

# Exploration of stress-induced immunosuppression in chickens reveals both stress-resistant and stress-susceptible antigen responses

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## Abstract

In the present study, depriving chickens of foraging material was shown to induce stress. The impact of this type of stress on the immune response was compared with feeding of corticosterone (1.5 mg per bird per day), a hormone known to be immunosuppressive and to be the major stress hormone of chickens. Corticosterone feeding induced stress as revealed by higher heterophil/lymphocyte (H/L) ratios, longer tonic immobility (TI) reaction, reduced body weight gain and reduced egg production. Blood corticosterone levels were increased. Corticosterone feeding decreased the antibody response to tetanus toxoid and SRBC, DTH to PPD from *Mycobacterium tuberculosis* and the inflammatory response to PHA. Housing chickens on slats also induced chronic stress, as evidenced by increased H/L ratios, prolonged TI duration and decreased egg production. Corticosterone levels were slightly but not significantly enhanced. This novel form of chronic stress strongly suppressed humoral and cellular immune responses as evidenced by lower antibody titers to sheep red blood cells (SRBC) and tetanus toxoid (TT) decreased DTH reaction to PPD and inflammatory reaction to PHA in the skin. In contrast, the antibody response to human serum albumin (HSA) was neither influenced by corticosterone feeding nor by keeping the birds on slats. Even the combination of corticosterone feeding and housing the birds on slats did not significantly impair antibody responses to HSA. In conclusion, the present study showed that chronic stress induced by depriving the birds of foraging material led to a similar impairment of humoral and cell-mediated immunity as did feeding with corticosterone. More importantly, it showed for the first time that depending on the antigen tested, there are stress-resistant and stress-susceptible antigen responses.

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**Abbreviations:** H/L, heterophil:lymphocyte; TI, tonic immobility; DTH, delayed-type hypersensitivity; HAS, human serum albumin; TT, tetanus toxoid; AU, arbitrary units; ANOVA, analysis of variance  
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## 1. Introduction

The immune response to a given antigen or pathogen is subject to modulation by both genetic or environmental factors. For example, it has been put forward that stress downregulates an immune response, thereby

jeopardizing anti-microbial resistance (Berczi, 1998; Dantzer, 1997; Dohms and Metz, 1991; Glaser et al., 1998; Magnusson et al., 1998; Sheridan et al., 1998). Stress induces a complex neuroendocrine response with an activation of the hypothalamus–pituitary–adrenal gland axis, and involvement of the sympathetic nervous system. The supporting evidence, however is based to a large extent on the use of artificial stress models. Moreover, in most studies, a humoral response to a single antigen or pathogen was measured, and the impact of stress upon cell-mediated immunity has not been addressed (Boa-Amponsem et al., 2000; Hester et al., 1996). Although stress was induced experimentally, it has not been verified in most models that the desired effects on stress were indeed achieved, as evidenced by hormonal, behavioral or physiological parameters.

In this study, a novel way to induce stress has been compared with corticosterone treatment of birds with regard to its immunosuppressive properties. Birds of defined genetic background were housed on slats without access to litter material, and a variety of stress parameters were compared with responses induced by chronic corticosterone feeding, and with those of control birds housed on litter. That both corticosterone feeding and housing birds on slats induced stress was verified by determining four established indicators of stress in birds; increase in heterophil:lymphocyte (H/L) ratios, blood corticosterone level, tonic immobility (TI), and impaired general performance (e.g. egg production). Humoral immune responses to three defined antigens were determined at various intervals after immunization. Cell-mediated immunity was determined by a DTH reaction to mycobacterial antigens following immunization, and by the swelling of wattles upon injection of PHA. The latter response was shown to represent cutaneous basophil hypersensitivity (Corrier and DeLoach, 1990; McCorkle et al., 1980). Five out of six parameters representing adaptive immunity were strongly impaired by stress induction, whereas one response (antibody formation to human serum albumin) was not influenced. Thus, while providing solid evidence in support of the concept that stress impairs adaptive immunity in chicken, this study reveals for the first time the existence of both stress-resistant and stress-susceptible antibody responses.

## 2. Materials and methods

### 2.1. Animals, housing conditions and corticosterone treatment

At 11 weeks of age, a total of 251 white laying hens (Lohman Selected Leghorn hybrids) were randomly assigned to groups of 15 or 16 individuals and distributed among 16 pens of identical size (265 cm × 90 cm, height 235 cm) built side by side along a corridor.

Housing conditions and the application of corticosterone in the feed were varied between the pens (2 × 2 factorial design, Fig. 1A). In eight pens, part of the floor was covered with deep litter consisting of wood shavings, chaff and long-cut straw, as foraging material ('litter' condition). In the other eight pens, the whole floor area was made of slats (width 1 cm, 1.5 cm apart; 'slats' condition). From the onset of the experiments, the birds had for 10 h light per day throughout. In four of the slats pens and four of the litter pens dietary corticosterone (Sigma; 1.5 mg per bird per day, 'corticosterone' condition) was offered from 11 to 19 weeks of birds age. In the remaining eight pens, the birds received a diet devoid of corticosterone ('no corticosterone' condition) (for more details see El-Lethey et al., 2002). The chosen rearing and feeding conditions resulted in four different treatments ('litter/corticosterone', 'litter/no corticosterone', 'slats/corticosterone', 'slats/no corticosterone'), each randomly assigned to four pens (Fig. 1A). The experiment was subjected to the Swiss authorization procedure prescribed by the Swiss Animal Legislation (Application No. 85/99).

### 2.2. Blood sampling

At 14 weeks of age (Fig. 1B), blood samples (1.5 ml) were taken from the right wing veins of eight birds per pen for determination of both blood corticosterone concentrations and heterophil/lymphocyte ratios (H/L). For the latter, one drop of blood was smeared onto a glass slide using a cover glass technique (Campbell, 1988). The smears were stained using Diff-Quik (Dade AG, Duedingen, Switzerland). One hundred leukocytes, including both granular (heterophils, eosinophils, basophils) and non-granular (lymphocytes, monocytes) cells, were counted once

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