fresh blood was smeared on a clean microscope glass slide. The dried smear slides were stained with Giemsa for 2 min. Heterophils and lymphocytes were enumerated in 100 cells per field, and their ratio was calculated.

The serum was then centrifuged (5,000 x g) for 10 min at 4°C and stored at -20°C until analyses. Glucose and cholesterol levels were determined using a spectrophotometer (Randox Laboratories, UK). Maternal antibody titers against Newcastle disease (ND), infectious bronchitis (IB), and infectious bursal disease (IBD) were determined using commercial ELISA kits (Idexx Laboratories, Inc., USA). After blood



Table 1 – Dietary ingredients and chemical composition of starter diets

Ingredients, g/kg	
Corn	542.6
Soybean meal	361.0
Palm oil	54.0
Dicalcium phosphate	23.0
Ground limestone	7.20
DL-methionine	2.30
Salt	3.00
Vitamin premix ¹	2.50
Trace mineral mix ²	0.50
Choline chloride 60	1.00
Sodium bicarbonate	2.90
Calculated analysis	
ME, kcal/kg	3100
Crude protein, %	22.0
Lysine, %	1.20
Methionine, %	0.55
Threonine, %	0.84
TSAA, %	0.90
Calcium, %	1.00
Non-phytate phosphorus %	0.45

¹Vitamin-mix is supplied in the following per kg of diet: Retinyl acetate, 3.41 mg; cholecalciferol, 0.07 mg; DL- α -tocopheryl acetate, 27.5 mg; menadione sodium bisulphate, 6 mg; riboflavin, 7.7 mg; niacin, 44 mg; pantothenic acid, cyanocobalamin, 0.02; choline 496 mg; folic acid, 1.32 mg; pyridoxine HCl, 4.82 mg; thiamine mononitrate, 2.16 mg; D-biotin, 0.11 mg. ²Mineral-mix is supplied in the following per kg of diet: manganese, 67 mg; zinc, 54 mg; copper, 2 mg; iodine, 0.5 mg; iron, 75 mg; and selenium, 0.2 mg. ME, metabolizable energy; TSAA, total sulfur amino acids.

Table 2 – Effect of age and stocking density on the performance of broiler chicks reared at four stocking densities from 0-14 days of age

Treatments	Performance			
	Body weight gain (g/d)	Feed intake (g/d)	Feed conversion ratio (g: g)	Mortality (%)
Stocking density (SD)				
30 ^a	32 ^a	38 ^a	1.14 ^b	0.51
60	30 ^{ab}	36 ^{ab}	1.14 ^b	0.22
90	28 ^b	34 ^b	1.17 ^{ab}	0.39
120	24 ^c	30 ^c	1.23 ^a	0.66
SEM	0.789	1.019	0.021	0.180
LSD	2.255	2.912	0.059	0.510
Age (A) (days)				
2	14 ^d	11 ^e	0.78 ^e	0.44
5	22 ^c	22 ^d	0.99 ^d	0.23
8	33 ^b	39 ^c	1.20 ^c	0.22
11	36 ^a	48 ^b	1.34 ^b	0.64
14	36 ^a	55 ^a	1.56 ^a	0.71
SEM	0.882	1.139	0.023	0.200
LSD	2.521	3.256	0.066	0.570
Probability	P > F			
SD	**	**	*	ns
A	**	**	**	ns
A*SD	**	**	**	ns

Stocking density: number of birds per m²; Mean of three replicates per treatment; SEM, standard error of the mean; LSD, least significant difference; ^{ab}Means in the same column bearing different superscripts are significantly different (*p<0.05; **p<0.01; ns, not significant).

collection, chicks were killed by cervical dislocation, and the bursa, spleen and thymus were removed and weighed. Their absolute and relative weights (calculated as a percentage of body weight) were subjected to statistical analysis.

Statistical analysis

Data were statistically analyzed by two-way analysis of variance using the General Liner Models procedure of SAS statistical package (SAS Institute, 2004). The following model was used:

$$Y_{ij} = \mu + A_i + S_j + AS_{ij} + e_{ijt}$$

Where Y_{ijk} is the individual observation; μ is the experimental mean; A_i is the effect of the i th age; S_j is the effect of the j th stocking density; AS_{ij} is the effect of age by stocking density interaction; e_{ijt} is the random error. Means were compared by Duncan's multiple range test at 5% probability level. Percentage data were subjected to arcsine transformation prior analysis; however, actual percentages are reported.

RESULTS

Performance, mortality, lymphatic organ, and antibody titer results of broiler chicks reared at four SD from d 1-14 are shown in Tables 2 and 3. Stocking density significantly (p<0.01) affected the



Table 3 – Effect of age and stocking density on maternal antibody titers (\log_{10}) against infectious bronchitis (IB), Newcastle disease (ND) and infectious bursal disease (IBD), and on stress indicators of broiler chicks reared at four stocking densities from 0-14 days of age

Treatments	Relative liver and lymphoid organ weights (%)				Maternal antibody titers (\log_{10})				Stress indicators			
	Liver weight: BW	Bursa weight: BW	Spleen weight: BW	Thymus weight: BW	IB	IBD	NDV	Heterophil (%)	Lymphocyte (%)	Hi: L Ratio	Glucose (mg/dl)	Cholesterol (mg/dl)
Stocking density (SD)												
30 ^o	4.35 ^a	0.14	0.09	0.44	3.03	3.61	3.50	28.93 ^b	58.89 ^a	0.50 ^d	208	131 ^b
60	4.19 ^{ab}	0.14	0.08	0.45	3.00	3.60	3.48	31.27 ^b	56.69 ^b	0.56 ^c	209	134 ^{ab}
90	4.18 ^{ab}	0.14	0.08	0.42	3.01	3.61	3.50	32.73 ^a	56.06 ^b	0.60 ^b	212	139 ^a
120	4.06 ^b	0.14	0.08	0.41	2.94	3.57	3.49	34.02 ^a	54.15 ^c	0.64 ^a	212	141 ^a
SEM	0.069	0.005	0.004	0.014	0.035	0.024	0.027	0.555	0.489	0.015	4.666	2.557
LSD	0.188	0.013	0.011	0.038	0.093	0.066	0.075	1.539	1.354	0.003	12.016	6.470
Age (A) (days)												
0	2.59 ^{ve}	0.14 ^{ab}	0.06 ^c	0.43 ^{bc}	3.46 ^{ab}	3.88 ^a	3.83 ^{ab}				232 ^a	248 ^a
3	4.80 ^a	0.13 ^b	0.08 ^{ab}	0.57 ^a	3.57 ^a	3.89 ^a	3.92 ^a	23.99 ^d	61.87 ^a	0.39 ^d	218 ^a	150 ^b
6	4.56 ^b	0.15 ^a	0.08 ^b	0.48 ^b	3.20 ^b	3.69 ^b	3.63 ^b	29.85 ^c	55.58 ^b	0.54 ^c	221 ^a	139 ^c
9	4.04 ^c	0.145 ^{ab}	0.09 ^a	0.37 ^c	2.73 ^c	3.49 ^c	3.32 ^c	34.45 ^b	55.32 ^b	0.63 ^b	191 ^b	131 ^d
12	3.39 ^d	0.14 ^{ab}	0.08 ^{ab}	0.29 ^d	2.47 ^d	3.32 ^d	3.10 ^d	38.66 ^a	53.03 ^c	0.74 ^a	210 ^a	126 ^d
SEM	0.075	0.007	0.005	0.014	0.040	0.028	0.030	0.555	0.488	0.021	6.021	3.385
LSD	0.216	0.019	0.014	0.064	0.115	0.079	0.086	1.544	1.362	0.041	13.349	7.306
Probability												
SD	*	ns	ns	ns	ns	ns	ns	**	**	**	ns	**
A	**	**	*	**	**	**	**	**	**	**	**	*
A*SD	ns	ns	ns	ns	ns	*	*	ns	ns	ns	ns	ns

Stocking density: number of birds per m²; SEM, standard error of the mean; LSD, least significant difference; ^{ab}Means in the same column bearing different superscripts are significantly different (*p<0.05; **p<0.01; ns, not significant).



performance, serum cholesterol concentration, blood heterophil and lymphocyte counts and H:L ratio, and relative liver weight ($p < 0.05$) of broiler chicks during the experiment.

Birds stocked at lower densities gained more weight ($30 = 60 > 90 > 120$, birds/m², respectively), consumed less feed ($30 > 90 > 120$ birds/m², respectively), and converted feed more efficiently ($30 = 60 > 90 = 120$ birds/m², respectively). Chicks reared at higher densities presented significantly ($p < 0.05$) lower relative liver weight ($120 < 30 = 60 = 90$ chicks/m²) and higher ($p < 0.01$) serum cholesterol levels ($120 = 90 > 60 = 30$ chicks/m², respectively). The blood heterophil percentage of chicks significantly increased ($p < 0.001$) as SD increased ($30 = 60 < 90 = 120$ chicks/m²). Opposite to heterophils, lymphocyte percentage significantly decreased ($p < 0.001$) as SD increased ($30 > 120$ chicks/m², respectively). Consequently, H:L ratios significantly increased ($p < 0.001$) as SD increased ($30 < 90 = 120$ chicks/m²). The relative weights of the bursa, spleen and thymus, maternal antibody titers against IB, IBD, and ND, and serum glucose concentration were not affected by SD. On the other hand, as expected, BWG, FI, and FCR significantly increased with age ($p < 0.01$), as well as heterophil percentage and H:L ratio. High maternal antibody titers against IBV, IBD, and NDV were observed until day 3, and decreased thereafter ($p < 0.01$). Age significantly ($p < 0.01$) affected serum glucose and cholesterol concentrations, and the relative weights of the liver, bursa, thymus, and spleen ($p < 0.05$). Mortality was not affected by age or SD. This experiment shows that, as expected, age of birds significantly ($p < 0.001$) increased body weight, gain, feed intake and feed efficiency of broiler chicks.

There were significant interactions ($p < 0.01$) between age and SD for BWG, FI and FCR (Figures 1 to 3, respectively), and for maternal antibody titers against IBD and NDV ($p < 0.05$) (Figures 4 and 5, respectively). The effect of SD on live performance (BWG, FI and FCR) was significant ($p < 0.01$) in older broilers, but not at early ages (on d 2 and 5 vs. 8, 11 and 14; on d 2, 5, and 8 vs. 11 and 14; and on d 2, 5, and 8 vs. 11 and 14 of age for BWG, FI and FCR, shown in Figures 1, 2 and 3, respectively). Broilers presented better performance with age when reared under lower SD. Stocking density significantly ($p < 0.05$) influenced maternal antibody titers against IBD and NDV on d 3 and 9, but not on d 0, 6 and 12 d (Figures 4 and 5, respectively). On d 3 and 9, higher maternal antibody titers against IBD and NDV were obtained at lower SDs compared with higher SD ($30 > 120$ and $90 > 120$ chicks/m²).

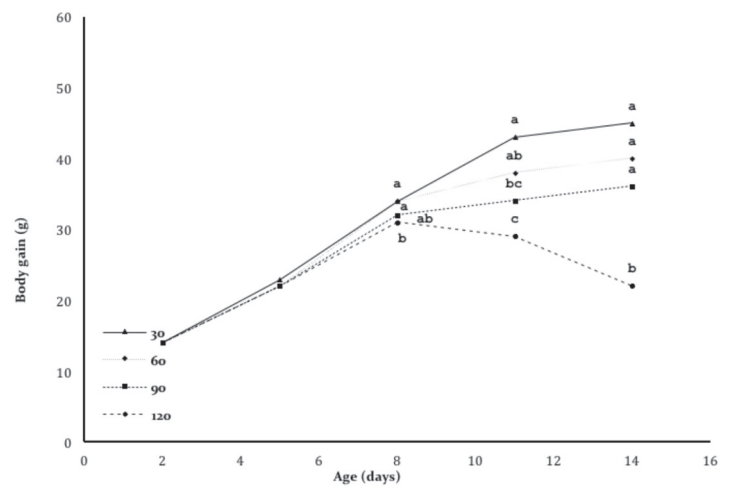


Figure 1 – The effect of age and stocking density on the body weight gain of broiler chickens (SEM = 0.45)

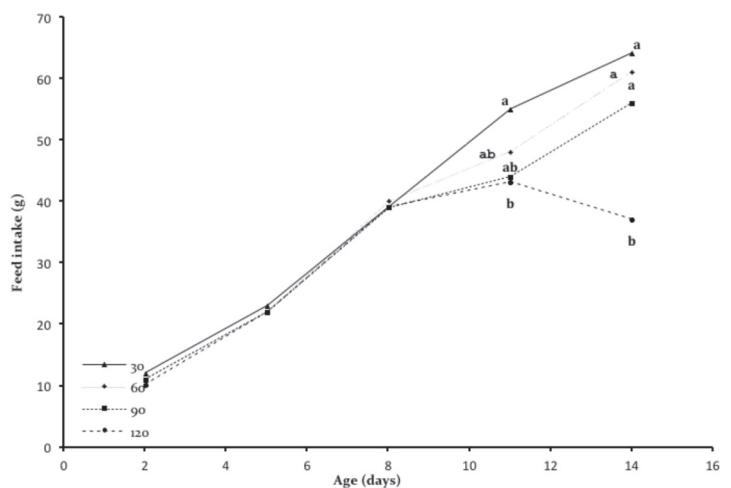


Figure 2 – The effect of age and stocking density on the feed intake of broiler chicks (SEM = 0.552)

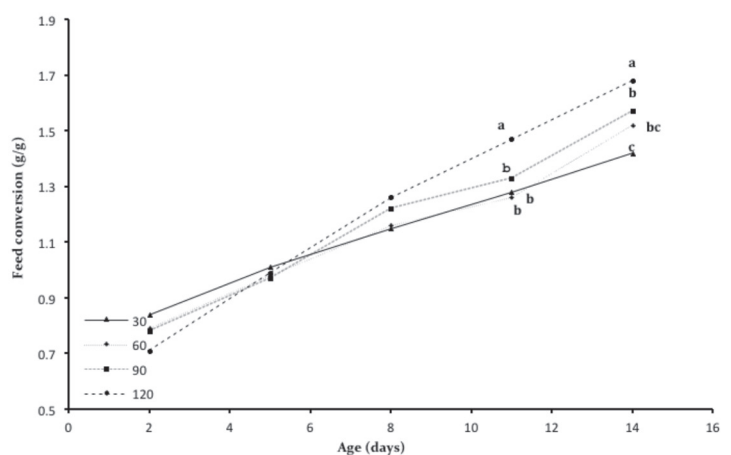


Figure 3 – The effect of age and stocking density on the feed conversion ratio of broiler chicks (SEM = 0.015)

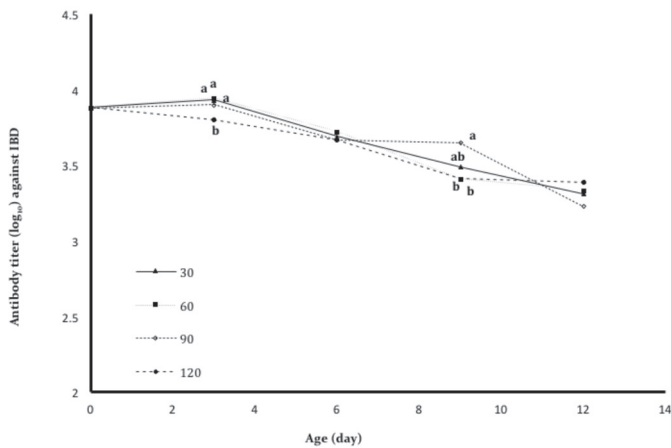


Figure 4 – The effect of age and stocking density on maternal antibody titers (\log_{10}) of broiler chicks against infectious bursal disease virus (SEM = 0.024)

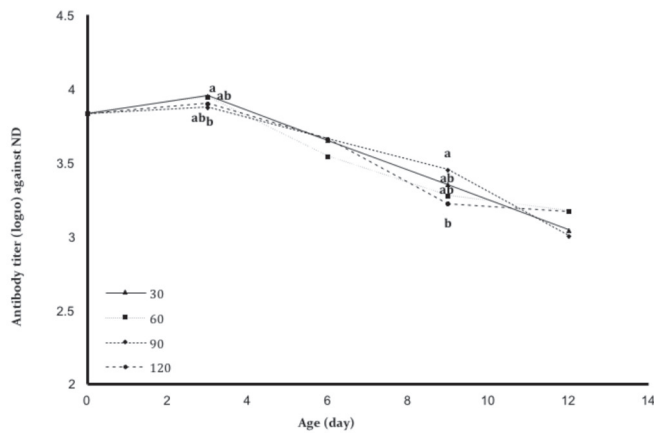


Figure 5 – The effect of age and stocking density on maternal antibody titers (\log_{10}) of broiler chicks against Newcastle disease virus (SEM = 0.026)

DISCUSSION

Most studies published in scientific literature evaluated broiler stocking density at the end of the grow-out cycle. Therefore, there are no performance or immunity data available to compare the current results obtained in younger broilers. However, the results clearly showed that increasing SD significantly reduced BWG and FI, resulting in worse FCR, but this effect was age-dependent. This finding is in agreement with the SD effects reported by other authors for market-age broilers (Elwinger, 1995; Puron *et al.*, 1995; Edriss *et al.*, 2003; Dozier *et al.*, 2005; Abudabos *et al.*, 2013a). However, other studies did not find any influence of reducing SD on broiler performance (Thomas *et al.*, 2004; Zhang *et al.*, 2011), nor any negative impacts (Feddes *et al.*, 2002).

In the present study, birds reared at SDs of 30 and 60 birds/m² performed better than those stocked at

90 and 120 birds/m². When reared at SDs of 30 and 60 birds/m², birds consumed more feed than those stocked at 90 and 120 birds/m². Increasing the number of water line and/or feeder space may have improved the performance of the birds stocked at high SDs, but further studies are needed to test this assumption. On d 2, 5, 8, and 11, the body weights measured at all SD levels used in this trial were higher than the targets of the genetic company manual (Cobb 500). On d 14, the body weights of chicks maintained at SDs of 30 and 60 birds/m² were also higher than the targets.

The results clearly showed that increasing SD significantly reduced the weights of the lymphoid organs in an age-dependent manner. This indicates that, under stressful conditions, such as high SD, the higher the SD as the birds grow, the more stress is suffered by the birds, resulting in less developed lymphoid organs. This result is in agreement with the SD effects at market age reported by Ravindran *et al.* (2006), whereas other authors did not find any influence of SD reduction influence on the development of lymphoid organs (Heckert *et al.*, 2002; Thaxton *et al.*, 2006; Buijs *et al.*, 2009; Houshmand *et al.*, 2012). In agreement with Houshmand *et al.* (2012), relative bursa and spleen were not affected by SD. However, Ravindran *et al.* (2006) reported that relative bursa and spleen decrease as housing density increases. High SD can be stressful and compromise broiler immunity (Heckert *et al.*, 2002; Thaxton *et al.*, 2006; Estevez, 2007; Abudabos *et al.*, 2013b).

Some studies reported that physiological indicators of stress were not affected by stocking density (Dozier *et al.*, 2006; Thaxton *et al.*, 2006; Buijs *et al.*, 2009). Our results on H:L ratio agree with most of those reported in the literature (Martrenchar *et al.*, 1997; Feddes *et al.*, 2002), particularly in the study of Beloor *et al.* (2010), who found broiler that H:L ratio is independent of SD at 15, 20 and 25 birds/m². In agreement with Skrbic *et al.* (2009), blood glucose was not affected by SD. Contrary to our findings, Virden & Kidd (2009) reported that blood glucose increased due to stress caused by high stocking density. On the other hand, as the stress load increases, the demand for blood glucose increases, adrenalin is secreted, and glycogen body reserves are mobilized, resulting in low blood glucose levels (Donaldson, 1995; Summers, 2006). Additionally, in agreement with Dozier *et al.* (2006), serum cholesterol concentration significantly increased in the broilers reared at higher SDs in the present study. On the other hand, Skrbic *et al.* (2009) did not find any effect of stocking density stress on



blood cholesterol concentration. Thaxton *et al.* (2006) found that blood glucose and cholesterol levels and H:L ratio of broilers reared at different densities ranged between 200-250 mg/dL, 100-200 mg/dL and 0.8-1.6, respectively.

Age affected broiler performance, as well as H:L ratios and maternal antibody titers against IBV, IBD, and NDV. These results are in agreement with other studies (Sekeroglu *et al.*, 2011; Zuowei *et al.*, 2011). Also, as expected, the weight of the lymphoid organs increased with age, in agreement with Khalil *et al.* (2009). The significant effect of age on maternal antibody titers is consistent with the findings of Kowalczyk *et al.* (1985), who reported that maternal antibody titers increase in the first 2 to 4 days of life, when the yolk is absorbed, and then decline and are catabolized by the offspring over the first 14 days post-hatch (Ahmed & Akhter, 2003; Grindstaff *et al.*, 2003). Talebi *et al.* (2005) had previously shown that age affected the absolute counts of heterophils and lymphocytes, as well as H:L ratios of broilers.

The decline in serum glucose and cholesterol levels as broilers aged observed in the present study was reported in other studies. Raji (2000) showed the blood glucose levels of Japanese quails was affected by age. Other authors verified high cholesterol and low glucose levels in the sera of newly-hatched chicks (Latour *et al.*, 1996; Vieira & Moran, 1999; Peebles *et al.*, 2004), which may be explained by the transition from yolk-derived lipid to dietary-based carbohydrate as primary source of energy in broilers chicks during first 2-3 days after hatch. Vieira & Moran (1999) demonstrated that the yolk sac contents are rich in fats and proteins, but are very poor in carbohydrates.

The interaction between age and stocking density showed that the performance of broilers reared at high SD was impaired as they aged. The BWG results obtained on d 8, 11 and 14, and FI and FCR on d 11 and 14 were significantly worse as both SD and age increased. This result is in agreement with those of Zuowei *et al.* (2011), who observed adverse effects of high SD on live performance as broilers aged. The interaction between broiler age and SD significantly affected anti-IBD and NDV maternal antibody titers.

Mortality is considered an end-point in animal welfare and therefore, the ultimate indicator of stress (Buijs *et al.*, 2009). Although mortality was not influenced by SD or age in the present study, Hall (2001) reported that mortality significantly increased in broilers reared at increasing stocking densities.

CONCLUSIONS

In summary, the results of the present study indicate that age significantly influences broiler performance and immunity. As expected, performance significantly improved with age. The evaluated stocking densities had significant effects on broiler performance and physiological indicators of stress (except for serum glucose). However, stocking density did not influence relative lymphoid organ weights or maternal antibody titers. As expected, performance worsened as stocking density increased. However, further research is needed to model the effect of stocking density on performance. We suggest evaluating shorter stocking density intervals than those applied in the present study to obtain more precise results. Broiler body weight, body weight gain, feed intake, feed conversion ratio, and anti-IBD and anti-NDV maternal antibody titers were significantly influenced by the interaction between age and stocking density. In conclusion, the results indicate that, under the conditions of this experiment, stocking density had an adverse effect on the performance and immune status of broilers from 1 to 14 days of age, although the recorded performance was better compared with that of the genetic company manual (Cobb 500). However, the intervals between stocking densities applied in the present study need to be shortened in order to produce better models.

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REFERENCES

- Abudabos AM, Samara EM, Hussein EO, Al-Ghadi MAQ, Al-Atiyat RM. Impacts of stocking density on the performance and welfare of broiler chickens. *Italian Journal of Animal Science* 2013a;12:66-71.
- Abudabos, AM, Samara EM, Hussein EO, Al-Ghadi MAQ, Al-Atiyat RM, Al-Haidary A. Influence of stocking density on welfare indices of broilers. *Italian Journal of Animal Science* 2013b;2:213-218.
- Afolayan M, Afolayan MO. An innovative use of boxboard in the northern guinea savanna zone of Nigeria. *Journal of Applied Sciences Research* 2008;4:1588-1595.
- Ahmed Z, Akhter S. Role of maternal antibodies in protection against infectious bursal disease in commercial broilers. *International Journal of Poultry Science* 2003;2:251-255.
- Al-ghamdi ZHH. Effect of some environmental stress on broiler production performance in eastern region of the kingdom of Saudi Arabia [thesis]. Damman: Girl College of Science; 2005.



- Al-Murrani W, Kassab A, Al-Sam H, Al-Athari A. Heterophil/lymphocyte ratio as a selection criterion for heat resistance in domestic fowls. *British Poultry Science* 1997;38:159-163.
- Altan O, Altan A, Cabuk M, Bayraktar H. Effects of heat stress on some blood parameters in broilers. *Turkish Journal of Veterinary and Animal Sciences* 2000;24:145-148.
- Beloor J, Kang H, Kim Y, Subramani V, Jang I, Sohn S, et al. The effect of stocking density on stress related genes and telomeric length in broiler chickens. *Asian-Australasian Journal Animal Science* 2010;23:437-443.
- Bruzual J, Peak S, Brake J, Peebles E. Effects of relative humidity during the last five days of incubation and brooding temperature on performance of broiler chicks from young broiler breeders. *Poultry Science* 2000;79:1385-1391.
- Buijs S, Keeling L, Rettenbacher S, Van Poucke E, Tuytens F. Stocking density effects on broiler welfare: Identifying sensitive ranges for different indicators. *Poultry Science* 2009;88:1536-1543.
- Cobb. Cobb 500 Broiler performance and nutrition supplement. Available from: <http://cobb.vantress.com>.2012.
- Cravener T, Roush W, Mashaly M. Broiler production under varying population densities. *Poultry Science* 1992;71:427-433.
- Davis A, Maney D, Maerz J. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology* 2008;22:760-772.
- Davison TF, Kaspers B, Schat KA. *Avian immunology*. 6th ed. London: Elsevier/AcademicPress; 2008.
- Demeke S. Sustainability of hay-box brooding technology to rural household poultry production system. *Livestock Research for Rural Development* 2007;19.
- Dohms JE, Metz A. Stressmechanisms of immunosuppression. *Veterinary Immunology and Immunopathology* 1991;30:89-109.
- Donaldson W. Early poult mortality: the role of stressors and diet. *Turkey World* 1995:27.
- Dozier W, Thaxton J, Branton S, Morgan G, Miles D, Roush W, et al. Stocking density effects on growth performance and processing yields of heavy broilers. *Poultry Science* 2005;84:1332-1338.
- Dozier W, Thaxton J, Purswell J, Olanrewaju H, Branton S, Roush W. Stocking density effects on male broilers grown to 1.8 kilograms of body weight. *Poultry Science* 2006;85:344-351.
- Edriss M, Davoodvandi S, Pourreza J. The Effect of stock density on the production of performance and carcass traits in broiler chickens. *Proceedings of the 16 th European Symposium on the Quality of Poultry Meat*; 2003; Paris. France; 2003.
- Elwinger K. Broiler production under varying population densities: a field study. *Archive fur Geflugelkd* 1995;59:209-215.
- Estevez I. Density allowances for broilers: Where to set the limits? *Poultry Science* 2007;86:1265-1272.
- Feddes J, Emmanuel E, Zuidhof M. Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities. *Poultry Science* 2002;81:774-779.
- Grindstaff JL, Brodie ED, Ketterson ED. Immune function across generations: integrating mechanism and evolutionary process in maternal antibody transmission. *Proceedings of the Royal Society of London. Series B. Biological Sciences* 2003;270:2309-2319.
- Gross W, Siegel H. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases* 1983;972-979.
- Gross W, Siegel P. Effects of early environmental stresses on chicken body weight, antibody response to RBC antigens, feed efficiency, and response to fasting. *Avian Diseases* 1980;24(3):569-579.
- Hall A. The effect of stocking density on the welfare and behaviour of broiler chickens reared commercially. *Animal Welfare* 2001;10:23-40.
- Hamal K, Burgess S, Pevzner I, Erf G. Maternal antibody transfer from dams to their egg yolks, egg whites, and chicks in meat lines of chickens. *Poultry Science* 2006;85:1364-1372.
- Hassan A, AbdelAzeem HM, Reddy P. Effect of Some Water Supplements on the performance and immune system of chronically heat-stressed broiler chicks. *International Journal of Poultry Science* 2009;8:432-436.
- Heckert R, Estevez I, Russek-Cohen E, Pettit-Riley R. Effects of density and perch availability on the immune status of broilers. *Poultry Science* 2002;81:451-457.
- Houshmand M, Azhar K, Zulkifli I, Bejo M, Kamyab A. Effects of prebiotic, protein level, and stocking density on performance, immunity, and stress indicators of broilers. *Poultry Science* 2012;91:393-401.
- Huff G. Thinking about stress and disease in turkeys. *Avian Advice* 2001;3:11-12.
- Idexx Laboratories. *Idexx IBV, IBD and NDV Test Laboratories*. Westbrook: Rechte Vorbehalten; 2012.
- Johnson EO, Kamilaris TC, Chrousos GP, Gold PW. Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. *Neuroscience & Biobehavioral Reviews* 1992;16:115-130.
- Jones T, Donnelly C, Dawkins MS. Environmental and management factors affecting the welfare of chickens on commercial farms in the United Kingdom and Denmark stocked at five densities. *Poultry Science* 2005;84:1155-1165.
- Khalil M, Sultana S, Rahman M, Mannan S, Ahmed S, Ara Z, et al. Study of prenatal and postnatal development of spleen of Gallus Domesticus (deshi chicken). *Mymensingh Medical Journal* 2009;18:169.
- Kowalczyk K, Daiss J, Halpern J, Roth T. Quantitation of maternal-fetal IgG transport in the chicken. *Immunology* 1985;54:755.
- Latour MA, Peebles ED, Boyle CR, Doyle SM, Pansky T, Brake J. Effects of breeder hen age and dietary fat on embryonic and neonatal broiler serum lipids and glucose. *Poultry Science* 1996; 75:695-701.
- Lin H, Jiao H, Buysse J, Decuyper E. Strategies for preventing heat stress in poultry. *World's Poultry Science Journal* 2006;62:71-86.
- Martrenchar A, Morisse J, Huonnic D, Cotte J. Influence of stocking density on some behavioural, physiological and productivity traits of broilers. *Veterinary Research* 1997;28:473.
- Montgomery RD, Boyle CR, Maslin WR. Influence of antigen concentration, inoculation interval, number of exposures, type of housing, and placement concentration on the tear antibody response to Brucella abortus in chickens. *Avian Diseases* 1991;606-614.
- Muniz E, Fascina V, Pires P, Carrijo A, Guimaraes E. Histomorphology of bursa of Fabricius: effects of stock densities on commercial broilers. *Revista Brasileira de Ciencia Avicola* 2006;8:217-220.
- Panda A, Reddy M. Setting chicks off to a good start-Boosting the chick's immune system through early nutrition-Research shows that chicks that feed soon after hatching are healthier and get off to a better start. *Poultry International* 2007;46:22-27.
- Pandurang L, Kulkarni G, Gangane G, More P, Ravikanth K, Maini S, et al. Overcrowding stress management in broiler chicken with herbal antistressor. *Iranian Journal of Applied Animal Science* 2011;1:49-55.



- Patterson P, Siegel H. Impact of cage density on pullet performance and blood parameters of stress. *Poultry Science* 1998;77:32-40.
- Peebles ED, Keirs RW, Bennett LW, Cummings TS, Whitmarsh SK, Gerard PD. Relationships among post-hatch physiological parameters in broiler chicks hatched from young breeder hens and subjected to delayed brooding placement. *International Journal of Poultry Science* 2004;3:578-585.
- Pettit-Riley R, Estevez I. Effects of density on perching behavior of broiler chickens. *Applied Animal Behaviour Science* 2001;71:127-140.
- Pope CR. Pathology of lymphoid organs with emphasis on immunosuppression. *Veterinary Immunology and Immunopathology* 1991;30:31-44.
- Puron D, Santamaria R, Segura JC, Alamilla JL. Broiler performance at different stocking densities. *The Journal of Applied Poultry Research* 1995;4:55-60.
- Raji K. Effect of age and sex on the blood sugar profile in Japanese (Coturnix coturnix japonica). *Biology* 2000;31:53-56.
- Randox Laboratories. Randox Reagent of Glucose (GLUC-PAP). United Kingdom. Available from: <http://www.randox.com/>.
- Ravindran V, Thomas DV, Thomas DG, Morel PC. Performance and welfare of broilers as affected by stocking density and zinc bacitracin supplementation. *Animal Science Journal* 2006;77:110-116.
- SAS Institute. SAS user's guide statistics. Version 9.1 ed. Cary; 2004.
- Scott T, Dunnington E, Siegel P. Brucella abortus antibody response of white leghorn chickens selected for high and low antibody responsiveness to sheep erythrocytes. *Poultry Science* 1994;73:346-349.
- Sekeroglu A, Sarica M, Gulay MS, Duman M. Effect of stocking density on chick performance, internal organ weights and blood parameters in broilers. *Journal of Animal and Veterinary Advances* 2011;10:246-250.
- Skomorucha I, Muchacka R, Sosnowka-Czajka E, Herbut E. Response of broiler chickens from three genetic groups to different stocking densities. *Annals of Animal Science* 2009;9(2):175-184.
- Skrbic Z, Pavlovski Z, Lukic M, Peric L, Milosevic N. The effect of stocking density on certain broiler welfare parameters. *Biotechnology in Animal Husbandry* 2009;25:11-21.
- Smith TW. Brooding chickens and quail [cooperative extension service]. Mississippi: Mississippi State University; 1996. p.1-4.
- Summers J. Early chick and poultry mortality. *Ceva Animal Health Pacific* 2006;5:1-2.
- Talebi A, Asri-Rezaei S, Rozeh-Chai R, Sahraei R. Comparative studies on haematological values of broiler strains (Ross, Cobb, Arbor-acres and Arian). *International Journal of Poultry Science* 2005;4:573-579.
- Thaxton J, Dozier W, Branton S, Morgan G, Miles D, Roush W, Lott B, Vizzier-Thaxton Y. Stocking density and physiological adaptive responses of broilers. *Poultry Science* 2006;85:819-824.
- Thomas D, Ravindran V, Thomas D, Camden B, Cottam Y, Morel P, et al. Influence of stocking density on the performance, carcass characteristics and selected welfare indicators of broiler chickens. *New Zealand Veterinary Journal* 2004;52:76-81.
- Turkyilmaz M. The effect of stocking density on stress reaction in broiler chickens during summer. *Turkish Journal of Veterinary and Animal Sciences* 2008;32:31.
- Vieira S, Moran JRE. Effects of egg of origin and chick post-hatch nutrition on broiler live performance and meat yields. *World's Poultry Science Journal* 1999;55:125-142.
- Viriden W, Kidd M. Physiological stress in broilers: Ramifications on nutrient digestibility and responses. *The Journal of Applied Poultry Research* 2009;18:338-347.
- Yang X, Li W, Feng Y, Yao J. Effects of immune stress on growth performance, immunity, and cecal microflora in chickens. *Poultry Science* 2011;90:2740-2746.
- Zhang HF, Jiao H-C, Song ZG, Lin H. Effect of alum-amended litter and stocking density on ammonia release and footpad and hock dermatitis of broilers. *Agricultural Sciences in China* 2011;10:777-785.
- Zuowei S, Yan L, Yuan L, Jiao H, Song Z, Guo Y, et al. Stocking density affects the growth performance of broilers in a sex-dependent fashion. *Poultry Science* 2011;90:1406-1415.

