Pathology of Oviduct in Sub Optimally Producing Commercial Layer Chicken

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Abstract: Pathology of oviduct in sub optimally producing commercial layer chicken were investigated in five flocks. Suboptimal production was commonly noticed between 23rd to 45th wk of age. The production drop ranged from 5 to 12 per cent below the expected egg production. The morbidity and mortality rates varied from 5 to 11 and 0.2 to 2 percent, respectively. The antibody HI titres for NDV (128-445.7) IB (45.3-128) and EDS -76 (2 to 4) were within the normal range. ELISA titre for MG and MS was below 269. The heterophil : lymphocyte (H:L) ratio was above the normal level in all the five flocks and it was more elevated in younger (0.97) compared to older (0.63) flocks. Bacteriological and virological examination revealed no organisms of any etiological significance. Grossly, regression, deformation and loss of hierarchy in ovarian follicles were noticed. The left oviduct was, short, narrow and thread like structure in few birds and in the remaining cases partial atrophy with demarcation were noticed. On opening, the mucosal surface of the affected oviduct lost its glistening appearance and the folds were markedly reduced in height and width. Histopathologically, deciliation of surface epithelium, degeneration and involution of the glandular tissues with well developed connective tissue matrix and mild to moderate mononuclear cells infiltration in all the parts of oviduct were noticed. Absence of etiological agents and elevation of H:L ratio suggest that the atrophy of oviduct in sub optimally producing flocks may be due to non specific stress experienced by the commercial layer chickens.

Key words: Oviduct, pathology, Suboptimal production, layer chicken

INTRODUCTION

The avian oviduct is a tubular organ responsible for the secretion of the components surrounding the yolk and transport of the egg. Fully functional and healthy oviduct is essential for both quantity and quality of egg, offspring fitness, maternal transfer of immunoglobulins from mother to offspring (Saino et al., 2002). Any disorder that affects the reproductive system will have a great bearing on production potential and incur a heavy loss. Namakkal is the most thickly populated poultry zone in India and occupying second place in egg production at national level and it has a layer population of approximately 45 million which produce 35 million eggs per day. Even though the flocks were free from infections some of the flocks have not attained expected production potential.

Stressors exert their effects directly or indirectly on the general well-being of the domestic animals including poultry species (Debasilo et al., 2002). Birds have limited body resources for growth, reproduction, response to environmental changes and defense mechanism (Rosales, 1994). Under the stress conditions, there is redistribution of body resources including energy and protein at the cost of decreased growth, reproduction and health (Beck, 1991). Physiological response to stress includes the rapid secretion of catecholamines from the adrenal medulla and glucocorticoids from the adrenal cortex and their levels have often been used to assess the level of stress in birds (Harvey et al., 1984). The half life of these hormones is short (minutes to hours). Another index to stress is the ratio of heterophil and lymphocyte (H:L) in blood (Gross and Siegel, 1983; Maxwell, 1993). Leukocyte numbers change more slowly (30 min to 20 hr) in response to stress than does corticosterone (Cunmick et al., 1994). These changes are less variable and longer lasting than the corticosterone response and multiple stressors usually have an additive effect (McFarlame and Curtis, 1989). The H:L ratio may be more persistent indicator of stress associated with reproductive cycle compare to corticosterone (Carol et al., 2000).

Abnormalities of the female genital organs of poultry have been reported (Batra and Singh, 1978) however, the information available in the literature regarding the production performance and H:L ratio in commercial layer chicken experiencing non specific stress and effect on reproductive tract is scanty. Hence the present paper describes the prevalence of suboptimal production in commercial layer chicken, profile of heterophil to lymphocyte ratio as a indices of stress and pathology of reproductive tract.

MATERIALS AND METHODS

Data collection: The study was carried out over a period of three years (2005 to 2008). A total of 85 commercial...
layer flocks, above 20 weeks of age belonging to white leghorn breed in and around Namakkal district, Tamil Nadu, India were investigated. All the flocks were maintained in cage system of management and vaccinated against Marek’s disease, Newcastle disease, Infectious bronchitis, Infectious bursal disease, fowl pox and Infectious coryza according to a standard vaccination schedule. Flocks were inspected and the records were verified for production performance.

**Serum antibody measurement:** Ten serum samples were collected from each flock and analysed for the antibody titer against Newcastle disease (Alexander, 1988), Infectious bronchitis (Alexander and Chettle, 1977) and Egg drop syndrome-76 virus (Shakya and Dhawedkar, 1991) by HI test. A commercial indirect enzyme linked immunosorbent assay (Hester Pharmaceuticals Limited, India) test kit was used to detect specific antibodies against *Mycoplasma galisepticum* and *Mycoplasma synoviae*. The titer value of 0-269, 270-743 and 744 and above were interpreted as negative, suspicious and positive, respectively.

**Blood sampling:** Blood smears were made from a small puncture in the comb of ten birds from each flock. One drops of blood was taken, smeared on glass slides. The smears were stained with Giemsa stain and differential count on white blood cells such as heterophils (H), lymphocytes (L), monocytes (M), basophils (B) and eosinophils (E). The H:L ratio was determined according to the procedure described by Gross and Siegel (1983).

**Bacterial and viral agents screening:** Heart blood and oviduct swabs were collected from dead birds and screened for bacterial agents. The samples were placed in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours and cultured aerobically in Blood agar for isolation of bacteria. Bacterial isolates were identified on the basis of sugar fermentation and biochemical characteristics. Oviduct, spleen, caecal tonsil, kidney and trachea were collected from atrophied oviduct cases and subjected to Haemagglutination (HA) test for detection of Newcastle Disease Virus (NDV), Infectious Bronchitis Virus (IBV) and EDS -76 virus.

**Pathology:** A detailed post mortem examination was carried out on dead birds of flocks having sub optimal production performance and gross lesions were recorded. Oviducts from the five flocks were removed and a slit along the dorsal longitudinal aspect was made to examine the mucosal surface. Materials for histopathology were collected from different parts of oviduct and fixed in 10% neutral buffered formalin. After fixation, samples were processed by following the routine histological procedures, embedded in paraffin, sectioned at 5 μm thickness and stained with hematoxylin and eosin for histopathological examination.

**RESULTS AND DISCUSSION**

**Flock performance:** Out of 85 layer flocks investigated, five showed suboptimal production with atrophied oviducts. The production drop ranged from 5 to 12 percent below the expected level of egg production in the respective age group (Table 2). The morbidity and mortality varied from 5 to 11 and 0.2 to 2 per cent respectively. Sharma and Singh (1968) also reported underdeveloped ovary and oviduct (30.9 per cent) as major factor for non- laying in 6 to 12 months old hens. On flock examination, sub optimally producing birds showed pale and shriveled combs, yellowish discoloration of beak and shank areas and one to two finger distances between the pelvic bones. In commercial layers, the peak production occurs between the age of 25th to 50th wk, during this period the birds are subjected to high production stress. In the present study suboptimal production was commonly noticed between 23rd to 45th wk age, that might be due to production stress experienced by some of the birds in the flock. Similar findings were also reported by Odihambo Mumma et al. (2006) in birds with increased level of plasma corticosteroid, causing atresia of ovarian follicles and reduction in egg production.

**Serum antibody titre:** The antibody titre for NDV, IBV, EDS-76, MG and MS were shown in Table 1. The antibody titre for NDV in the sub optimally producing layer chicken was ranged from 128 to 445.7. Raghul (2003) observed that, a HI titre of 128 and above was sufficient to protect the oviduct from NDV induced direct damage. In Namakkal area vaccination against Newcastle disease using mesogenic and killed vaccines was performed at 16-18 wk of age, followed by revaccination regularly at every three months intervals after 40th wk. Hence the antibody titre found in this study i.e., from 128 to 445.7 was within the normal range due to vaccination.

The IBV antibody titre in the present study ranged from 45.3 to 128. IB in Namakkal area was controlled primarily by using attenuated live virus vaccine (H120 and Ma41 strain) as well as inactivated oil emulsion vaccine. The minimum and maximum age of layer flocks affected in this investigation was 23rd and 45th wk. In Namakkal areas vaccination against IBV was performed on 7th day, 5th and 14th wk. IBV antibodies can be detected as soon as four days pi, reaches a peak at about 21 days and remain in high titre in the serum for many weeks. If the flock has more IBV antibody titre it was better protected against the oviduct damage (Mockett and Darbyshire, 1981).
The HI titre against EDS -76 is 2 to 4. The HI titre of 8 and below should be considered as negative due to the presence of non specific HI antibodies to haemagglutinating adenoviruses (Calnek, 1978). All the blood serum samples tested for MG and MS was found to be a titer value of less than 269, hence all the serum samples were negative for MG and MS. The possible reason is adaptation of good farm management and biosecurity measures and regular use of anti mycoplasmal agents in feed at regular intervals leading to the low chance of MG and MS infection (Kleven, 1996). Trawinski et al. (2003) also observed low percentage of positivity for MG (5.0%) and MS (4.3%) in serological monitoring cobb line reproductive hens with ELISA and stated the use of Tilmicosin completely eliminate the serum positive titers for MG and partially eliminate it for MS as measured by ELISA titres but do not completely cure the birds from the infections.

Heterophil: Lymphocyte ratio: Grey et al. (1969) and Maxwell (1983) reported that heterophil : lymphocyte ratio was a less variable indicator of stress in birds and more reliable than corticosteroid level in plasma. Hence in the present study the H:L ratios were used to measure the level of stressful condition in the flock. The average number of heterophils and lymphocytes in adult white leghorn was approximately 33 and 54 respectively per 100 cells which gives an H:L ratio of 0.61 (Sastry, 1997). In the present study H:L ratio was above the normal level in all the five flocks (Table 2) and it was more elevated in younger (0.97) compared to older (0.83) flocks. In young birds initiation and peak egg production periods produce considerable physiological and hormonal stress, where as the older birds adapted to stress conditions. Heterophils are the primary phagocytic leukocyte and proliferate in circulation in response to infections, inflammation and stress (Harmon, 1998) while lymphocytes are involved in a variety of immunological functions such as immunoglobulin production and modulation of immune defence (Campbell, 1996). Glucocorticoids causes an influx of heterophils into the blood from bone marrow and attenuate the egress of heterophiles from the blood to other compartments (Bishup et al., 1968). In contrast, in response to glucocorticoids, circulating lymphocytes adhere to the endothelial cells of blood vessels and subsequently undergo transmigration from circulation into other tissues, for example lymphnodes, spleen, bone marrow and skin, where they are sequestered and caused significant reduction (Fauci, 1975; Dhabhar et al., 1996). Alteration in the migration pattern of heterophils and lymphocytes due to stress induced corticosterone may be the cause for the elevation of H:L ratio in the present study.

Etiological identification: Bacteriological examination of heart blood and oviduct swabs collected from the sub optimally producing layers revealed no organisms of any etiological significance. Tissue samples collected for virological examination also found to be negative in haemagglutination and haemagglutination inhibition test against NDV and IBV. The absence of antibodies against EDS-76 and Mycoplasma organisms suggest that the atrophy of oviduct in sub optimally producing flocks might be due to non specific stress experienced by the commercial layer chickens. This finding was in agreement with Pradhan (1979), who also reported that the atrophied ovaries and oviducts was due to nonspecific causes.

Gross and histopathology: On necropsy examination, body condition of the affected birds varied from fair to poor. Visceral organs were reduced in size. Ovarian follicles were regressed, deformed and the hierarchy was lost. The left oviduct was, short, narrow and appeared as a thread like structure without any

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Table 1: Antibody titre against NDV, IBV, EDS-76, MG and MS in sub optimally producing commercial layer chicken

<table>
<thead>
<tr>
<th>Age (Weeks)</th>
<th>ND HI titre (GM value)</th>
<th>IB HI Titre (GM value)</th>
<th>EDS 76 HI Titre</th>
<th>MG Titre</th>
<th>MS Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>415.7</td>
<td>128</td>
<td>4</td>
<td>2&lt;269</td>
<td>2&lt;269</td>
</tr>
<tr>
<td>28</td>
<td>194.0</td>
<td>78.8</td>
<td>2</td>
<td>&lt;269</td>
<td>&lt;269</td>
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<tr>
<td>34</td>
<td>147.0</td>
<td>64.0</td>
<td>0</td>
<td>&lt;269</td>
<td>&lt;269</td>
</tr>
<tr>
<td>37</td>
<td>128.0</td>
<td>48.5</td>
<td>0</td>
<td>&lt;269</td>
<td>&lt;269</td>
</tr>
<tr>
<td>45</td>
<td>128.0</td>
<td>45.3</td>
<td>2</td>
<td>&lt;269</td>
<td>&lt;269</td>
</tr>
</tbody>
</table>

Table 2: Flock details and H:L ratio in sub optimally producing commercial layer chicken

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Flock size</th>
<th>Production (%)</th>
<th>Mortality</th>
<th>Mortality</th>
<th>H (%)</th>
<th>L (%)</th>
<th>H:L ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>20000</td>
<td>Actual</td>
<td>Expected</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>0.97</td>
</tr>
<tr>
<td>28</td>
<td>25000</td>
<td>90</td>
<td>97</td>
<td>0</td>
<td>1.0</td>
<td>40.1</td>
<td>0.91</td>
</tr>
<tr>
<td>34</td>
<td>15000</td>
<td>91</td>
<td>98</td>
<td>8</td>
<td>0.75</td>
<td>37.6</td>
<td>0.79</td>
</tr>
<tr>
<td>37</td>
<td>10000</td>
<td>90</td>
<td>98</td>
<td>6</td>
<td>0.50</td>
<td>38.3</td>
<td>0.76</td>
</tr>
<tr>
<td>45</td>
<td>10000</td>
<td>89</td>
<td>94</td>
<td>5</td>
<td>0.20</td>
<td>33.8</td>
<td>0.63</td>
</tr>
</tbody>
</table>
demarcation between the different parts of oviduct in few birds and the remaining cases partial atrophy with demarcation were noticed. On opening, the mucosal surface of the affected oviduct lost its glistening appearance and the folds were markedly reduced its height and width. The results of the present study are in agreement with the findings of Vaisala and Sivadas (1970).

Histopathological lesions observed in the oviduct of sub optimally producing commercial layers were deciliation of surface epithelium, degeneration and involution of the glandular tissues with well developed connective tissue matrix and mild to moderate mononuclear cells infiltration in all the parts of oviduct. Eroschenko and Wilson (1974) stated that the degenerative cellular changes observed in the atrophied oviduct was due to insufficient sex steroid secretion and decreased stimulation of oviduct. The first significant change observed in chicken with stress was the increase in corticosteroid level due to activation of hypothalamic-pituitary-adrenal axis to mobilize body energy stores. Increased corticosterone levels are associated with inhibition of the hypothalamic-pituitary-gonadal axis (Williams et al., 1985) leads to decline in Leutinising and Follicular stimulating hormone level in the peripheral circulation. These hormones are required for the development of follicies, ovulation and secretion of sex steroids especially progesterone and estrogens. The growth differentiation and secretory functions of the oviduct are primarily regulated by sex steroids (Palmiter and Wrenn, 1971), hence the atrophy of the oviduct follows the loss of ovarian steroid support.

Conclusion: A study on the sub optimally producing commercial layer chicken showed an elevation of H:L ratio of blood, absence of etiological agents of any infection and atrophy of oviduct which might be due to nonspecific stress experienced by these birds. It affects the production efficiency of modern commercial layer and cause heavy economic loss to the poultry industry. It is important to have an appropriate management programme to overcome this stress related production problem in modern commercial layer to avoid follicular atresia and atrophy of oviduct since it is also one of the cause responsible for the drop in egg production.

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