ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 2 (5): 357-360, 2003

Physiological Responses of Laying Hens to the Alternative Housing Systems

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Abstract: Measurements of differential leucocyte count, H/L (Heterophil to Lymphocyte) ratio and Ab (Antibody) titres to commercial used vaccines, ND (Newcastle Disease) and IB (Infectious Bronchitis) were employed to investigate whether the exposure of laying hens to different housing systems was associated with haematological-immunological changes. Layers were kept in three different housing systems: conventional battery cages, modified cages and an intensive free-range housing system. Differential leucocyte count and H/L ratio were used as indicators of stress response and sensitive biomarkers crucial to immune function, whereas the Ab levels to IB and ND vaccines were measured to assess humoral-mediated immunity. This study indicated that in hens exposed to the three various housing conditions H/L ratio was found to be significantly different, 0.58, 0.43 and 0.38, respectively. The results show that in hens kept in battery cages heterophils were raised, while lymphocytes decreased. Although, differences in H/L ratio suggest that hens of different housing systems should have a reduced antibody response and several investigators have recommended that environmental stressors decrease Ab production this was not demonstrated in this survey. Ab titre presented as $\log_{10}$ was unaffected by any housing system. A slight negative correlation between H/L ratios and antibody levels was also observed. The results would suggest that housing conditions and social stress might have a great effect on the stress response (H/L ratio) while humoral response seems unaltered.

Key words: H/L ratio, antibody production, layers, housing system

Introduction

The purpose of a poultry house is to confine birds and protect them from environmental extremes, which might increase mortality or reduce growth, immunocompetence and fertility or egg production. In the past few years the keeping of laying hens in cages has been criticized around the world. However, in most developed countries about 90% of hens are kept in cages (Tauson, 1998). The cage system for laying hens offers several behavioural and economic advantages, but there is evidence of welfare problems (Nicol, 1987; Appleby, 1993, 1998; Baxter, 1994). According to a new EU-directive in 1999 and recent developments in poultry well-being mainly in North Western Europe, North America and Australia, immediate welfare improvements could be made in laying hen managements by changing housing systems and stocking densities. For that reason, a new layer-house was built in 1998 at a research and training farm in Hannover, where 5200 layers were kept each year in three different housing systems: 1500 hens in conventional battery-cages for layers, 1500 hens in modified cages and 2200 hens in intensive free range housing system.

In birds as in other vertebrates, the physiological responses to the environment can be specific and non-specific leading to a state of general stress. There is evidence that environmental stressors, in general, reduce immune responses and cause immunomodulation initiated by the hypothalamus-pituitary-adrenal cortical pathways (Dohms and Metz, 1991). Stress may lower immunity and cause chain reactions that decrease immune antibody responses (Siegel, 1985). The same immune endpoint should be applied to decisions regarding all areas of poultry environmental management, housing, lighting, space, temperature, diet, vaccinations, feed additives and therapeutics (Cheville, 1979; Dohms and Saif, 1983; Gross, 1985; McFarlane and Curtis, 1989; Maxwell *et al*., 1992).

Regarding to the measurement of stress-induced immune alteration, appropriate haematological and immunological tests have been recommended. H/L ratio has been used as a sensitive haematological indicator of stress response among chicken populations (Gross and Siegel, 1983) and as a general biomarker relevant to immune function (Dietert *et al*., 1996). Otherwise, Dohms and Saif (1983) suggested the criteria that might be considered to determine changes to the im munological responses and proposed that Ab

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responses to commonly used vaccines should be evaluated. Likewise, it was documented that poultry in stressful environments have less antibody activity against a variety of particulate antigens, including vaccinations (Siegel, 1985).

There is, however, little known about the evaluation of haematological and humoral changes of laying hens in various housing systems and few experiments have attempted to correlate stress-induced changes in circulating leucocytes with those in antibody titres.

The present study, therefore, was designed to assess the effect of various housing systems on stress and humoral response, in order to be able to observe any relationship between housing systems and physiological response of laying hens. Ab responses to IB and ND vaccines were measured to assess humoral-mediated immunity, while the differential leucocyte count and H/L ratio were used as sensitive indicators of stress responses relevant to immune function. The relationship between H/L ratios and Ab titre levels was also observed.

Materials and Methods

Laying hens (Brown layers) kept in three different housing systems: 1500 hens in battery-cages with 4 hens per cage with a density 690 cm^2 per bird; 1500 hens kept in modified cages, in small compartments with perch's, a nest box and a scratch tray with 10 hens per compartment with a density 750 cm^2 per bird and over 600 cm^2 useable floor space; as well as 2200 hens housed in intensive free range combined with a floor system and a density of 9 birds per one m^2 were used in this study. The egg laying period started in April 2000 when the hens were 15 wk of age and ended in March 2001. Production and welfare characteristics for all groups were recorded for 47 weeks. In each housing system layers were kept in the same ground area and had identical feeding. Water was automatically available *ad libitum*. The maintenance and care management was the same for all animals and the same personnel were responsible for all keeping systems. Commercially available ND and IB vaccines were administered via drinking water prior to the laying period and a booster was given 3 weeks before blood samples were taken.

Ninety laying hens, thirty for each housing system, teen for each replicate were chosen at random for blood collection. Animals were up to 35 wk of age at the time of measurement.

For blood samples hens were carried to a separate room and blood was collected immediately via the wing vein. Non-heparinized blood was collected for measuring ND and IB Ab titres and blood smears were prepared to count leucocytes and H/L ratios. Serum was separated by centrifugation and stored at -20 ^{0}C until analysis. To obtain a blood leucocytes profile and H/L ratio the stained-slide-method was used (Dumonceaux and Harrison, 1994; Campbell, 1998). Three blood smears for each hen were prepared and fixed with methanol. Then, smears were stained immediately with Wright's stain 100% and rinsed with distilled water. They were allowed to air dry. 100 cells per slide were counted and classified using oil immersion microscopy at 100X. Differential leucocytes count: heterophils-, lymphocytes-, eosinophils-, monocytes-, basophils-percentages of total leucocytes; and the H/L ratios were calculated for each hen. Commercially available ELISA (Enzyme-linked Immunoabsorbent assay) test kits were used to evaluate Ab responses to ND and IB vaccines from a single serum sample.

Data on heterophils, lymphocytes, eosinophils, monocytes, basophils, H/L ratios and Ab titres were subjected to a one-way ANOVA (SAS Institute, Inc., 1996). Data for antibody titres were logarithmically transformed prior to analyses to achieve homogeneity of variance and were presented as $\log_{10}$ antibody titre. Significance was set at $P<0.05$ and when found, multiple means were compared by Duncan's multiple range test. Relationships between three variables (H/L ratio and antibody titre values for ND and IB vaccines) were ascertained by means of Pearson's correlation coefficient. This method eliminates the individual variability and assesses the relationships between two sets of variables.

Results

Mean values indicating the effect of the housing system on differential leucocyte count and H/L ratio are summarized in Table 1. In hens kept in conventional cages H/L ratios were significantly higher ($P<0.001$), than in hens housed in modified cages and intensive free-range system (0.58, 0.43 and 0.38 respectively). The same significance was found for heterophils ($P<0.001$) and lymphocytes ($P<0.001$) in hens in the conventional cages (59.08 and 33.99). On the other hand, the count of monocytes differed slightly and basophils count did not differ significantly ($P>0.05$) between the housing systems. The count of eosinophils was higher ($P<0.001$), in hens in conventional cages (1.60) and modified cages (1.31) than in the intensive free-range system (1.16).

The means of Ab production ($\log_{10}$ antibody titre against ND and IB vaccinations) for hens in the three different housing systems are shown in Table 2. The Ab production seemed to be slightly affected by the housing systems. A higher antibody level ($P<0.001$) was observed, against IB vaccine in hens in the free-range system (4.32) than in the two other systems (4.27 and 4.28). However, Ab production against IB and ND vaccines was unaffected by the various keeping systems. Table 3 shows the correlation coefficients between H/L ratios and Ab titre values for ND and IB vaccines. Most of the correlations between Ab production

Table 1: Differential leucocyte counts and H/L ratios[1] of laying hens in three different housing systems

Housing systems	H/L ratios	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Conventional battery cages	0.576±0.006 [a]	33.99.±0.28 [a]	59.08±0.26 [a]	3.620±0.079 [a]	1.596±0.031 [a]	1.710±0.040 [a]
Modified cages	0.429±0.007 [b]	28.06±0.29 [b]	65.43±0.34 [b]	3.393±0.073 [b]	1.310±0.050 [b]	1.756±0.043 [a]
Intensive free-range system	0.381±0.006 [c]	25.77±0.30 [c]	67.71±0.28 [c]	3.583±0.069 [ab]	1.168±0.047 [c]	1.680±0.041 [a]

[1]Values are means (±S.E.M.) n = 30. Means within a column with differing letters (a, b, c) differ significantly (P<0.05)

Table 2: ND and IB mean (±S.E.M.) antibody titres[1] of laying hens in three different housing systems

Housing systems	n	ND Ab titre (log_{10})	IB Ab titre (log_{10})
Conventional battery cages	29	4.148±0.013[ab]	4.269±0.010[b]
Modified cages	29	4.131±0.008[b]	4.278±0.007[b]
Intensive free-range system	28	4.167±0.005[a]	4.321±0.007[a]

[1]Antibody titres were measured by ELISA. Means within a column with differing letters (a, b, c) differ significantly (P<0.05)

Table 3: Correlation between H/L ratios and antibody titres (log_{10}) against ND and IB vaccines

Correlation coefficients	Conventional battery cages	Modified cages	Intensive free-range system
Between H/L ratios and ND	-0.227 (P<0.236)	-0.065 (P<0.736)	0.222 (P<0.247)
Between H/L ratios and IB	0.324 (P<0.086)	0.236 (P<0.217)	0.096 (P<0.625)
Between ND and IB	-0.019 (P<0.920)	0.301 (P<0.103)	-0.178 (P<0.364)

P = Probability

and H/L ratios were negative and/or not significant.

Discussion

According to our results, housing systems had a mild influence on the differential circulating leucocytes and H/L ratio, but no influence on Ab responses in laying hens. An increase in the H/L ratios of hens in conventional cages observed in this study showed that these hens have been exposed to an optimal degree of stress (H/L ratio: 0.58), but this does not indicate that immune response is also altered. The Ab production in response to ND and IB vaccines was not affected and there was no correlation between H/L ratio and the antibody titre to IB and ND vaccines.

Present results demonstrated a relationship between the different housing systems and an increase in the potential non-specific immune reactive cells, such as heterophils. As non-lymphoid cells with phagocytic potential in avian species (Maxwell and Robertson, 1998), heterophils provide a non-specific immunological defense. As a consequence of stressful conditions the stimulation arises from bone marrow interactions with the hypothalamic-pituitary-adrenal cortical axis is responsible for the emergence of the heterophils into circulation. With respect to environmental factors and disease resistance, the H/L ratio quantifies the balance between the nonspecific, fast-acting defenses of heterophils and the antigen specific, slower-acting defenses of lymphocytes.

Several investigators have used H/L ratio as a very sensitive indicator of stress, but uncommonly with no changes in lymphocyte count. However, H/L ratios cannot be relied on in some circumstances to provide an accurate assessment of stress and to illumine a reduction in immunological response. Laying hens might demonstrate heterophilia and lymphopenia as a natural defense mechanism against bacterial infection (Gross and Siegel, 1993; Maxwell and Robertson, 1998). Furthermore, there may be situations in which the leucocyte response in poultry is mild but is manifest by heterophilia and a correspondingly raised H/L ratio. However, many authors accept H/L ratio as a less variable indicator of stress in birds than individual cell numbers, but as being more reliable than corticosteroid levels in plasma (Grey *et al.*, 1989; Maxwell, 1993). With extreme stress basophilia becomes evident in avian species (Maxwell *et al.*, 1992). This was not of significance in our study.

The data of Ab titres to ND and IB vaccines in the present study were in accordance with conditions of layers health for each housing system, but high titres are not necessarily equal to protection (Siegel, 1985). It was concluded that immunological response was not affected by any of the housing systems and the H/L ratios and other immune response measurements (Ab) do not correlate. As said above, most of the correlations between Ab production and H/L ratios were negative and not significant. Even those that were significant were relatively low (P<0.2), indicating the relative independence of the neuroendocrine and immune control of these two components of the immune system. In conclusion, H/L ratio seemed to be appropriate as an indicator of stress response for evaluating hens exposed to multiple stresses in alternative housing systems but there is not in this stage any suppression of Ab production. However, to consider the total immune

competence of a hen it is also necessary the measuring of more than one humoral and/or cellular immune parameter. Additional research is needed to determine the relationship between different housing systems of layers, immune function and increased susceptibility to disease.

Acknowledgements

The financial support of Mrs. Ingeborg Gieselmann is greatly appreciated. The assistance and advice on statistical analyses of Mr. Allan Lisle from University of Queensland, Australia, are also gratefully acknowledged.

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