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Histomorphology of Bursa of Fabricius: Effects of Stock Densities on Commercial Broilers

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■ Keywords

Bursa of Fabricius, stock density, commercial broilers.

ABSTRACT

During the past few years, there has been considerable interest on the effects of stocking density on broiler behavior and immunity. Stress may cause immunodeficiency by affecting cell and humoral responses, as well as body weight decrease, and foot-pad dermatitis. The aim of this study was to study histomorphological changes of the bursa of Fabricius in broilers submitted to three different stocking densities (10, 15, and 20 birds/m^2) from one to 42 days of age. Three birds from each group were sacrifieced on days 7 and 42. The bursa was collected, fixed, and processed for histomorphometric assessment using a Kontrom KS 400 image analyzer. Data were analyzed by Biostat 3.0 (Tukey Test). The results of average cortical area percentage in bursal follicles of 6-week-old birds were 45.12a (10 birds/m^2), 30.43b (15 birds/m^2), and 23.77b (20 birds/m^2). Average body weight was 2.58a kg (10 birds/m^2), 2.56a Kg (15 birds/m^2), and 2.47b Kg (20 birds/m^2), respectively. The percentage of foot-pad dermatitis in 6-week-old birds was 3.33a (10 birds/m^2), 17.76b (15 birds/m^2), and 49.17c (20 birds/m^2). These differences were statistically significant at a $P<0.05$ level. Under these experimental conditions,, it was concluded that the best stocking density to produce broilers is between 10-15 birds per square meter.

INTRODUCTION

Broilers must be raised under excellent environmental conditions. Any deviation may impair performance. Several studies measured the impact of stocking density on broiler performance (Feddes *et al.*, 2002); however, broilers are typically housed in stocking densities varying from 9 to 16 birds/m^2 aiming at reducing fixed costs and maximizing profitability.

Carcass damage is used as indication of poultry welfare. Recent data show that the extent of damage is depends on poultry housing conditions and handling methods (Bruce *et al.*, 1990). When the quality of carcasses is examined at the processing plant, it is possible to perceive differences among flocks raised in different stocking densities. Flocks reared in high stocking densities present cellulitis, foot-pad dermatitis, and hock burns, resulting in an increase of condemnation rates during sanitary inspections (Bilgili & Hess, 1995). In Sweden, the correlation between foot-pad dermatitis and stocking density is used as a parameter of animal welfare (Ekstrand & Carpenter, 1998).

Stress significantly influences the immune system of animals, and consequently may result in an increase in the incidence of diseases. Therefore, immune response in poultry is considered of economic, social, and scientific importance (Dohms & Metz, 1991). Today, it is known that the process of lymphoid tissue depletion is mediated by intensification of the programmed cell death – also known as apoptosis

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– mainly in the bursa of Fabricius, and this is why it has become one of the most important organs for studying this type of cellular death in poultry lymphoid systems (Paramithiotis & Ratcliffe, 1994). Studies on the bursa of Fabricius in poultry submitted to heat and cold stress or thermal comfort conditions concluded that thermal stress affects the development and maturation of the bursa, reducing the amount of parenchyma (Guimarães *et al.*, 2001). Furthermore, thermal stress increases the index of lymphocyte apoptosis in the bursa of Fabricius in growing poultry (Guimarães *et al.*, 2001).

The objective of this experiment was to assess and to compare histomorphological characteristics of the bursa of Fabricius of commercial broilers submitted to different stocking densities, as well as to determine body weight changes, and foot-pad dermatitis rates.

MATERIALS AND METHODS

Eight-hundred and ten Ross 308 male day-old chicks were housed in an environmentally controlled broiler house until 6 weeks of age. The floor was covered with a 10-cm layer of wood shavings. Inside the house, nine 6 m^2 pens housed 10, 15, and 20, birds/m^2, corresponding to 60, 90, and 120 birds per pen, respectively. Pens measured 2 X 3 m. Feed and water were offered *ad libitum* throughout the study. The house was equipped with automatic feeders, nipple drinker, and automatic heating and ventilation systems. Commercial diets, based on corn and soybean meal, were supplied. The starter diet was offered until wk 3, and contained 3,100 kcal ME/kg and 24% crude protein; the grower diet was supplied until wk 5, and contained 3,200 kcal ME/kg and 21% crude protein, finisher diet was provided in last week contained 3,250 kcal ME/kg and 19% crude protein. Light was provided for 20 h/d, using a combination of natural and artificial lighting. Treatments consisted different stocking densities of 10 birds/m^2, 15 birds/m^2, and 20 birds/m^2. Each treatment was replicated in three different pens.

Samples from one bird per pen were randomly taken at the end of week 3 and 6. Birds were sacrificed, and approximately 0.5-mm thick bursa samples were taken, and submitted to histopathological examination.

The sacrificed bird was replaced by another one of the same age, identified by a wing band, in order to maintain stocking density in each treatment. These birds were not used to collect bursal samples.

On the first and sixth week of the experiment, three bursas per treatment were collected, corresponding to the three replicates per treatment. After collection, bursal samples were placed in formaldehyde at 10%, dehydrated in alcohol, cleared in xylene, and imbedded in paraffin. Tissue blocks were then cut in a microtome into 5-mm thick sections, which were stained with hematoxylin-eosine (Luna, 1968). This material was processed in the Laboratory of the Veterinary School of UFM. Images were captured in JVC-Color Video TK1270 high resolution microscopy, and were analyzed using the KS400 software, version 3.0, of a Kontron Elektronic GMBH image analyzer. The cortex percentage of lymphoid follicles was determined using 10 X planapochromatic lens images of 12 complete bursal follicles on three slides, corresponding to three birds per treatment. The follicle section was selected for analysis when the cut passed through the central area of the follicle. Total follicular area was calculated by a line surrounding the selected follicles. Medullar follicular area was determined by a line crossing the basal membrane that divided the two follicular areas. The percentage of follicular cortex was determined as total follicular area minus medullar area in each follicle. This material was analyzed in the Laboratory of Veterinary Pathology of EMBRAPA CNPGC, Campo Grande, MS. Individual body weights and foot-pad dermatitis rates were measured in 42-day-old broilers.

Mean values of percentages of follicular cortical area, body weight, and foot-pad dermatitis were submitted to analysis of variance using the General Linear Model procedure (GLM) of BIOSTAT 3.0 software (Santos *et al.*, 2003). Significant differences among treatments were identified by the test of Tukey test at a level of $P<0.05$.

RESULTS AND DISCUSSION

Body Weight

Body weight results and foot-pad dermatitis percentages in 42-day broilers are illustrated in Figure 1. Average body weights were 2.58 kg, 2.56 kg, and 2.47 kg for stocking densities of 10, 15, and 20 birds per m^2, respectively. These experimental results are consistent with literature data, which show that broiler body weight is inversely proportional to stocking density (Feddes *et al.*, 2002). Significant body weight differences were found between densities of 20 birds/m^2 as compared to 10 and 15 birds/m^2, showing that the density of 20 birds/m^2 had strong impact on final body weight. On the other hand, the treatment 10 birds/m^2 was not statistically different from the treatment of 15 birds/m^2 in terms of body weight.

Foot-Pad Dermatitis

Foot-pad dermatitis was observed in all the birds in the present experiments. Incidences were 3.33%, 17.76%, and 49.17% for stocking densities of 10, 15, and 20 birds/m^2, respectively, and were significantly different among treatments. This parameter is widely used for measuring poultry behavior, and thus other lesions, such as breast and hock burns, may not be required to measure behavior. These problems are partially caused by the same factors related to litter quality, which also result in foot-pad dermatitis (Bruce *et al.*, 1990). High condemnation rates indicate unsuitable environment for rearing poultry. The results of the present experiment clearly demonstrate a proportional relationship between stocking density and foot-pad dermatitis percentage, indicating that the number of birds per square meter interferes with the condition of the litter. Therefore, the frequency of foot-pad dermatitis, as well as other types of dermatitis, depends mainly on stocking density. According to the percentage of foot-pad dermatitis found in the present study, the best stocking density is 10 birds/m^2, as it presented significantly lower incidence of this condition as compared to the other two studied densities.

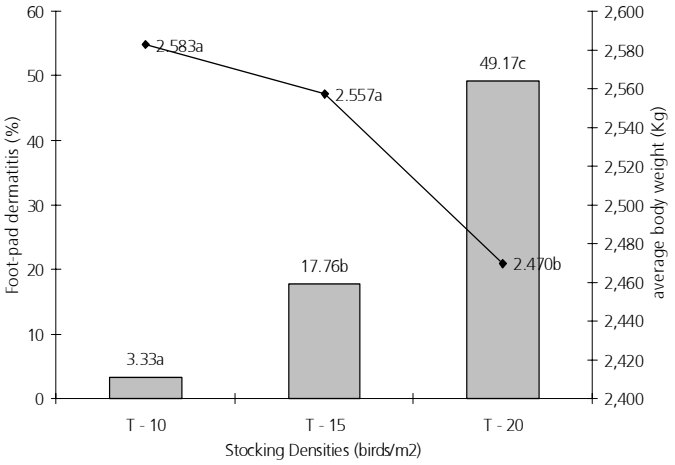

Figure 1 – Effect of stocking density on foot-pad dermatitis rate, and on average body weight of broilers at 42 days of age.

Histomorphology of Bursa of Fabricius

The morphological results of bursa of Fabricius on one- and six-week-old broilers are shown in Figure 2. The histomorphometric processing of bursal samples clearly showed the effects of high stocking densities on cortex percentage of bursal follicles. As expected, during the first week, there were no differences among treatments. At this age, the environmental effect of the different stocking densities was probably not sufficient to induce the retraction of the lymphoid tissue. However, on the sixth week, statistical differences between the treatment of 10 birds/m^2 as compared to 15 or 20 birds/m^2. This result indicates that, at this age, the immune system of broilers housed in low stocking density is able to react faster to infectious challenges. This was also demonstrated in broilers submitted to heat and cold stress, which the percentage of cortical area in bursal follicles was significantly reduced, evidencing the effects of temperature on the bursa (Guimarães *et al.*, 2001). On the other hand, there was no significant difference between stocking densities of 15 and 20 birds/m^2, which indicated that the immune potential of broilers reared under both densities is not different. Therefore, the immune response capability, based on the cortex percentage of bursal follicles, suggests that the best population density for rearing broilers is 10 birds/m^2.

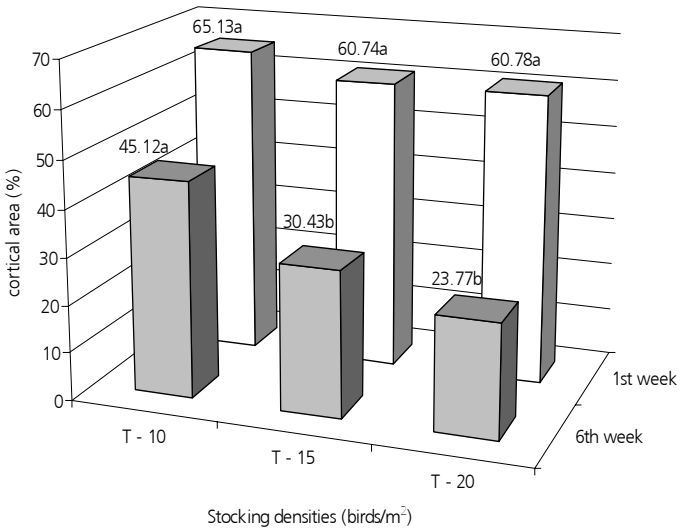

Figure 2 – Average percentage of cortical area in lymphoid follicles of bursa of Fabricius of one-six-week-old broilers.

In addition to the reduction of the cortical area of bursal follicles, the qualitative assessment of the follicles showed morphological changes related to the different stocking densities studied. The bursal epithelium of broilers housed in densities of 15 and 20 birds/m^2 was plaited, indicating a retraction of this organ. Lymphoid follicles presented lower volume; however, the bursal epithelium of broilers in densities of 10 birds/m^2 was not plaited, suggesting that the bursal area was not reduced. In these birds, the lymphoid follicles were completely filled by lymphocytes, with clear delimitation

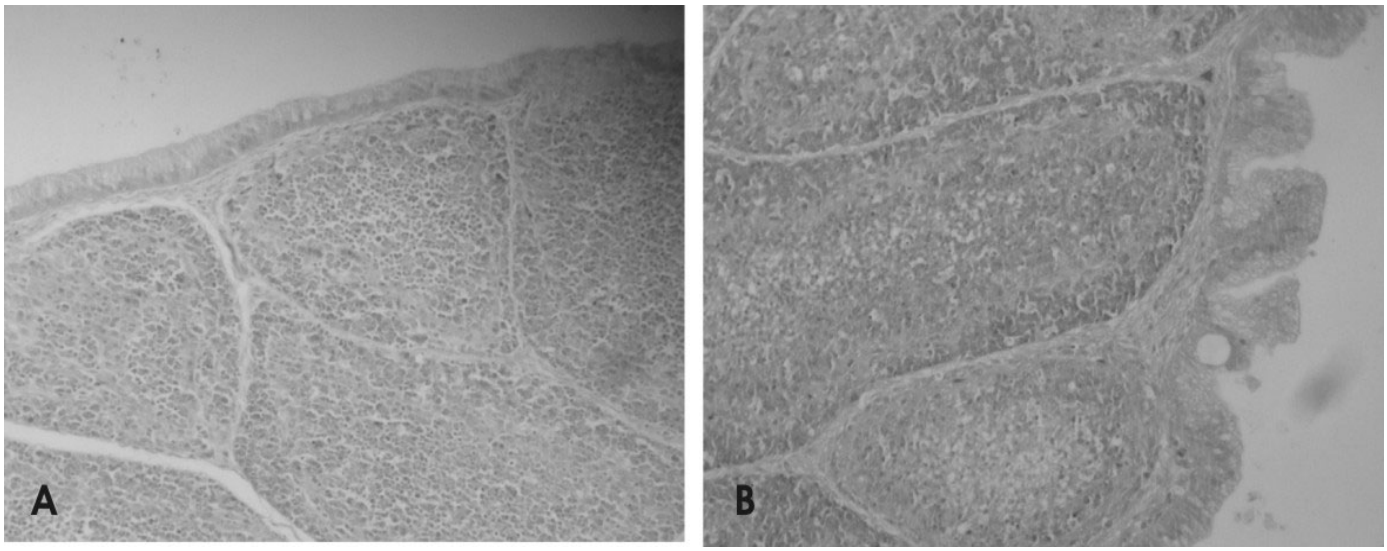

Figure 3 – Histological view of the bursa of Fabricius in 42-day old broilers. (A) Lymphoid tissue of broiler reared at a density of 10 birds/ m^2, with extended epithelium. (B) Lymphoid tissue of broiler reared at a density of 20 birds/ m^2 with plaited epithelium (HE 200X).

of the cortical and medullar regions. These differences are illustrated in Figure 3.

CONCLUSIONS

The analyses of the results of the present study show an association among body weight, foot-pad dermatitis rate, and histomorphology of the bursa of Fabricius. It is possible to state that higher stocking densities cause detrimental effects on the lymphoid tissue, and on foot-pad dermatitis rate. As assessed by bursal histomorphology, the stocking density of 10 birds/m^2 has the least effect on the broilers immune system. Therefore, the correct decision as to stocking density would be between 10 and 15 birds/m^2, and this decision should take into consideration performance parameters, as well as market requirements. For consumers demanding higher animal welfare standards, producers should use the lowest density; however, for less demanding markets, the density of 15 birds/m^2 could be used if the aim is to obtain higher production volumes.

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