EFFECT OF STRESS ON THE PRODUCTION PERFORMANCE, HEMATOLOGY AND GASTROINTESTINAL ENZYMES IN BROILERS

Rekha P. Raghavan, Sreekumar K. Panicker and Beena Vakayil

ABSTRACT

The study evaluated the effect of overcrowding stress on production parameters, hematology, organs and gastrointestinal enzymes activity in broiler chicken. Twenty day-old broiler chicks were divided into two groups: unstressed group and stressed group, with 10 birds in each group. Stress was induced by reducing the floor space of each bird by 1/3 of the optimum requirement (1116 cm^2/ bird) at 30 days of age and the birds were subjected to stress for 15 days, until 45 days of age. Overcrowding stress lowered the erythrocyte and leukocyte counts but enhanced the heterophil to lymphocyte ratio. The reduction in bursal weight and hypertrophy of adrenals adversely affected the immune status and hematology of stressed birds. Even though only pancreatic lipase expressed lowered activity, the overall results indicated that overcrowding stress could culminate in poor digestion in broilers. The economics revealed that there is a net revenue loss to the farmers due to overcrowding.

Keywords: broiler, digestion, hematology, overcrowding, stress

INTRODUCTION

The poultry industry is gaining importance in India due to changing social strategies. Although broilers are the most preferred animals under the poultry meat category, commercial broiler production experiences lots of economic losses because of improper management. Among these management errors, overcrowding of birds is the most important, inducing stress on birds and leading to great economic loss (Dozier, 2005; Onbasilar and Aksoy, 2008) Although studies on the influence of overcrowding stress on the production performance and carcass quality of broilers have been carried out, little is known about its influence on digestive enzymes and organs involved with digestion, hematology, immune system and endocrinology. This study was undertaken to evaluate the impact of overcrowding stress on the hematology, body weight, feed consumption, slaughter weight,

Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Thrissur, India 680651 (email:kannoth_s@yahoo.com).

carcass weight, weight of giblet (heart, gizzard and liver), bursa, spleen, adrenals and activities of digestive enzymes such as protease, amylase and lipase in broiler chicken. The economics was also worked out to determine the loss or gain in revenue to farmers due to this practice.

MATERIALS AND METHODS

Twenty (20) day-old broiler chicks (Vencob strain) procured from a commercial broiler hatchery and reared under standard management conditions in battery brooder were used for this study. They were fed with commercial broiler starter ration for the first four weeks and then with finisher ration for the next two weeks (Bureau of Indian Standards). They were divided into two groups; G1 (unstressed group), and G2 (stressed group) comprising 10 birds in each group. Stress was induced in G2 by reducing the floor space of each bird by 1/3 of the optimum requirement (1116 cm^2/ bird) at 30 days of age. The birds were subjected to stress for 15 days (up to 45 days of age). G1 formed the birds of the control group and was maintained at the optimal floor space throughout the study period. Body weight and feed consumption were recorded at weekly intervals for both groups.

Hemoglobin concentration (Hb), volume of packed red cells (VPRC), total erythrocyte count (TEC), total leukocyte count (TLC) and heterophil-lymphocyte ratio (H:L) were evaluated one day before stress and after 15 days of stress, *i.e.* at the 29^{th} day and 45^{th} day of age, respectively. Blood samples were collected by wing vein puncture with EDTA as the anticoagulant. The estimation of TEC and TLC was done by the method suggested by Natt and Herrick (1952). VPRC and Hb concentration were estimated as per standard procedures (Feldman *et al.,* 2000). Blood smears were prepared using fresh blood at the time of blood collection. Air dried blood smear was stained with Leishman-Giemsa stain solution and different leucocytes were counted and H:L was derived (Gross and Siegel, 1983).

At 45 days of age, both G1 and G2 birds were sacrificed by decapitation after overnight fasting. The body cavity was exposed; the needed organs (bursa, spleen, adrenal, heart, liver, gizzard) and the whole gastrointestinal tract were excised out, thoroughly cleaned and weighed. The combined weight of heart, liver and gizzard was taken as the weight of the giblet. The pancreas was taken and weighed immediately. The proventricula and duodenum were exposed, washed out with ice cold normal saline and then the moisture was removed. The mucous membrane scrapings were homogenized with ice cold normal saline and the final volume of the homogenate of each sample was made up to 25 ml. Proventricular protease was estimated by the method described by Hawk *et al.* (1954), intestinal amylase as per the procedures described by King and Wootton (1959) and pancreatic lipase according to the protocol described by Boutwell (1962).

The data were analyzed for differences using analysis of variance and sample means were compared using Paired t-test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Hematological and physiological parameters are presented in Table 1. The effect on body weight of stressed birds expressed a similar trend, but not significantly different, agreeing with the reports of Dozier *et al.* (2005) and Samale *et al.* (2008), where stress due to overcrowding lowered the body weight of broiler chicken. Shivakumar *et al.* (2004) and Onbasilar and Aksoy (2008) also reported that overcrowding stress reduced body weight gain. Kuan *et al.* (1990) reported that increasing stocking density (stress) reduced feed consumption in broiler chicken. A similar trend was observed in the present study, even though it was also not statistically significant. Reduced floor space restricts physical mobility and leads to reduced feed consumption. The lack of statistical significance in the observed numerical reduction of body weight and feed consumption in the present study might be due to the small sample size.

Table 1. Effect of stress on physiohematological parameters (mean±SE, n=10) in broilers.

Parameter	Group	One day before stress	After 15 days of stress
Body weight (g)	G1	837.0±5.97^{a}	1505.0±97.47^{a}
	G2	806.5±33.50^{a}	1333.0±51.29^{a}
Feed consumption (g)	G1	625^{a}	1180^{a}
	G2	580^{a}	1110^{a}
Hb (g %)	G1	13.9±1.28^{a}	19.1±1.84^{a}
	G2	14.6±0.90^{a}	17.8±0.85^{a}
VPRC (%)	G1	35.0±2.28^{a}	26.7±0.75^{a}
	G2	28.4±1.13^{b}	27.5±0.93^{a}
TEC (10^6/µl)	G1	6.5±0.33^{a}	6.5±0.35^{a}
	G2	5.6±0.23^{a}	4.7±0.34^{b}
TLC (10^3/µl)	G1	24.0±0.43^{a}	30.7±0.43^{a}
	G2	25.8±0.39^{b}	24.0±0.27^{b}
H:L	G1	0.5±0.01^{a}	0.5±0.01^{a}
	G2	0.5±0.00^{a}	0.7 ±0.02^{b}

G1: Unstressed birds; G2: stressed birds.
Means with different superscripts between groups in the same column are different ($P<0.05$).

There was no significant difference ($P>0.05$) in Hb concentration between stressed birds and unstressed birds. Reddy (2003) and Karthiayini and Philomina (2008) also observed that overcrowding stress does not significantly affect the Hb concentration in broiler chicken. There was no significant difference in the VPRC values between unstressed birds and birds subjected to overcrowding stress. Reddy

(2003) also could not observe any significant change in the value of VPRC in broiler chicken subjected to stress by increasing the stocking density. Karthiayini and Philomina (2008) also reported that overcrowding stress induced by reducing the floor space by 50% did not produce any significant difference in VPRC values, which was similar to the present study. Fifteen days of stress induced a significantly ($P<0.05$) reduced TEC in stress birds compared to unstressed ones. This is in agreement with the study of Bedanova *et al.* (2006), where the reduction of floor space during crating decreased the total erythrocyte count. Reddy (2003) and Karthiayini and Philomina (2008) observed a significant difference in TEC between unstressed and stressed birds. In the present study, stressed birds expressed a lower spleen weight even though this was not statistically significant. Spleen is involved in hematopoiesis and, hence, this might have also contributed to the decreased TEC in stressed birds. TLC count in stressed birds was significantly ($P<0.05$) lower than in unstressed birds even though the level was significantly ($P<0.05$) higher before induction of stress. The elevation of corticosteroid level during stress could have contributed to the decline in the TLC. Mejo (2006) also reported that heat stress induced severe leukocytopenia associated with a significant reduction in absolute lymphocyte count in broiler chicken.

After fifteen days of overcrowding, the stressed birds had a significantly ($P<0.05$) increased H:L compared to unstressed birds. Kuan *et al.* (1990) reported that elevation of H:L was observed with increased stocking density. H:L of about 0.2, 0.5 and 0.8 are characterized as low, optimum and high levels of stress, respectively. Gross and Siegel (1983) and Zulkifli and Sti Nor Azah (2004) reported that heterophil to lymphocyte ratio was a reliable indicator of avian stress. The results revealed that overcrowding by 1/3 reduction of optimal floor space inflicted high stress in broilers.

The activities of digestive enzymes are presented in Table 2. There was no significant ($P>0.05$) difference between unstressed birds and stressed birds in the proventricular protease and intestinal amylase activity, even though stressed birds had numerically lower enzyme activity than unstressed birds. The lipase activity was significantly ($P<0.05$) decreased in stressed birds compared to unstressed birds. Overcrowding induces reduction in feed consumption and might contribute to a decreased enzyme activity in broilers. Kuan *et al.* (1990) reported that increasing

Table 2. Effect of stress on gastrointestinal enzymes parameters (mean±SE, n=10) in broilers.

Group	Protease (Pepsin units/g of tissue)	Amylase (Somogyi units/g of tissue)	Lipase (Lipase units/g of tissue)
G 1	237.2±20.41^{a}	17.0±0.40^{a}	23.2±0.57^{a}
G 2	164.0±10.20^{a}	15.1 ±1.28^{a}	15.8±0.64^{b}

G1: Unstressed birds; G2: stressed birds.
Means with different superscripts in the same column are different ($P<0.05$).

stocking density reduced the feed consumption in broiler chicken. Shivakumar *et al.* (2004), Dozier *et al.* (2005) and Samale *et al.* (2008) also reported that broiler chicken subjected to overcrowding stress had reduced feed consumption. In the present study, the feed consumption was lower in stressed birds compared to unstressed birds and this might have affected the activity of digestive enzymes.

The weight of carcass and organs are indicated in Table 3. The slaughter weight and carcass weight were lower in stressed birds compared to unstressed birds; however, the differences were not statistically significant. According to Feddes *et al.* (2002) increasing the stocking density lowered body weight and carcass quality, though no effect was observed in mortality, which is in agreement with the present results. There was a numerical decrease in weight of giblet and spleen and a significant ($P<0.05$) decrease of bursal weight in stressed birds compared to unstressed birds. The reports of Gill and Sharma (1992) and Puvadolpirod and Toxan (2000) indicated that stocking stress and elevated adrenal corticoids could induce decline in weight of giblet and spleen, respectively, in chicken.

Table 3. Effect of stress on weight of carcass and organs parameters (mean±SE, n=10) in broilers.

Parameter	G1	G2
Slaughter weight (g)	2100.0 ± 72.65^{a}	1740.0 ± 59.07^{a}
Carcass weight (g)	1255.0 ± 38.33^{a}	1107.5 ± 37.65^{a}
Giblet (g %)	7.0 ± 0.04^{a}	6.3 ± 0.34^{a}
Bursa (g %)	0.2 ± 0.03^{a}	0.1 ± 0.01^{b}
Spleen (g %)	0.2 ± 0.01^{a}	0.2 ± 0.02^{a}
Adrenals (g %)	0.01 ± 0.01^{a}	0.03 ± 0.01^{b}

G1: Unstressed birds; G2: Stressed birds
Means with different superscripts in the same row are different ($P<0.05$).

The adrenals showed a significant ($P<0.05$) increase in weight in stressed birds compared to unstressed birds, which was similar to the findings of Nayanathara *et al.* (2009), where chronic stress caused increase in the weight of adrenal glands of rats. This is due to the physiological response of stress for increased corticosteroid hormone synthesis leading to hypertrophy of the gland. There was a significant ($P<0.05$) reduction in bursal weight in stressed birds compared to unstressed birds. Puvadolpirod and Toxan (2000) observed a reduction in the weight of bursa and spleen in chicken subjected to high corticosteroids medication. Both these organs have a positive role in the immune mechanism of birds and the reduction in weight would adversely affect the protection of the birds against foreign organisms. This was clearly evidenced by the significant ($P<0.05$) fall in the TLC count in overcrowded stressed birds.

The mean difference of the body weight indicated that there was an approximate body weight gain of 668 g and 526.5 g in the unstressed and stressed birds, respectively. The cost of poultry meat was INR 70/kg; hence, the revenue generated due to this weight gain was INR 46.76 and INR 36.86 respectively, indicating a loss of INR 9.9/bird due to overcrowding stress. The feed cost was INR 18/kg; hence, the cost of feed for 1.18 kg consumed by the unstressed bird was INR 21.24 and that consumed (1.11kg) by stressed bird was INR 19.98, respectively, indicating a gain of INR 1.26/kg feed due to overcrowding stress. It could be observed that overall revenue is a loss of INR 8.84/ bird (INR 9.90 - INR 1.26), establishing that overcrowding causes net loss to the broiler farm.

This study showed that stress due to overcrowding would result in economic loss to the farmers and adversely affect certain hematological parameters, digestive enzyme, organs and immune status of broiler chicken. Thus, broiler chicken should be provided with adequate floor space to get the best performance and to mitigate stress-induced pathology and economic loss

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