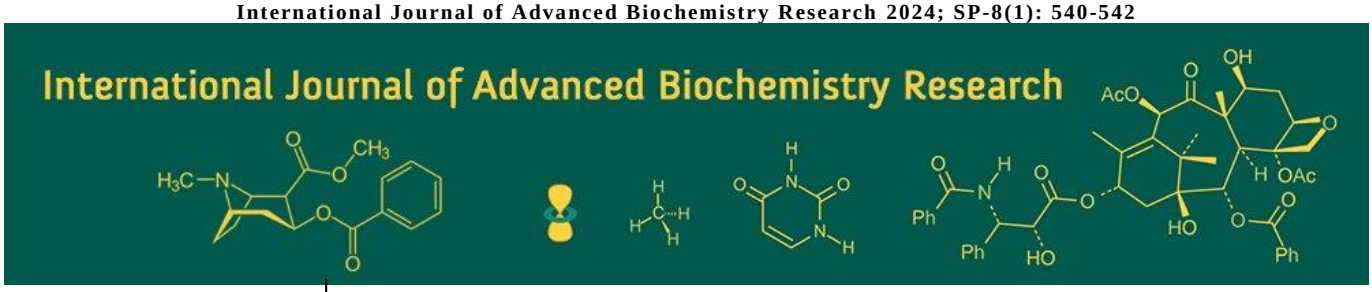

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Study of stocking density on Haemato-biochemical indices of broiler chicken under intensive poultry production system

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Abstract
Present investigation was conducted on broilers aged 6 weeks at poultry unit of Livestock farm complex, College of Veterinary and Animal Science, Navania, Udaipur (Rajasthan University of Veterinary and Animal Sciences) India. There was a significant difference ($p \leq 0.05$) in hemoglobin (Hb) concentration. The mean hemoglobin concentration was highest in D1 (11.05g) followed by control group D2 (10.47g) and D3 (9.25) respectively. Similarly heterophil sand lymphocytes (H/L) ratio was highest in D1 (0.34) followed by control group D2 (0.31) and D3 (0.27). A significant ($p \leq 0.05$) effect of H/L ratio was observed on all the three stocking densities. Whereas total protein, albumin, globulin level and albumin globulin ratio were unaffected by stocking density.

Keywords: Broiler, Haemato-biochemical indices, intensive system, stocking density

1. Introduction
Poultry all over the world serve as a good source of an animal protein to most people throughout the world. Poultry is the second most widely eaten meat in the world, accounting for about 30% of meat production worldwide, after pork at 38% (FAO, 2019) [4]. As per the 20th Livestock census (2019) [8] total Poultry population in India is 851.81 million that has been increased by 16.81% than previous census. Over 45.78% increase in backyard Poultry and total backyard Poultry is 317.07 millionin 2019. The total commercial Poultry is 534.74 million which has increased by 4.5%. Among the livestock sector Poultry industry contributes about 1% of national GDP and about14% of the livestock GDP (Mishra, 2020) [10].
Stocking density is considered to be one of the highest important environmental factors due to established effects on growth rate of broiler chickens. Inadequate stocking density and heat stress caused by climate change can lower blood homeostasis of animals. (Mortari *et al*. 2002; Park *et al*. 2018) [11, 13]. The negative effects of stocking density are more likely to cause foot-pad dermatitis, scratches, bruises, more feathery paws, and condemnation. (Simitzis *et al*. 2012; Rambau *et al*. 2016) [15, 14]. Stocking density has a major influence on the broiler production, especially during the final weeks of the growing period when body weight per unit area is high which leads to higher mortality, lower meat production. It is well documented that stocking density is a critical stressor in intensive Poultry production because inappropriate floor space is highly related to increase level of stress hormones which causes turbulences in Hemato-biochemical parameters like Hb, heterophil lymphocytes ratio (H/L), glucose, cholesterol, total protein, total albumin and triglyceride levels (Onbasular *et al*. 2008; Karthiayini *et al*. 2015) [12, 6].

2. Materials and Methods
The experiment was conducted at Poultry Farm of Livestock Farm Complex, College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur (Rajasthan University of Veterinary and Animal Sciences, Bikaner). One hundred and twenty (120) day old broiler chicks from a commercial hatchery in Ajmer, Rajasthan, were used for the study. In the brooding process, heat and light were provided by electrical hover brooders.

densities up to six weeks of age. Stocking densities were considered experimental design treatments. Four The period of brooding lasted for 2 weeks. A total of 120 birds were randomly assigned to three stocking replications were assigned to each treatment and every replication was allocated to eight chicks D1 (8 birds/m2), D2 (10 birds/m2) which served as control, D3 (12 birds/m2). Both sexes were reared together on deep litter floor. The experimental pens, drinkers, and feeding troughs were cleaned, disinfected, and sprayed against external parasites before the commencement of experiment. During the entire experimental period, all experimental chicks were handled identically and strict hygienic measures were taken as per standard practice. On the 4th and 14th days, broiler chicks were vaccinated against Ranikhet disease (F1 strain) and Infectious Bursal Disease.

2.1 Haemato-biochemical indices

Blood samples of approximately 3ml of wing vein from 2 representative birds of each replication were collected on the 42nd day of the experiment for haematological and serum biochemical parameters. Using an automated haematologya nalyzer, half of the blood was transferred to sterilized EDTA containing vacutainer tubes for the measurement of haemoglobin (Hb) and heterophils and lymphocytes H/L ratio. The residual blood sample was transferred to non-EDTA tubes for serum restoration. The serum was collected and processed for examination under deep freezing temperature, as per the normal protocol. Such samples were analyzed using an electronic biochemistry analyzer for total protein, serum albumin, albumin and globulin ratio. The content of serum globulin was calculated by subtracting the concentration of serum albumin from total protein levels. Data on haemato-biochemical traits were entered into M.S. Excel and analyzed with SPSS software Version 22.0 (SPSS, 2015) [17]. A statistical technique of one-way ANOVA was used to compare means and if the probability value was less than 0.05, the difference was pronounced statistically significant. Duncan's Multiple Range Test was used to distinguish significant ($P<0.05$) differences across variables (Steel *et al*. 1997) [18].

3. Results and Discussion

3.1 Haemato-biochemical indices

The effects of different stocking density on hemato-biochemical indices of broilers are presented in Table-1.

1.1 Hemoglobin concentration

The mean for the hemoglobin concentration (Hb) in the blood at the end of the experiment were 11.05, 10.47 and 9.25 g for treatmentgroup D1, D2 (control) and D3, respectively. The analysis of variance for the mean hemoglobin level between the treatment groups showed significant ($p<0.05$) differences. The highest mean hemoglobin level was observed for treatment group D1 followed by D2 (control) and D3. Our results corroborated with the findings of Mohammed *et al.* 2014 [9]., Bueno *et al.*, 2017 [2]., Abouelenien *et al.* 2016 [1] and Park *et al.* 2018 [13] who found that level of Hb significantly decreased with increasing stocking density in poultry.

1.2 Heterophils and lymphocytes ratio (H/L ratio): The mean for the heterophils and lymphocytes ratio (H/L) in the blood were 0.34, 0.31 and 0.27 percent for treatment group D1, D2 (control) and D3, respectively. The analysis of variance for the mean heterophils and lymphocytes (H/L) ratio between the treatment groups showed ($P<0.05$) differences. The highest mean H/L ratio was observed for treatment group D1 followed by D2 (control) and D3. Our results corroborated with the findings of Mohammed *et al.* 2014 [9]., Bueno *et al.*, 2017 [2]., Abouelenien *et al.* 2016 [1] and Park *et al.* 2018 [13] who found that level of H/L ratio significantly decreased with increasing stocking density in poultry.

1.3 Total protein: The mean for the total protein in the blood at the end of theexperiment were 2.65, 2.88 and 2.71 g/dl for treatment group D1, D2(control) and D3, respectively. The analysis of variance forthe mean total protein (g/dl) between the treatment groups showednon-significant differences. The highest mean (g/dl) was observed for treatment group D2 (control) followed by D3 and D1. Our finding are in line with Skrbic *et al.*, 2009 [16], Das, 2014 [3], Gupta *et al.*, 2016 [5] and Kumar *et al.*, 2017 [7] who found that stocking density had a non-significant impact on total protein.

1.4 Albumin

The mean for the albumin in the blood were 1.25, 1.59 and 1.45 g/dl for treatment group D1, D2(control) and D3, respectively. The analysis of variance for the mean albumin (g/dl) between the treatment groups showed non-significant differences. The highest mean (g/dl) was observed for treatment group D2 (control) followed by D3 and D1. Our results are in line with Skrbic *et al.*, 2009 [16], Das, 2014 [3], Gupta *et al.*, 2016 [5] and Kumar *et al.*, 2017 [7] who found that stocking density had a non-significant impact on albumin.

1.5 Globulin: The mean value for the globulin in the blood at the end of the experiment were 1.40, 1.28 and 1.25 g/dl for treatment group D1, D2 (control) and D3, respectively. The analysis of variance for the mean globulin (g/dl) between the treatment groups showed non-significant differences. The highest mean (g/dl) was observed for treatment group D1 followed by D2 (control) and D3. Our results are in line with Skrbic *et al.*, 2009 [16], Das, 2014 [3], Gupta *et al.*, 2016 [5] and Kumar *et al.*, 2017 [7] who found that stocking density had a non-significant impact on globulin.

1.6 Albumin and globulin ratio (A/G)

The mean for the albumin and globulin ratio in the blood at the end of the experiment were 0.88, 1.25 and 1.15 for treatment group D1, D2 (control) and D3 respectively. The analysis of variance for the mean globulin (g/dl) between the treatment groups showed non-significant differences. The highest mean (g/dl) was observed for treatment group D2 (control) followed by D3 and D1. Our results are in line with Skrbic *et al.*, 2009 [16], Das, 2014 [3], Gupta *et al.*, 2016 [5] and Kumar *et al.*, 2017 [7] who found that stocking density had a non-significant impact on A/G ratio.

Table 1: Mean±SE M effect of different stocking densities on haemato-biochemical parameters of broiler chickens

Parameters Groups	N	Haemoglobin (g)	Heterophil/Lymphocyte %	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Albumin/Globulin ratio
D1	32	11.05±0.35b	0.34±0.17b	2.65±0.11	1.25±0.09	1.40±0.02	0.88±0.05
D2	40	10.47±0.18b	0.31±0.00b	2.88±0.12	1.59±0.13	1.28±0.06	1.25±0.14
D3	48	9.25±0.46a	0.27±0.02a	2.71±0.20	1.45±0.18	1.25±0.03	1.15±0.13
P-value		*	*	NS	NS	NS	NS

*-Significant ($p<0.05$),**-Significant ($p<0.01$),NS-Non–significant,
a, b, c, Means with different superscript within the columns differ significantly with each other.

Hb level and H/L ratio are considered the most important parameters that used for assessing physiological stress in birds. Results showed that, there is a significant difference ($p<0.05$) in H/L ratio among the experimental groups. This may be due to damage to red blood cells, the reduction in the output of red cells, or the decrease in the number and size of red blood cells (Bueno *et al.*, 2017; Park and Park, 2017) [2, 13]. In humans and animals, a reduction in red blood cells and hemoglobin levels under normal environmental conditions may cause iron deficiency anemia. Even if there is no difference in plasma volume, chickens on high stress and High stocking density suffer from hem dilution due to increased water absorption. This can cause water to evaporate from cells, lowering the number of red blood cells and hemoglobin levels (Turkyilmaz, 2008; Park and Kim, 2017) [19, 13].

Conclusion

It was concluded from the present study that hematological parameters *viz*., Hemoglobin (Hb) concentration, heterophils and lymphocyte (H/L) ratio increased with decreasing density. The best results in terms of Hb and H/L ratio was observed in lowestdensity group and lowest values were observed in intermediate andhighest stocking densities respectively. It can be concluded that Hb concentration an dH/L ratio improved with decreasing stocking density. However other parameters *viz*., tota lprotein, albumin, globulin level and albumin globulin ratio was unaffected among all the three stocking densities in the present study.

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