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## Genetic Differences in Chicken Splenic Immune Gene Expression in Response to Dietary Immune Modulation

Sarah Beth Redmond  
*Iowa State University*

Rachel Tell  
*Iowa State University*

Derrick Coble  
*Iowa State University*

Carrie Mueller  
*Iowa State University*

Dušan Palić  
*Iowa State University*

*See next page for additional authors*

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## Authors

Sarah Beth Redmond, Rachel Tell, Derrick Coble, Carrie Mueller, Dušan Palić, Claire B. Andreasen, and Susan J. Lamont

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Sarah Beth Redmond, graduate research assistant;  
Rachel Tell, graduate research assistant;  
Derrick Coble, graduate research assistant;  
Carrie Mueller, undergraduate intern;  
Dušan Palić, assistant professor of biomedical science;  
Claire B. Andreasen, professor of veterinary pathology; and  
Susan J. Lamont, distinguished professor of animal science

### Summary and Implications

Chickens from broiler, Leghorn, and Fayoumi lines were fed diets with ingredients to affect immune function:  $\beta$ -glucans, ascorbic acid, or corticosterone. Splensens were tested for expression of genes involved in immune response: interleukin-4 (IL-4), IL-6, IL-18, and macrophage inflammatory protein-1 $\beta$  (MIP-1 $\beta$ ). Birds from the broiler line did not show any change in splenic gene expression associated with the dietary immunomodulators, perhaps due to the stringent selection of these birds for growth. The corticosterone diet was associated with increased expression of IL-4, indicative of an immune response relying primarily on humoral defenses. The Leghorn and Fayoumi lines showed opposing changes in expression of IL-4, IL-6, and IL-18 in response to the ascorbic acid and  $\beta$ -glucans enhanced diets, suggesting that processing of these immunomodulators and/or immune signaling in these lines are different. Our findings emphasize the need to further evaluate the effects of dietary immunomodulators before applying them in commercial settings.

### Introduction

Growing concern about the negative impacts of dietary antibiotics in poultry meat and egg production has increased interest in alternative mechanisms of enhancing disease resistance. Genetic resistance and use of dietary immune enhancers are two methods to decrease susceptibility to pathogenic diseases.  $\beta$ -glucans are polysaccharides found in the cell walls of fungi which imitates the surfaces of bacteria and stimulate immune cells to search for other signs of pathogens. Ascorbic acid increases the ability of white blood cells to phagocytose and kill bacteria. In contrast to these immune enhancers, corticosterone mimics the depression of the immune response that is associated with stress. Inclusion of these ingredients in poultry diets is known to alter immune response, and we hypothesized that expression of immune genes in the spleen would also be changed.

### Materials and Methods

Birds from broiler, Leghorn, and Fayoumi lines were randomly assigned to a basal diet or a diet enhanced with 0.1%  $\beta$ -glucans, 0.1% ascorbic acid, or 0.01% corticosterone. Diet treatments began at 8 weeks of age and lasted for 3 weeks. RNA was collected from spleen tissue of each bird. Gene expression assays were performed by quantitative PCR with SYBR Green and the 28s housekeeping gene. A standard curve was generated using a serial dilution of template for each primer set. C(t) values were adjusted to account for the amount of template and reaction efficiency and evaluated for significant changes using PROC MIXED in SAS.

### Results and Discussion

The analysis of C(t) values indicated that birds in the broiler line did not significantly alter gene expression in response to any of the enhanced diets. This may be a reflection of the stringent selection of broilers for growth, preventing changes in resource allocation for immune response. The immune suppressing corticosterone diet increased expression of IL-4, which is an indicator of a response which favors humoral mechanisms over cell mediated mechanisms. This type of response has been associated with poultry in stressful conditions. The corticosterone diet also significantly decreased body weight.

The Leghorn and Fayoumi lines showed divergent splenic expression of interleukin genes in response to the ascorbic acid diet. The Leghorn line showed higher levels of IL-6 and IL-18, while the Fayoumi line had higher expression of IL-4. Similarly, the  $\beta$ -glucans enhanced diet increased expression of IL-6 and IL-18 in the Fayoumi line in comparison to the Leghorn line. These differences likely reflect divergent mechanisms of immune response in these two genetic lines, and that immune modulators may have different effectiveness in different genetic lines of chickens.

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ANOVA model effects on splenic gene expression (*p*-values), bolded values indicate significant effects

	IL-4	IL-6	IL-18	MIP-1 $\beta$
Line	0.08	<b>0.01</b>	<b>&lt;0.01</b>	<b>&lt;0.01</b>
Diet	<b>0.02</b>	0.92	0.09	0.33
Sex	0.47	0.22	<b>0.01</b>	0.82
Line*Diet	<b>0.02</b>	<b>&lt;0.01</b>	<b>0.02</b>	0.63