

Original Research Article

Effect of Stocking Density on Stress Reaction and Mortality in Broiler Chickens

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ABSTRACT

The research was conducted to investigate the effect of stocking density (I.C.A.R., Venky's and Amrit recommendations) on stress reaction and mortality in broiler chicken. A 500 day-old straight run commercial broiler strain "VENCOB" chicks were divided in two batches, each containing 3 equal groups (i.e., I.C.A.R., Venky's and Amrit). The blood samples were collected at 4th, 5th, and 6th week of study and mortality were recorded every day. After May-Grunwald-Giemsa staining, H: L ratios were calculated. The results showed that, LYM and HET numbers decrease and increase respectively in response to stressors. The HET and LYM count (%) was significant ($P < 0.01$) in all the weeks of study. At week 4, H: L ratio under I.C.A.R. (00.67 ± 0.015) significantly ($P < 0.01$) higher than that of both Venky's (00.52 ± 0.009) and Amrit (00.52 ± 0.005) recommendations which did not differ each other. At week 5 the H: L ratio in Amrit (00.66 ± 0.012) recommendation was significantly ($P < 0.01$) more than rest recommendations which, however did not differ each other. At week 6 the highest and lowest H: L ratio were 0.71 ± 0.008 (Amrit) and 0.50 ± 0.005 (I.C.A.R.) respectively. All the three ratios differ significantly ($P < 0.01$). This can be concluded that H: L ratio was higher under Amrit's recommendation than those in Venky's and I.C.A.R. No significant difference was observed among mortality rates of broiler chickens under these three stocking densities during all the weeks studied.

Keywords

Stocking density, stress reaction, mortality, broiler chicken

Introduction

Archaeological discoveries in the Indus Valley suggest that chickens were probably domesticated from the red jungle fowl (*Gallus gallus*) as early as 5400 B.C. (West and Zhou, 1988). The total poultry population in India is 729.2 million, which is 12.39 percent higher than numbers in the previous census (Livestock Census, 2012). Poultry is one of the fastest growing sectors of Indian agriculture today, with annual

growth rates of 5.57 percent and 11.44 percent in egg and broiler production, respectively. The sector is providing direct or indirect employment to 6.5 million people. About 80 percent of the employment is generated directly by poultry farms; the rest by the feed, pharmaceutical, equipment and other support services required by poultry. The value of output from the poultry sector was US\$10 billion in 2014

(Rajendran *et al.*, 2014). Broiler production has been more vibrant than layer production within the poultry sector, with an annual growth rate of 11.44 percent, production of 3.725 million tons and employment of 4.29 million people (Index Mundi, 2015). India is the fourth largest producer of poultry meat in the world, valued at US\$ 6.6 billion. Poultry production accounts for about 0.66 percent of India's GDP and 7.72 percent GDP from the livestock sector (Prabakaran, 2014; Rajendran *et al.*, 2014). Poultry meat production increased from 0.069 million tons in 1961 to 3.725 million tons in 2014. The per capita availability of poultry meat is 2.8 kg; against recommended level of 11 kg (Prabakaran, 2014; Rajendran *et al.*, 2014; Index Mundi, 2015). The main reasons for improved broiler poultry production (Kalamkar, 2012; Prabakaran, 2012; SAPPLPP, 2009) are: modernization of production practices; import of pure lines/grandparent stock; least-cost feed formulation; vaccines against major diseases; provision of EAS and other input services; improved quality breeder management; developments in poultry processing; and private sector partnerships through Contract Broiler Farming.

Stocking density in general is a significant factor in broiler performance (Feddes *et al.*, 2002; Heckert *et al.*, 2002; Tablante *et al.*, 2003). The term "stress" is commonly used to describe the detrimental effects of a variety of situations on the health and performance of poultry (Moberg, 2000). The adaptation process due to stress causes the release of hormones and requires the redistribution of body reserves including energy and protein at the cost of decreased growth, reproduction, and health (Estevez, 2007). After extended or repeated periods of stress, birds become fatigued and weak; they often succumb to starvation and infectious diseases (Heckert *et al.*, 2002; Thaxton *et*

al., 2006; Estevez, 2007). In several studies, it has been determined that psychological and physical stressors such as fasting, frustration, water deprivation and crowding, increase the ratio of Heterophil to Lymphocyte (H/L) (Hacking, 1993; Dozier *et al.*, 2006; Buijs *et al.*, 2009). The H/L ratio has been shown to be highly heritable (al-Murrani, *et al.*, 1997) and a reliable index for determining stress in poultry (Heckert *et al.*, 2002). A majority of published studies have noted no effects of stocking density on mortality, even at levels that should be regarded as extreme (Shanawany, 1988). Feddes *et al.*, (2002) concluded that stocking density had no effect on mortality. However, Dozier *et al.*, (2005b) concluded that mortality was higher for densities above 30 kg/m² (3.6 vs. 7.5%) but was not significantly different. Pettit-Riley and Estevez (2001) found that mortality due to heat stress was significantly higher at densities above 0.066 m² (15 birds/m²). Hall (2001) reported that there was significantly higher daily mortality at the largest density toward the end of rearing but no differences in total mortality.

Materials and Methods

The aim of the present investigation was to examine the effects of various stocking densities on stress reaction and mortality of broiler chickens. The study was carried out at the Poultry Unit of Teaching Livestock and Poultry Farm of the College of Veterinary Science and Animal Husbandry, N.D.U.A.T. Kumarganj, Faizabad U.P. To carry out the present investigation, 500 day old straight run, commercial broiler strain "VENCOB" chicks were divided in two batches. Each batch was subdivided in 3 equal groups namely Gr 1, Gr 2 and Gr 3 randomly distributed among 3 pens. All the birds were raised on pucca floor with saw dust as litter material. Chicks were reared

under uniform managerial practices and were kept up to the age of 42 days for recording heterophil (HET) and lymphocyte (LYM) counts. A replication was made thereafter with the next batch of chicks. The floor space for Gr 1 follows as per I. C. A. R. recommendation (I.C.A.R. bulletin on animal housing and equipment; IS 5309, Part-II) whereas Gr 2 and 3 follow the recommendation of hatchery companies namely Venkateshwara Hatcheries Pvt. Ltd. (www.venkys.com, 2008) and Amrit Hatcheries Pvt. Ltd. (www.amritgroup.in, 2008) respectively (Table 1).

The blood samples were collected at 4th, 5th, and 6th week of the study. A total of 10 randomly selected chickens (identified and wing banded birds) from each group were gently removed from pens and blood samples (0.5 ml) were collected from the wing vein for heterophil (HET) and lymphocyte (LYM) counts. Blood smears were prepared on a dry, clean and grease free glass slide. The smears were stained with May-Grunwald –Giemsa stain.

To assess H: L ratios, 100 leucocytes were counted. H: L ratios were calculated by dividing the number of heterophils by the number of lymphocytes counted (Gross and Siegel, 1983). Daily mortality of birds was recorded in morning hours in each pen and each batch. The mortality rate was calculated weekly.

Statistical analysis

The recorded data of heterophyl and lymphocyte count were analyzed as per standard statistical procedure outlined by MMLS and ML computer programme PC-2, Harvey (1990). Least squares means were compared by Critical Difference Test. Mortality data of chicks were analysed by Chi-Square test.

Results and Discussion

Heterophyl and Lymphocyte count

The number of lymphocyte in the chicken blood samples decrease and the number of heterophyl increase in response to stressors. The high stocking density also causes social stress upon chickens. Table II shows variability in HET and LYM counts in broiler chickens at week 4, 5 and 6 under three recommended stocking densities, viz. I.C.A.R., Venky's and Amrit. At week 4th, L.S. means of HET count was significantly ($P<0.01$) higher under I.C.A.R. recommendation of stocking density than, those of Venky's and Amrit recommendations which did not differ from each other. That means at week 4 the broiler chickens under I.C.A.R. stocking rate exhibited comparatively more stress. At this week I.C.A.R. recommends 0.50 sq. ft./bird floor space where as other two recommendations provide 1 sq. ft./bird floor space. At week 5th L.S. means of HET count under Amrit recommendation was found to be significantly ($P<0.01$) higher than other two recommendations of stocking densities which did not vary significantly each other. The more stress in chickens under Amrit Stocking rates was exhibited due to lesser (1.25 sq. ft./bird) floor space recommendation as compared to others (2.25 sq. ft./bird, I.C.A.R. and 2.50 sq. ft./bird, Venky's). At week 6 more floor space (2.25 sq. ft./bird) have been provided under I.C.A.R. recommendation of stocking rate than rest two (1.50 sq. ft./bird in each). This indicates less stress in chicks under I.C.A.R. recommendation which have been executed in the Table 2. Here the L.S. means of HET count under I.C.A.R. rate was significantly ($P<0.01$) lesser (30.70+0.191 %) than other to stoking rates (34.76+0.302 % for Venky's, 35.15+0.279 % for Amrit). The almost similar but reverse pattern of

LYM count (%) have been observed like HET count (%) of broiler chickens under three stocking densities at week 4, 5 and 6. At week 4th L.S. means of LYM count under Venky's recommendation did not differ from that of Amrit. However, significant ($P<0.01$) difference was observed from I.C.A.R. On the other hand there was no significant difference recorded between I.C.A.R. and Amrit stocking rates in LYM count at these weeks. At week 5 lowest and highest L.S. means % of LYM count were recorded to be $51.65+0.568$ (Amrit) and $60.50+0.307$ (Venky's) respectively. The L.S. means of Venky's was significantly higher than other two recommendations of stocking rate which again differed significantly ($P<0.01$) each other. Here the higher LYM % in chicks under Venky's stocking rate indicate lesser stress due to higher (2.50 sq. ft./bird) floor space allowance than others (1.25 sq. ft./bird Amrit, 2.25 sq. ft./bird I.C.A.R.).

At week 6th LYM count under I.C.A.R. stocking rate was significantly ($P<0.01$) higher than other two densities which again differed statistically ($P<0.01$) from each other. The lowest and highest L.S. means of LYM count (%) were recorded to be $49.20+0.536$ (Amrit) and $61.45+0.497$ (I.C.A.R.) respectively. The result indicated that during 6th week the birds exhibited comparatively lower stress under I.C.A.R. recommendation of stocking density (2.25 sq. ft./bird) than others. However the floor space allowance per bird during 6th week in both Venky's and Amrit were same (1.50 sq. ft./bird) but higher LYM count in Venky's recommendation indicated lesser stress than that of Amrit's.

Heterophyl and Lymphocyte ratio

The L.S. means of H: L of broiler chickens at week 4, 5 and 6 under three recommended

stocking densities have been given in Table 3. In general, higher the ratio, higher will be the stress among birds. At week 4, H: L ratio under I.C.A.R. significantly ($P<0.01$) higher than that of both Venky's and Amrit recommendations which did not differ each other. At week 5 the H: L ratio in Amrit recommendation was significantly more than rest recommendations which, however did not differ each other. At week 6 the highest and lowest H: L ratio were $0.71+0.008$ (Amrit) and $0.50+0.005$ (I.C.A.R.) respectively. There were significant ($P<0.01$) differences among all the three ratios. The results obtained here was as per the hypothesis, which states that higher the floor space lower the H: L ratio which in return indicates lesser stress in birds.

Mortality rates under different stocking densities

The weekly mortality rates of broiler chickens under three stocking densities have been presented in Table 4. The overall mortality (%) was less than 5 % over weeks under each placement densities. No significant difference was observed among mortality rates of broiler chickens under these three stocking densities during all the weeks studied.

In present study higher H: L ratio has been recorded with increased stocking density during last 3 weeks under study. This observation was in evidence with the findings of El-Lethey *et al.*, (2000). However, Turkeyilmaz (2008) reported that H: L ratio was not stocking density dependent.

The discrepancy observed between the findings may be due to genotype, methodology used and relatively small population used.

Table.1 Floor space provided to three different groups

Weeks	Floor Space (Square feet / Bird)		
	Group 1 (I. C. A. R.)	Group 2 (Venky's)	Group 3 (Amrit)
1	0.50	0.25	0.25
2	0.50	0.33	0.50
3	0.50	0.50	0.75
4	0.50	1.00	1.00
5	2.25	2.50	1.25
6	2.25	1.50	1.50

Table.2 Least squares' means \pm S. E. of % heterophil and lymphocyte count of broiler chickens week wise under three stocking densities

H&L Count	Week	Stocking density			Level of significance
		I.C.A.R.	Venky's	Amrit	
HET	WK 4	33.75 \pm 0.451 ^a	31.15 \pm 0.395 ^b	30.75 \pm 0.202 ^b	P<0.01**
	WK 5	29.70 \pm 0.298 ^b	29.90 \pm 0.231 ^b	34.10 \pm 0.343 ^a	P<0.01**
	WK 6	30.70 \pm 0.191 ^b	34.76 \pm 0.302 ^a	35.15 \pm 0.279 ^a	P<0.01**
LYM	WK 4	50.00 \pm 0.785 ^b	59.20 \pm 0.517 ^a	58.55 \pm 0.265 ^{ab}	P<0.01**
	WK 5	58.40 \pm 0.436 ^b	60.50 \pm 0.307 ^a	51.65 \pm 0.568 ^c	P<0.01**
	WK 6	61.45 \pm 0.497 ^a	55.09 \pm 1.358 ^b	49.20 \pm 0.536 ^c	P<0.01**

N= 10 under each observation; Means having dissimilar superscripts within row differ significantly.

Table.3 Least squares' means \pm S. E. of heterophil: lymphocyte ratio of broiler chickens week wise under three stocking densities

H: L Ratio	Week	Stocking density			Level of significance
		I.C.A.R.	Venky's	Amrit	
H: L	WK 4	00.67 \pm 0.015 ^a	00.52 \pm 0.009 ^b	00.52 \pm 0.005 ^b	P<0.01**
	WK 5	00.51 \pm 0.008 ^b	00.49 \pm 0.005 ^b	00.66 \pm 0.012 ^a	P<0.01**
	WK 6	00.50 \pm 0.005 ^c	00.63 \pm 0.016 ^b	00.71 \pm 0.008 ^a	P<0.01**

N= 10; Means having dissimilar superscripts within row differ significantly.

Table.4 Week wise mortality (%) of birds under different stocking densities

Weeks	Stocking density			Total
	I.C.A.R.(n=168)	Venky's(n=166)	Amrit(n=166)	
WK 1	1.19	1.81	1.81	4.81
WK 2	0.60	1.23	1.23	3.06
WK 3	1.21	1.24	0.62	3.04
WK 4	1.23	0.63	0.62	2.48
WK 5	0.00	0.00	0.00	0.00
WK 6	0.00	0.00	0.00	0.00

Figures in the parentheses represent respective no. of birds under each density

In this context, Keeling and Duncan (1991) also founded that aggressiveness is higher in small flocks than in large flocks. On the other hand, environmental factors, such as temperature, humidity, and gas emissions (CO₂, CO, NH₃, and H₂S), which cannot be controlled, may cause different levels of stress in broilers. Statistically higher H: L ratio are reported (Cravener *et al.*, 1992) to be correlated with increased stress in avian species. According to this study birds housed under higher densities (.05 and .07 m² per bird) had similar H: L ratios, which were significantly lower than those housed under higher densities (.09 and .11 m² per bird). The increasing H: L ratio with decreasing density suggests the complex social dynamics of avian species may necessitate caution when interpreting this ratio. Stressed birds show an increase in basophils and heterophils and a decrease in lymphocytes and, as a result, an increase in the heterophil: lymphocyte (H: L) ratio (Gross and Siegel, 1983). With time, the H: L ratio often returns to normal, but basophils remain elevated and thus could be used to assess prolonged stress (Maxwell *et al.*, 1990). Zulkifli *et al.*, (2003) reported that the heterophil to lymphocyte ratio (H: L) is a reliable indicator of avian stress. Broiler exposed to heat stress in summer show an increase in heterophil and a decrease in lymphocytes, which lead to an increase H: L ratio. McFarlane and Curtis (1989) reported that the H: L ratio increase with heat stress in broiler chicks. Elevation of the heterophil to lymphocyte ratio (H: L) with increasing stocking density was reported to indicate that high stocking density in broiler production is stressful (Feddes *et al.*, 2002). Spinu *et al.*, (2003) reported that there was no difference in the H: L ratio between different stocking densities in broiler breeders in summer. No difference was found in the H: L ratio between the 3 stocking density groups. Mean H: L ratio on

day 42 was 0.41, 0.43, and 0.45 for 15, 20, and 25 birds/m² respectively. There was no significant difference in H: L ratio between the weeks (Turkyilmaz, 2008). Stocking density had not significant effect on mortality in broilers in the present study. Iscan *et al.*, (1996) reported that mortality for 15 and 20 birds/m² was 7.1 and 6.4 %, respectively. As in the present study, stocking density in broilers had no significant effect on mortality, which was also similarly suggested by Cravener *et al.*, 1992 and Heckert *et al.*, 2002. Increasing mortality can be explained by decreased animal welfare, such as bad air and litter quality, poor immune response and poor feed intake.

The heterophil and lymphocyte count (%) was significant in all the weeks under study. Higher HET count (%) was observed under Amrit followed by Venky's and I.C.A.R. floor space recommendation during last three weeks. LYM count (%) was observed to be higher under Venky's followed by I.C.A.R. and Amrit. The H: L ratio was significant among three densities at 3rd week of study. Higher H: L ratio was observed mostly under Amrit recommendation followed by Venky's and I.C.A.R. Effect of stocking density on mortality (4.25%) of chicks was non-significant among three stocking densities.

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