Differential Immunological Gene Expression after *Escherichia coli* Infection in Chickens

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Summary and Implications

Chickens infected with avian pathogenic *Escherichia coli* (APEC) have reduced production and livability. Gene expression in response to APEC infection was assessed in 4 genes; interleukin-1β (IL-1β), IL-6, IL-10, and Granzyme A (Gzm A). Differences in expression were deduced between challenged and non-challenged birds, and between birds with a mild and severe response to infection. These genes may be useful candidates for future research into breeding for resistance against APEC.

Introduction

APEC causes millions of dollars in lost production every year. Current methods of control include utilizing good biosecurity practices and treating birds with antibiotics. Concern over antibiotic use in food animals has led to a greater push to increase disease resistance through genetics and breeding. Birds with differing responses to infection exhibit differences in their expression of immunological genes. Interleukins are signaling molecules that are produced by, and act on white blood cells. Granzyme A is a protease that helps trigger programmed cell death in infected cells.

Materials and Methods

Commercial male broiler chicks were purchased at 1 day of age. At 4 weeks of age, non-vaccinated birds were either challenged with APEC or given a control treatment. Necropsy took place at two timepoints, 1 and 5 days post challenge. At necropsy, internal lesion scores were assessed to determine pathology of challenged birds, mild or severe infection. This created six unique groups; non-challenged day 1, non-challenged day 5, challenged mild day 1, challenged mild day 5, challenged severe day 1, and challenged severe day 5.

Spleen samples were collected and RNA isolated from 4 replicates of the 6 groups, 24 samples in total. Gene expression was assessed by quantitative PCR using SYBR Green and 28s ribosomal RNA as a housekeeping gene. A standard curve was generated using a serial dilution for each gene. Observed C(t) values were adjusted to correct for the starting amount of RNA and reaction efficiency. Two models were used in this study, one utilizing all samples to assess challenge, and one utilizing only challenged samples to assess pathology. Replicate was included as a random effect. Tests were performed using the Fit Model procedure in JMP. Interactions with *p*-value less than 0.10 were excluded.

Results and Discussion

Challenged birds had a significant increase in expression of IL-1β and IL-6, indicative of an increased pro-inflammatory response due to infection. Birds with severe pathology had significantly higher levels of expression of IL-6 and IL-10 than birds with mild pathology. Gene expression on day 1 was higher than on day 5 for both IL-6 and IL-10 in the challenge model. This trend was also seen in IL-6 and IL-10 in the pathology model. The only significant interaction was between pathology and day for Gzm A, with severe pathology higher at day 1 and mild pathology higher at day 5.

Increased knowledge about gene expression patterns in response to infection allows for more detailed research into breeding for disease resistance. These results demonstrate differences in expression can be detected between mild and severe pathology groups in a commercial population.

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Table 1. Effect of challenge and day on spleen gene expression (*p*-values) using challenged and non-challenged samples.

	IL-1β	IL-6	IL-10	Gzm A
Challenge	**0.006**	**0.003**	0.117	0.319
Day	0.169	**0.004**	**0.017**	0.267
Rep (Random)	0.182	0.280	0.756	0.797

Table 2. Effect of level of pathology and day on spleen gene expression (*p*-values) using 16 challenged samples.

	IL-1β	IL-6	IL-10	Gzm A
Pathology	0.249	**0.031**	**0.018**	0.785
Day	0.432	**0.003**	**0.011**	0.231
Rep (Random)	0.036	0.124	0.073	0.623