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Influence of Stocking Density on Immune Response of Broilers Against Newcastle Disease Virus

Muhammad Yasser Mustafa, Muhammad Akram Muneer, Aftab Ahmad Anjum and Mansoor-ud-Din-Ahamd
Department of Microbiology, University of Veterinary and Animal Sciences, Lahore-Pakistan

Abstract

Influence of various levels of stocking densities (06 square inch/chick = group A; 09 square inch/chick = group B; 12 square inch/chick = groups C, D (non-vaccinated) and 13 sq. inch/bird = group E) on white blood cell counts of broiler chicks was non-significant ($P \geq 0.05$). At 36th day of age titer of antibodies against NewCastle disease virus (NDV) measured by haemagglutination inhibition (HI) test was significantly ($P \leq 0.05$) lower in group A (GMT 67) than groups B (GMT 96) and C (GMT 102). The chicks in group D (GMT 07) showed negligible HI titer whereas in group E (GMT 185) titer was significantly ($P \leq 0.05$) higher. There were non-significant ($P \geq 0.05$) differences in wattle thickness (cm) in groups A (1.44±0.07) and C (1.43±0.10) while group E (1.64±0.31) showed significant ($P \leq 0.05$) increase in wattle thickness. At 56th day of age (06 days post challenge) HI antibody titers in all groups raised gradually except in group D. The post-NDV-challenge GM HI titers recorded in groups A, B, C and E at the age of 56 days were 86, 121, 132 and 210, respectively. Mortality recorded in non-vaccinated group (D) was 100% by virulent ND virus challenge showing clinical signs of Newcastle disease. Post challenge mortality in groups A, B, C and D were recorded as 25/40, 11/40, 07/40 and 40/40 chicks, indicating that provision of less space caused an increase in the mortality rate.

Key Words: Stocking density, GMT, NDV, humoral response, cellular response and mortality

Introduction

In Pakistan commercial broiler farming was initiated in early 1960's with the import of Shaver broiler chicks (Qureshi, 1993). Ever since, poultry industry is progressing and growing at a rate of about 10 percent annually. Poultry industry is regarded as a vital source of eggs and meat for common man. Factors like stress, stocking density, abnormal behavior, poor feeding, toxins in feed, poor ventilation, high ammonia level, frequent rough handling, dehydration and starvation interfere in the optimal growth and performance of chickens (Saxena, 1997).

Corresponding Author: Aftab Ahmad Anjum
Department of Microbiology,
University of Veterinary and Animal Sciences,
Lahore-Pakistan
Email: drraza70@yahoo.com

Poultry farming when practiced as semi-commercial or commercial enterprise in the rural or urban areas is influenced by stress factors like vaccination, irregular lighting, extreme weather and occurrence of many infectious diseases (Jaffery, 1989).

The presence of any stress factor may lead to the induction of immunosuppression which is not so clearly defined in the domestic chicken and it requires many experimental observations that link various defined stress causing agents with the altered physiological responses that affect the specific immune responses and ultimately lead to increased susceptibility of the stressed bird (s) to various infectious disease agents (Dohms and Mertz, 1991).

The stressed birds have limited body resources, response to environmental changes and defensive immune response mechanisms they are confronted with various stress conditions. There is redistribution of body resources including energy and cost of decreased growth and health (Brake, 1987). Upon exposure to the long term stress conditions, the exposed birds become fatigue and this condition of bird may lead to its starvation and being infected with various disease agents (Dohms, 1990).

Keeping in view poultry farming conditions in Pakistan, influence of stocking density on immune response of broilers against Newcastle disease virus was assessed.

Materials and Methods

Work plan

A total of 400 day-old Hubbard X Hubbard broiler chicks procured from M/S Sabir's Poultry Breeders, Lahore were divided in five groups (A, B, C, D and E) having 80 chicks each. The birds of all groups except group D were inoculated with the live virus vaccines of NewCastle disease virus (NDV) and infectious bursal disease virus (IBDV). Birds in group A were reared at a stocking density of 6 sq.

inch/bird; those in group B at 9 sq. inch/bird; in group C and D 12 sq. inch/bird and the birds in group E at stocking density of 13 sq. inch/bird.

Humoral immune response:

Serum samples were collected from various treatment groups at regular interval (07 days) to monitor humoral immune response against NDV vaccine by haemagglutination inhibition (HI) test following the protocol described by Allan and Gough (1976). Geometric mean titers were calculated.

Cellular immune response:

Cellular immune response of birds in all groups was assessed using DTH, a Skin Test (Bachaman and Mashaly 1987) by injecting PHA-P (Red kidney bean, Sigma-Aldrich, Catalog # L8754). Pre-treatment and post-treatment wattle thickness was measured and recorded. White blood cell counts were also recorded.

Challenge protection:

At 50th day of age half birds from each group were administered challenge of virulent NDV having an $\mathbf{LD_{50}}$ of 3.5 x 10^6 per ml (Reed and Muench, 1938). The post virulent NDV challenge morbidity and mortality were recorded in each group to determine the protection level of NDV vaccines kept under a stressed condition. Data collected was analyzed statistically (Steel and Torrie,1980).

Results and Discussion

Stocking density has critical implications on the broiler production. It is believed that higher returns can be obtained with the less number of birds per unit area. Assigned densities have been primarily driven by cost-benefit analysis, but economic profit may reduce at the cost of lower bird performance, health and welfare if densities are excessive. Increasing the number of birds per unit area has been found to depress body weight and feed intake and have adverse effect on the bird health in order to report the most efficient stocking density in open shed system.

The present investigation was undertaken to determine the effects of various stocking densities on the immune function and mortality pattern of broiler chicks.

Humoral immune response

The effect of stocking density on the development of haemagglutination inhibition (HI) antibody in chicks in various experimental groups against Newcastle Disease Virus (NDV) vaccination is presented in table 1. The NDV geometric mean titers (GMT HI) in treatment groups A, B, C, D and E on day 36 were recorded as 67, 96, 102, 07 and 185, respectively (table-1). These GM titers indicated that the NDV vaccinated chicks exposed to space stress at the 6 sq inch/bird had significantly ($P \leq 0.05$) lower HI GMT than the vaccinated birds reared in a stocking density of 12 and 13 square inch/bird. The NDV unvaccinated chicks in group D had quite negligible HI titer indicating some non-specific exposure to cross reacting antigen having some mimicry with NDV. At the age day 56 (06 days post challenge) the HI antibody titers in all groups showed a rise except in group D. All the chicks in group D died indicating clinical signs of Newcastle disease. The post challenge GM HI titers recorded in groups A, B, C and E at the age of 56 days were 86, 121, 132 and 210, respectively (table 1-a).

From the above findings, it can be concluded that GM titer showed significant ($P \leq 0.05$) differences among groups against NDV. The chicks in group D (GMT 07) showed significantly ($P \leq 0.05$) lower GM titer while group E (GMT 185) showed significantly ($P \leq 0.05$) higher GM titer than other groups.

It is well documented that the nervous and immune systems are integrated in their response to stress (Besedovsky *et al*., 1983; Trout and Mashaly, 1994). Although birds at higher densities appear to be stressed, no comprehensive evaluation of the immune status of the density stressed birds has been reported despite the recognized fact that the stress can have a negative impact on the immune system of the chicken. Very few studies under the field conditions on the effect of rearing density on the immune mechanism of birds are reported (Cravener *et al*., 1992).

Cellular immune response

The cellular immune responses of the chickens in groups A, B, C and D recorded on day 56 of the experiment are presented in table 2. The post PHA injection wattle thickness in the groups A, B, C, D and E were recorded as 1.44±0.07**,** 1.10±0.20, 1.43±0.10, 1.07±0.30 and 1.64±0.31, respectively.

From the above findings, it can be concluded that cellular immune response showed significant ($P \leq 0.05$) differences among groups but no differences in groups A (1.44 ± 0.07) and C (1.43 ± 0.10). The chicks in group D (1.07 ± 0.30) showed significantly ($P \leq 0.05$) lower wattle thickness while group E (1.64±0.31) showed significantly ($P \leq 0.05$) higher wattle thickness than other groups.

There were no differences among the wattle thickness of chicks in the group A (6 sq inch/bird) **a**nd those in group C (12 sq inch/bird). Although birds at higher densities appeared to be stressed (Cravener *et al*., 1992), no comprehensive evaluation of the immune status of the density stressed birds has been reported despite the recognized fact that the stress can negatively impact the immune system, there have been very few controlled studies under field conditions to indicate the effect of rearing density on the immune status of birds with industry standard genetics.

Challenge protection

As per experimental design, the 50% of chicks in each treatment group were challenged with the virulent NDV to observe the effect of stocking density on the overall mortality. This study indicated occurrence of mortality in the NDV vaccinated and unvaccinated chicks. Since the chicks in group D were not vaccinated all the NDV challenged chicks (n=40) died showing signs of respiratory distress, in-coordination of legs while walking, torticollis, dropped wings and leg paralysis etc. However, the overall mortality in the NDV vaccinated group A chicks (provided stocking density of 6 square inch/chick) was the highest than the mortality of chicks in groups B (9 sq inch/bird) and C (12 sq inch/bird). The post challenge mortality in groups A, B, C and D were recorded as 25, 11, 07 and 40 chicks, indicating that provision of less space caused an increase in the mortality of chicks. The overall mortality recorded in chicks in groups A, B, C, D and E was 58, 30, 17, 48 and 8 chicks, respectively. The chicks reared at a stocking density of 12 square inch/chick suffered the lowest post challenge mortality.

From the above findings, it can be concluded that the mortality pattern in challenge with virulent strain of NDV showed significant ($P \leq 0.05$) differences amongst groups. The overall mortality in group A (6 square inch/chick) (58) showed significantly increased mortality than group B (9 sq. inch/bird) (30) and C (12 sq. inch/bird) (17). In challenge with virulent NDV virus group C (12 sq. inch/bird) (07) showed significantly ($P \leq 0.05$) lower and group D (12 sq inch/bird) (40) showed significantly ($P \leq 0.05$) higher mortality than other groups.

High rearing densities in broilers are associated with an increased incidence of leg problems (Sorensen et al., 2000; Saotra *et al*., 2001), which may be related to the reduced level of activity observed with increasing housing densities (Estevez, *et al*., 1997). Shanawany (1988) observed that high stocking density did not significantly affect the mortality in broiler chicks. Bhat *et al*., (2006) did not observe any effects of bird density (15 to 20 birds per deck) on mortality of broiler chicks. It has been suggested that providing broilers access to perches, under high housing density conditions, may be an effective mean of increasing bird activity, therefore potentially reducing the incidence of leg problems, while at the same time increasing air flow among the birds (Le Van Fiscus *et al*., 2000; Pettit-Riley and Estevez, 2001).

Table 1(a). Effect of Stocking Density on the Geometric Mean NDV HI Titer of Broiler Chicks in Various Treatment Groups[g] at day 36

Groups[g]	Geometric Mean HI Titer
Group-A	67^{e}
Group-B	96^{d}
Group-C	102^{c}
Group-D	07^{a}
Group-E	185^{b}

Groups[g] = A, stocking density of 6 square inch/bird; B, stocking density of 9 square inch/bird; C, stocking density of 12 square inch/bird; D. 12 square inch/bird and group E. stocking density 13 sq. inch/bird.

[a, b, c, d, e] Any two means carrying the same superscript are not significantly different from each other at 5% probability level using LSD test.

Table 1(b). Effect of NDV challenge on HI antibody in chickens in Various Treatment groups[g] at day 56

Groups[g]	Geometric Mean HI Titer
Group-A	86^{d}
Group-B	121^{c}
Group-C	132^{b}
Group-D	All chicks died
Group-E	210^{a}

Groups[g] = A, stocking density of 6 square inch/bird; B, stocking density of 9 square inch/bird; C, stocking density of 12 square inch/bird; D. 12 square inch/bird and group E. stocking density 13 sq. inch/bird.

[a, b, c, d] Any two means carrying the same superscript are not significantly different from each other at 5% probability level using LSD test.

Table 2. Effect of Stocking Density on the Cellular Immune Response of Broiler Chicks in Various Groups

	Wattle thickness(cm) Pre PHA-P Injection	Wattle thickness(cm) Post PHA-P Injection	Difference in Wattle thickness(cm)
	Mean ± S.E	Mean ± S.E	Mean ± S.E
Group-A	1.09±0.03	1.44±0.07	0.35±0.06[b]
Group-B	0.81±0.18	1.10±0.20	0.29±0.09[c]
Group-C	1.11±0.03	1.43±0.10	0.33±0.11[b]
Group-D*	1.05±0.25	1.07±0.30	0.02±0.05[d]
Group-E	1.05±0.14	1.64±0.31	0.59±0.21[a]

Groups[g =] A, stocking density of 6 square inch/bird; B, stocking density of 9 square inch/bird; C, stocking density of 12 square inch/bird; D. 12 square inch/bird and group E. stocking density 13 sq. inch/bird.
*Chicks in group D were not injected PHA-P, instead inoculated with sterile normal saline only.
[a, b, c, d] Any two means carrying the same superscript are not significantly different from each other at 5% probability level using LSD test.

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