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Effect of Stocking Density and Bird Age on Air Ammonia, Performance and Blood Parameters of Broilers

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ABSTRACT

Current study was conducted to recompense this deficiency through detection of ammonia (NH_3) in air samples taken from different groups at different SD and different ages. Additionally the effect of stocking density on stress indicators and broiler performance were studied. A total of 200 unsexed one day old chicks, purchased from a commercial hatchery at Kafr El-Sheikh city, Egypt, were used in this study. The birds were randomly divided into four groups according to SD. These experimental groups were divided as follows: the first group (G1) contains 10 bird/ m^2, the second group (G2) contains 14 bird/ m^2, the third group (G3) contains 17 bird/ m^2 and the fourth group (G4) contains 60 chicks was kept in an area of 3 m^2 with SD of 20 bird/ m^2. The chicken in all groups (from day 7 to day 42) was raised on deep litter system and received the same standard management, hygienic and environmental conditions. Comparing ammonia concentrations of different SD, the highest ammonia concentration was found in G4 (maximum of 31.4 mgL^{-1} at 36 day age) while the lowest were found in G1 (10 bird/m^2) (0.7, 3.1, 3.5 and 3.5 mgL^{-1} at 14, 21, 28 and 36 day age respectively). Increased SD tended to reduce the final Body Weight and Body Weight Gain significantly. On the other hand, the relative weights of spleen and bursa were increased significantly ($P < 0.05$) with increasing SD. In the current study there was a significant difference ($P < 0.05$) in Total Leukocitic Count, Heterophils %, Lymphocytes %, and H/L ratio between different SD. H/L ratio was highest in G4 (0.192%) and lowest in G1 (0.018%) which showed Increasing in H/L ratio with increase in SD that reflects increase in stress level.

Key words: Broiler, Socking density, Air ammonia, Boiler performance, Bood parameter

INTRODUCTION

Chicken has become one of the most famous meats consumed in the world (Watt Executive Guide, 2010). In Egypt the poultry industry is one of the main agricultural industries, where investment in this industry is estimated to be worth LE18 billion. The size of the labor force is about 1.5 million permanent workers and about one million temporary workers Elnagar et al (2007). The industry contributes a large part of the country's supply of animal protein (national agricultural income, Economic Affairs Sector (EAS) ministry of agricultural and land reclamation) Poultry production differs from other animal production activities in several ways, as broilers shows high growth rates and feed efficiency (Duclos et al., 2007).

Although the use of high Stocking Densities (SD) can adversely affect the individual health and performance (Sorensen et al., 2000; Feddes et al., 2002; Al Homidan et al., 2003; Dawkins et al., 2004 and Buijs et al., 2009), this has not always been a motivation for producers to lower the stocking densities because the economic benefit per square meter is often still higher if the chickens are stocked more densely (Cravener et al., 1992 and Feddes et al., 2002).

Intensive production with high stocking density usually associated with many environmental pollution problems. These problems include reduced air quality with high concentrations of organic and inorganic dust, pathogens and other micro-organisms as well as harmful gases such as ammonia, nitrous oxide, carbon dioxide, hydrogen sulphide, and methane (Ellen, 2005 and Gates et al., 2008).

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So, the poultry producers must make tremendous adjustments to meet the increasing demand for cheap and safe supply of meat and eggs. Increasing stocking density of broilers is a management practice used for reducing costs associated with labor, housing, fuel and equipment. Intensive broiler chicken production increased from year to year over the world attracting accusations of poor welfare.

Air pollutants have public health and economic importance for livestock production. When both the emission rate and the concentration level is high in livestock buildings, it causes health problems among housed animals. The public health importance of air pollutants is predicated on the diseases they may cause in livestock workers when their levels are high in the livestock pens. Most of studies in harmful gases of livestock buildings have concentrated on ammonia because of its toxicity and its role in acid rain formation (Okoli et al., 2004).

Aerial pollutant emissions in poultry farms may influence on bird performance that Threaten health of birds as a source of environmental pollutants. The sustainability of poultry production has to determine through monitoring of aerial pollutant emissions (Wathes, 1998). Effect of density on broiler mortality was not significant (Skomorucho et al., 2009).

Under the conditions used for the broilers industry in Egypt, the air released from the production buildings is polluted by microorganisms, dust, toxic gases and substances with an unpleasant odor (Baykov and Stoyanov, 1999). Most of the previous studies focused on the effects of stocking density on broiler performance and welfare parameters (Shanawany1988; Cravener et al., 1992; Martrenchar et al., 1997; Feddes et al., 2002; Adeyemo et al., 2016 and Shailesh et al., 2016). Ravinderan et al. (2006) studied the effect of adding in-feed antibiotic in relation to stocking density. None of these studies has examined the effects of stocking density in relation to age and air ammonia concentration. So, this study was carried out to make focusing on the relationship between different Stocking Densities (SD), bird ages and air hygiene. This aim could be achieved through, detection of ammonia (NH_3) in air samples taken from different groups at different SD and different ages, broiler performance of experimental broiler farm under different SD and finally monitoring of some blood parameters and hormones related to stress.

MATERIALS AND METHODS

The experiment was started on February 23, 2015 and lasted for 6 weeks at the research poultry farm unit, faculty of veterinary medicine, Kafr El-Sheikh University, Egypt.

Animal ethical approval

Animal ethics committee, faculty of veterinary medicine, Kafr El-sheikh University, Egypt, approved the protocol and conduct of the study.

Experimental birds

A total of 200 unsexed day-old (avian 48 type breed) chicks, purchased from a commercial hatchery at Kafr El-Sheikh city, Egypt, were used in this study. The birds were randomly divided into four groups according to SD. The first group (G1) contains 47 chicks were kept in an area of approximately 4.7 m^2, with SD of 10 bird/ m^2; the second group (G2) contains 42 chicks were kept in an area of 3 m^2, with SD of 14 bird/ m^2; the third group contains (G3) 51 chicks were kept in an area of 3 m^2, with SD of 17 bird/ m^2; the fourth group (G4) contains 60 chicks were kept in an area of 3 m^2 with SD of 20 bird/ m^2.

Housing and management

All chicks were brooded for seven days at the same room (deep litter system) and under the same condition at 33°C by using gas heater before they divided into four groups. The chicken in all groups (from day 7 to day 42) were raised on deep litter system and received the same standard management, hygienic and environmental conditions. The ventilation was provided with inlet and outlet ventilators and 1m ×1 m in size side windows, heating with a central thermogen, and artificial lighting with 16 regularly distributed bulbs.

Feed and water were provided *ad labitum* using manual plastic feeders and drinkers. Used ration was formulated (Table 1) to meet the nutrient requirements for broilers according to NRC (1994). Water was changed three times daily (at 8 AM, 4 PM and 12 PM).

Chickens were vaccinated against New Castle disease by oral administration in drinking water at days seven and eighteen using Hitchner and LaSota strains according to manufacturer recommendations, respectively. The light program was set as 24h continuous light during the first three days and twenty three hours light and 1h dark (23L/1D) till the end of the experiment using red bulb.

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Table 1. Composition of the diet used in the experiment from the day one to the day 42.

Ingredient	Experimental diet%
Yellow corn	55.5
Soybean meal	32.6
Sun flower oil	5.35
Fish meal	3.25
Dicalcium phosphate	1.5
Lime salt	1.0
L.lysine	0.1
Dl.methionine	0.1
Salt (NaCl)	0.3
Premix	0.25
Nutrient composition of ration	
Energy(kcal/kg)	3200.71
Crude protein (%)	21

Sampling and analysis

Air sampling and analysis: The air samples for ammonia measurement were collected by liquid impinger containing diluted sulfuric acid yielded aqueous solution of NH3. Ammonia was determined by the colorimetric method using Nessler's reagent according to Marr and Cresser (1983).

Blood sampling and analysis: From each group, five blood samples (5 ml each) were collected from the brachial vein at day 28th of the experiment and from bleeding during euthanize at the day 42. For making total and differential leucocytes count and to estimate of Heterophil / Lymphocyte (H/L), from each set of samples, 1ml whole blood was taken in Ethylenediaminetetraacetic acid (Edta) tubes ratio (Dein, 1984). Serum was prepared from the other 4 ml blood by centrifugation at 3000 rpm for 10 min. Serum samples were stored at -20°C until used for stress enzymes (Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (APK)) assessments. Stress enzymes were measured. The analyses were performed using commercial analytical kits according to the manufacturer's recommendations (Jian Cheng Bioengineering Institute, Nanjing, China).

Broiler performance

It was recorded from day 0 to 42, chickens were individually weighed weekly. Average live Body weight (BW) and Body Weight gain (BWG) were calculated. Feed Intake (FI) and Water Intake (WI) were calculated daily for each group. Feed Conversion Ratio (FCR) and WI/FI ratio were calculated weekly for all groups.

At day 42, five birds of an average BW from each group humanly euthanized via exsanguinations (from a neck cut that severed the carotid artery and jugular vein). The birds were kept for five mins for bleeding and then dipped in a hot water bath for two mins to facilitate the process of de-feathering. Manual evisceration was performed to obtain carcass, gizzard, heart, liver, spleen and bursa weight. Absolute weight and relative weight of carcass and internal organs to final BW were calculated.

Statistical analysis

Data were tested for distribution normality and homogeneity of variance. Data was reported as mean ± standard error of the mean and analyzed by ANOVA using SAS (Statistical Analysis Software), Institutes INC (2005). The significance of difference among the different treatments was evaluated by Tukey's test. The significance level was set at P<0.05.

RESULTS AND DISCUSION

Effect of different stocking density on gaseous pollutants of broiler house

Ammonia: Ammonia is the most famous pollutant in poultry houses. Elevated concentrations harmfully affect bird performance, welfare and human health (Costa et al., 2012). Figure1a and figure 1b illustrate the effect of SD on ammonia concentration in air of experimental farm at different ages. Figure1a: Ammonia concentration was 0.733, 1.434, 1.838, and 1.95 mg/L in G1, G2, G3, and G4 respectively at age 14 days. Figure1b showed no significant difference between G2, G3, and G4, but they are significantly differs from G1 (P<0.05). Figure1a indicated ammonia concentration was 3.13, 3.55, 5.41, and 6.86 mg/L in G1, G2, G3, and G4 respectively at age 21 days. Fgure1b showed no significant difference between G1 and G2, but they are significantly differs from G3 and G4 (P <0.05).

Ammonia concentration was at 3.53, 4.2, 7.5, and 20.26 in G1, G2, G3, and G4 respectively at age 28 days: Figure 1b showed no significant difference between G1 and G2, but they are significantly differs from G3 and G4 (P<0.05). Figure1a indicated that ammonia concentration was 3.47, 9.04, 11.1, and 31.4 in G1, G2, G3, and G4 respectively at age 36 days. Figure1b showed no significant difference between G2 and G3, but they are significantly differs from G1 and G4 (P < 0.05). Comparing ammonia concentrations of different SD. Highest ammonia concentration was found in G4 (SD 20 bird/m^2) (1.95, 6.86, 20.28 and 31.4 mgL^{-1} at 14, 21, 28 and 36 day age respectively) while lowest ones were found in G1 (10 bird/m^2) (0.7, 3.1, 3.5 and 3.5 mgL^{-1} at 14, 21, 28 and 36 day age respectively).

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From the obtained results, it was found that there was an increase in ammonia emission with increasing SD and with age. This result was in agreement with Harper et al. (2010) and Meda et al. (2011) who stated that ammonia emission was increased after the third week of broiler age. Ammonia concentration obtained in this study was much higher than that obtained by other surveys of ammonia concentrations in different poultry systems showed mean concentrations of 12.3 mgL^{-1} in perchery systems (Wathes, 1998; Groot Koerkamp et al., 1998; Kristensen and Wathes, 2000). Additionally Lima et al. (2011) stated that the concentrations of ammonia in poultry houses are usually around 20 ppm. As ammonia is the product of microbial mineralization of urea and uric acid in poultry litter mixed with chicken manure (Nahm, 2003). The obtained result could be explained as follows; by increase in SD and age, the amount of chicken manure increased and concomitantly ammonia emission increased. The obtained results are in agreement with those obtained by Tasistro et al. (2007); Zhao et al. (2015) and Brouček and Čermák (2015).

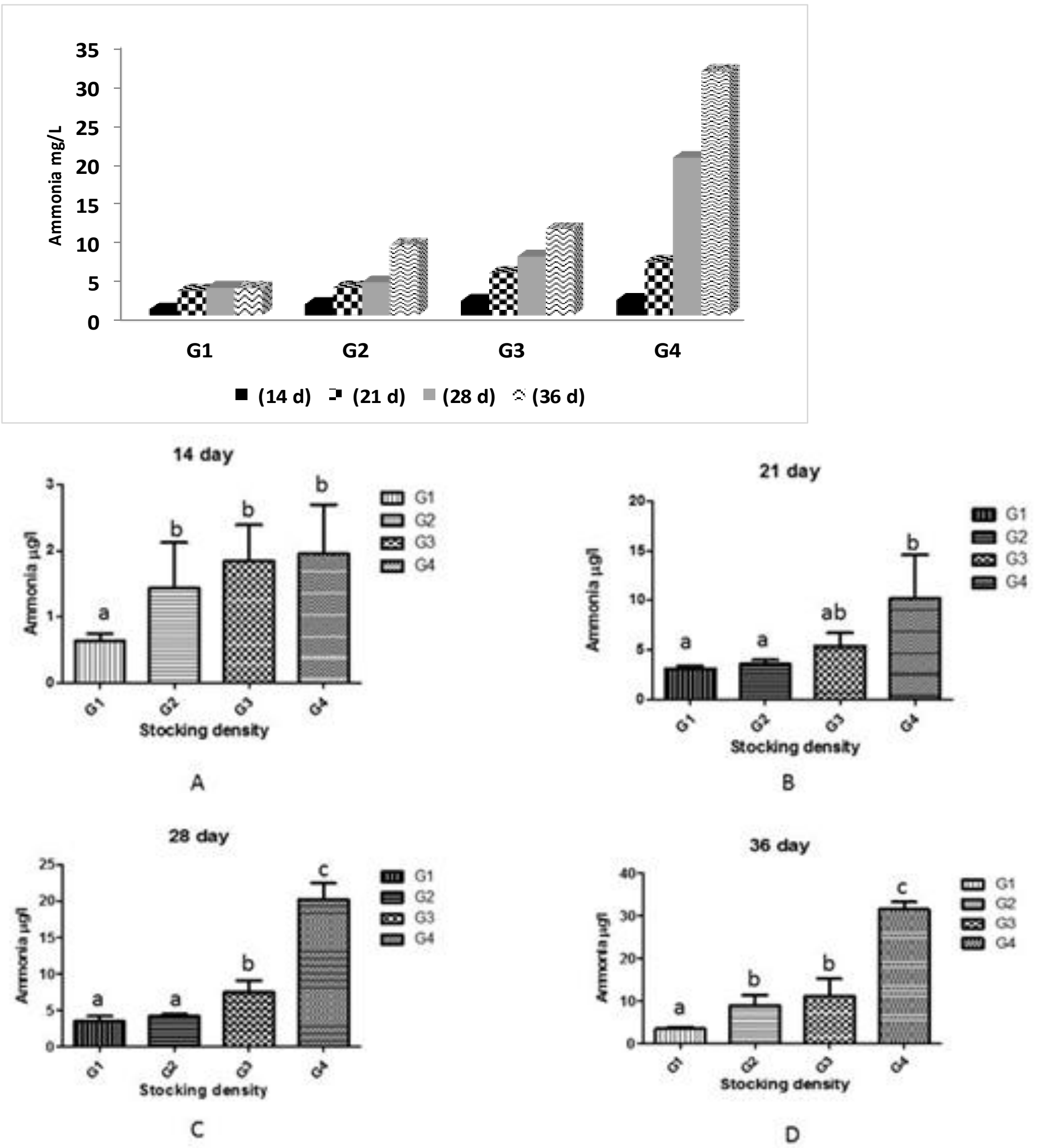

Figure1. a: Ammonia concentration in air samples of the broiler farm during day 14, 21, 28 and 36 in different groups; **b**: Detailed ammonia concentration (mgL^{-1}) with statistical differences between groups in air of the broiler farm in different ages (14 day, 21 day, 28 day, and 36 day). *Means ± Standard error which superscripts with different small letters (a-c) within the same diagram differ significantly at P<0.05.

Effect of stocking Density on boiler performance

Only two birds died during the experiment at 10 days age (data not shown). There were no significant differences in the initial BW among the experimental groups. However, increased SD tended to reduce the final BW and bodyweight gain significantly ($P < 0.05$) (Table 2).

The effect of SD on performance of broiler chickens over the 42 days trial period is shown in table 2. Stocking density had effect on the BW of birds. We found that birds raised at SD of 10 and 14 birds m^{-2} had significantly higher BW of 2530±41.44 and 2395±103.2 respectively than reared at stocking density of 17 and 20 birds m^{-2} 2178±46.63 and 2214±91.52 respectively ($P < 0.01$) The results are in contrast to Feddes et al. (2002), who reported similar BW of birds reared at densities of 12, 18, and 24 birds/m^2. Also Buijs et al. (2009) found no difference in final BW at 39 days of age as SD increased.

FCR was at 2.15, 1.74, 1.77 and 1.87 % for 10, 14, 17 and 20 birds m^{-2} respectively (Table 2). It was found that there is a significant difference between G1 and other groups ($P < 0.01$) but no significant difference between other 3 groups (14, 17 and 20 birds m^{-2}). Even though daily feed intake decreased significantly ($P< 0.05$) (Table 2) as SD increased. The result was similar to the report in another experiment (Dozier et al., 2005), who found a depression in daily weight gain, and the cumulative feed consumption was decreased as the SD increased.

Table 2. Effects of stocking density on body weight gain, feed intake, water intake, eviscerated weight and feed conversion ratio from first day to day 42 in broiler chicken

Parameter	G1	G2	G3	G4	P- value
BW (g)	2530±41.44^a	2395±103.2^{ab}	2178±46.63^b	2214±91.52^b	0.0155
BWG (g)	2460.11	2960.11	2264.71	2208.81	
Eviscerated Weight. (g)	1908±91.47^a	1715±58.74^a	1555±44.94^b	1526±60.88^b	0.0031
FI / week (g)	337.4±31.36^a	326.3±15.00^{ab}	255.2±11.50^b	263.1±13.38^b	0.0050
WI/ week (ml)	155.9±6.633^a	183.6±8.259^b	139.1±5.311^a	142.2±6.618^a	0.0001
FCR	2.15	1.74	1.77	1.87	

G1, 10 bird/m2; G2, 14 bird/m2; G3, 17 bird/m2; G4, 20 bird/m2; BWG=Body weight gain; FI=Feed intake; WI=Water intake; FCR=Feed conversion ratio; *Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at P<0.05.

Lymphoid organs

Liver, spleen and bursa are used as anatomical indicators of stress (Pope, 1991and Freire et al., 2003) as it is well known that the average weights of the lymphoid organs (spleen and bursa) change in response to stress (Ravindran et al., 2006). In the present study, the relative weights of spleen 2.42, 2.79, 2.85 and 3.69 g in G1, G2, G3 and G4 respectively and bursa (1.01, 1.8, 1.49 and 2.44 g in G1, G2, G3 and G4 respectively) were increased significantly ($P < 0.05$) with increasing SD (Table 3). These findings are in agreement with Muniz et al. (2006) who stated that higher SD cause detrimental effects on the lymphoid tissue. But the current results are different than that obtained by Heckert et al., (2002); Onbaşılar et al. (2008) and Tong et al. (2012) who concluded that increasing SD did not significantly affect the spleen and bursa weights. Additionally liver weight increased with increase in SD and stress level, this result was in line with Puvadolpirod and thaxton, 2000 and Sekeroglu et al. (2011).

Table 3. Effects of stocking density on body weight of lymphoid organs from first day to day 42 in broiler chicken

Parameters	G1	G2	G3	G4	P- value
Liver Weight (g)	52.26±2.922^a	56.80±3.108^{ab}	56.18±3.297^{ab}	66.26±1.843^b	0.0202
Spleen Weight (g)	2.422±0.1603^a	2.792±0.2910^a	2.848±0.1211^a	3.692±0.1428^b	0.0018
Bursa Weight (g)	1.010±0.1720^a	1.802±0.1498^b	1.490±0.2441^a	2.442±0.1899^c	0.0007

*Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at P<0.05.

Effect of stocking Density on Blood parameters

The effects of SD on some blood parameters are shown in table 4 it was found that there is a significant difference in the amount of blood hemoglobin and RBCs. This result was in contrast to that obtained by Sekeroglu et al. (2011). On the other hand significant differences were found in other blood parameters (PCV%, MCV (fl), MCHC (g/dl)) ($P > 0.05$). The results were in agreement with Thaxton et al. (2006), who found that SD did not result a significant difference in some blood parameters.

Table 4. Effects of stocking density on blood parameters and stress enzymes from first day to day 42 in broiler chicken

Parameters	G1	G2	G3	G4	P- value
Haemoglobin (g/dL)	11.60±0.2608^a	11.18±0.1463^a	10.82±0.1428^b	10.80±0.2168^b	0.0365
RBCs (×10^6/µl)	3.004±0.1966^a	2.918±0.03169^{ab}	2.560±0.04393^{ab}	2.524±0.09563^b	0.0151
PCV (%)	36.74±2.167	37.92±0.9173	36.04±1.059	35.80±1.305	0.7325
MCV (fl)	141.0±2.481	137.8±2.918	140.7±2.204	137.7±2.060	0.6601
MCHC (g/dL)	30.02±0.4224	29.98±0.2354	30.06±0.4250	30.44±0.4057	0.8164
TLC (x10^3/cmm)	106.1±3.261^a	108.4±2.948^a	103.3±2.134^{ab}	91.42±4.557^b	0.0114
Heterophils (%)	1.6±0.245^a	3.4±0.245^a	9.6±0.511^b	14.2±0.860^c	0.0001
Lymphocytes (%)	91.40±1.400^a	84.60±2.657^a	76.40±1.077^b	74.00±1.225^b	0.0001
H/L ratio	0.018±0.003^a	0.041±0.004^a	0.126±0.007^b	0.192±0.012^c	0.0001
LDH (IU/L)	41.00±4.461^a	506.2±19.62^b	601.0±47.52^b	872.8±44.61^c	0.0001
APK (IU/L)	3946±714.2^a	4632±314.2^a	5751±298.7^b	6091±286.6^b	0.0119

*Means ± Standard error which superscripts with different small letters (a-c) within the same row differ significantly at P<0.05.

In the current study there was a significant difference ($P < 0.05$) in TLC, hetrophils %, lymphocytes % and H/L ratio. Mohammed et al. (2014) stated that TLC and H/L ratio are considered the most important parameters that used for assessing physiological stress in birds. Results in table 4 showed that, there is a significant difference ($P < 0.05$) in TLC, heterophil (H%), lymphocyte (L%) and H/L ratio among the experimental groups. From these results, it was found that G4 (SD, 20bird/m^2) was represented by the lowest values of TLC (91.42×10^3/cmm), and highest (108.4×10^3/cmm) at G2 (SD, 14bird/m^2). In table 4 Heterophil H% was increased with increase in SD to reach highest percent in G4 (14.2%), on contrast and lymphocyte L% was decreased by increase in SD with highest percent in G1 (91.4%). H/L ratio was highest in G4 (0.192%) and lowest in G1 (0.018%), which showed increasing in H/L ratio with increase in SD which reflects increase in stress level, this result was in line with Puvadolpirod and Thaxton (2000) and Onbaşılar et al. (2008).

LDH is generally associated with cellular metabolic activity, which is inhibited or elevated under oxidative stress (Das et al., 2004). LDH and APK activity observed in the present study were increased laniary with increase in SD (Table 4). This result could be explained as increasing in SD resulted in increase in air pollution especially ammonia which increase level of stress and consequently increase the activity of LDH (Wang et al., 2014).

CONCLUSION

In conclusion, this study has declared that ammonia emissions from broiler house increased significantly with increasing in SD and age. This results pays attention to the need for improved ventilation and managemental procedures especially with increase in SD. Increased SD tended to reduce the BW and BWG significantly. On the other hand, the relative weights of spleen and bursa were increased significantly ($P < 0.05$) with increasing SD. H/L ratio was highest in G4 (0.192%) and lowest in G1 (0.018%), which showed increasing in H/L ratio with increase in SD which reflects increase in stress level.

Competing interests

The authors have no competing interests to declare.

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