Genetic Sensitivity to Pathogenesis: Response to *E. maxima* Challenge in High and Low Antibody Selection Lines of Chickens

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**ABSTRACT**: Pathogen exposure often causes a reduction in voluntary food intake or anorexia. The duration of anorexia depends on pathogen type and dose, and likely animal genotype. Chicks from two lines of White Leghorns, divergently selected for 37 generations for high (HAS) or low antibody response to sheep red blood cells, were challenged with two doses of *E. maxima* at 8 d post-hatch. We hypothesized that HAS chicks would express a greater magnitude of anorexia, but for shorter duration. At 6 d post-inoculation, jejunal lesions were moderate regardless of dose, and higher in HAS chicks. In both lines, weight gain and feed intake declined due to pathogenesis. Anorexia occurred later, and persisted less, in HAS chicks. Its magnitude was similar across lines, and dose independent. Our supposition of heightened immune-responsiveness to a pathogen from selection for high antibody titer appears doubtful.

**Keywords**: chicken; antibody selection lines; genetic sensitivity; coccidiosis

**Introduction**

Exposure to a pathogen challenge often results in a reduction in voluntary food intake, henceforth called anorexia (Kyriazakis et al. (1998)). The extent and duration of anorexia depends on pathogen type and dose (Sandberg et al. (2006)), although the physiology of an animal’s response and recovery to such a challenge is poorly understood. Nutrition and genotype likely play roles (Kyriazakis (2010)).

Since their selection profile is well defined, genetic lines established from a panmictic founder population provide a useful resource to study the interaction between environment, including pathogen challenge, and genotype. At Virginia Tech, White Leghorn chickens originating from the Cornell Randombred population have been selected for high or low antibody response to sheep red blood cells (SRBC) for 40 generations (Kuehn et al. (2006); Zhao et al. (2012)). There has been substantial direct response to selection with antibody titers (log₂) differing by 9-fold between lines. As an outcome, this population provides a useful animal model to understand the interrelationship between pathogenesis and genotype on anorexia.

Beyond causing differences in antibody titers, selection within the two lines has affected allocations of biological resources. Chickens in the low as compared to high antibody line reach sexual maturity at an earlier age (Siegel et al. (1982); Zhao et al. (2012)), and have greater intensity of lay and higher fertility (Albrecht et al. (2012a)). However, selection for improved immunocompetence is not necessarily detrimental to other production and fitness traits. For instance, egg quality in the high antibody line was generally superior to that of the low antibody line (Albrecht et al. (2012b)). Nonetheless, as anorexia leads to a problem of partitioning scarce resources (Coop and Kyriazakis (1999)), the consequences of intense selection for a single trait on animal responses come into question. With limits in resources available for other physiological functions (Gross et al. (2002)) animals’ capacities to respond to novel challenges may be compromised.

The objective of this study was to test the sensitivity of lines of chickens selected for high and low antibody response to SRBC to a pathogen. The test was based on challenging immune-naive young chickens with either a higher or lower dose of coccidian inoculation of *E. maxima*. Sensitivity was measured through changes in food intake and body weight gain. Our hypothesis was that selection for high antibody titers will lead to a greater magnitude of anorexia, but of a shorter duration.

**Materials and Methods**

**Chickens.** At Virginia Tech, White Leghorn chickens have been divergently selected for antibody response 5 d after an intravenous injection with 0.1 mL of a 0.25% suspension of SRBC administered at between 41 and 51 d of age (Zhao et al. (2012)). High (HAS) and low (LAS) antibody response selection lines have been established.

Chickens from the 37th generation were used. At selection age (approximately 6 wk), the antibody titers (on log₂ basis) were 18.2 ± 6.1 and 2.0 ± 1.3, respectively, in the HAS and LAS line pullets; in roosters, these antibody titers were 19.4 ± 3.1 and 2.2 ± 1.4, respectively (Zhao et al. (2012)). Chickens were pen mated within line. Individual parentages were therefore unknown. Eggs were collected, stored and set to achieve a target hatch date.

**Eimeria inoculata.** Oocysts of *E. maxima* (Tyson’s strain) were acquired from the USDA Animal Parasit-
nic Diseases Laboratory (Beltsville, MD), where they had been maintained by passage through 2- to 3-wk-old chicks. Following cleaning, sporulated oocysts were microscopically counted and dilutions were freshly prepared prior to inoculation on d 8 post-hatch.

Design. At hatch, 42 chicks from each line were vaccinated for Marek’s disease and co-housed. On d 4 post-hatch, chicks from both lines were randomly placed in individual cages in a single battery. On d 8 post-hatch, 14 chicks per line were orally gavaged with either a lower (5000 oocysts) or higher (7500 oocysts) coccidian dose suspended in 0.5 ml of water. An uninfected control of 14 chicks per line received the same amount of water only. On d 14 (d post-inoculation), 6 chicks per treatment group were dispatched, their sex determined, blood samples collected, immediately spun and the resulting plasma stored.

Body weights (BW; g) of chicks were recorded at hatch and daily thereafter for up to 24 d. Starting on d 4, daily food intakes (DFI; g/d) were also recorded. At the end of the experiment (16 d post-inoculation), the remaining chicks were weighed, and their sex determined. Chicks were offered free access to a standard soya/corn based diet (260 g crude protein/kg) without a coccidiostat and water throughout.

Measures. At dispatch, the jejunum was scored for intestinal lesions using the system of Johnson and Reid (1970). A score of 0 corresponded with no gross lesions and a score of 4 corresponded with extensive thickening and discoloration of the intestinal wall. Nitrate ($NO_2^-$) and nitrite ($NO_3^-$), metabolites of nitric oxide (NO), concentrations in plasma were assessed using a colorimetric kit (Cayman Chemical Company, Ann Arbor, MI). Plasma carotenoid concentrations were obtained using procedures described by Allen (1997).

Statistical Analysis. All analyses were conducted using Genstat 16th Edition (VSN International (2013)). Non-parametric tests were used to define the distributions of the data collected, and for editing. Lesion score was analyzed using a generalized linear model with a multinomial distribution fitted with a Poisson distribution and logarithmic link function. Nitric oxide concentrations followed a gamma distribution, and were normalized with a cube-root transformation. Treatment means and standard deviations for BW and DFI increased proportionally (scaled) with age, and were log transformed. Residual maximum likelihood procedure was used to analyze plasma measures, BW and DFI.

The models fitted included line (HAS, LAS), treatment (0, 5,000, or 7,500 oocysts inoculation), and their interaction. For analyses of BW and DFI in chicks dispatched at d 14, and for those retained to the end of the experiment, day was included as a repeated measure; its inter-actions with line and treatment were also fitted. An ant-dependence model with heterogeneous variances among days was used. A covariance structure with equal spacing yet non-equal correlations between days was assumed. Combination of chick and day became the residual term. Non-significant interactions were removed from the final models fitted. Sex did not define variation in any response variable ($P > 0.1$), and was excluded.

Results and Discussion

There was no mortality or any clinical signs (e.g., loose or bloody feces) associated with the E. maxima challenge.

Lesion score. At 6 d post-challenge, there was no interaction between line and treatment for lesion score ($P = 0.13$). Although lesion scores increased appreciably in coccidian inoculated as compared to control chicks ($P = 0.001$), no difference was detected between the lower and higher dose levels. Back-transformed mean lesion scores were $0.25 \pm 0.14$ (uninfected control), $2.33 \pm 0.44$ (5000 oocysts), and $2.08 \pm 0.42$ (7500 oocysts). However, a greater proportion of chicks were assigned a lesion score of 4 at the higher (50%) than lower (33%) dose. The HAS line had higher mean lesions scores than their LAS counterparts ($P < 0.001$). Mean back-transformed lesion scores were $2.28 \pm 0.36$ and $0.83 \pm 0.22$, respectively, for HAS and LAS line chicks. This is somewhat surprising, as it suggests a lower resistance in the HAS line. At an earlier stage of selection in these lines (generation 12), when challenged with E. tenella, LAS chicks had more severe cecal lesions than HAS chicks (Dunnington et al. (1992)). In that experiment, chicks were inoculated at an older age (21 d post-hatch).

Nitric oxide metabolites and carotenoid concentration. With Eimeria infection of immune-naïve animals, plasma concentrations of NO are expected to rise while those of carotenoids are expected to fall. Those responses reflect redox reactions and damage to the absorptive mucosa of the intestine, and may be dose-dependent during primary infection (Allen et al. (2004)). In this study, plasma NO concentrations increased in the presence of coccidian infection ($P < 0.001$). However, it was not dose dependent. Mean plasma concentration for NO, expressed on the observed scale, were $3.5 \pm 2.9$ (uninfected control), $28.3 \pm 2.9$ (5000 oocysts) and $25.8 \pm 2.9$ (7500 oocysts) mM nitrate. There was no line by treatment interaction for plasma NO concentration ($P = 0.66$) or line differences ($19.9 \pm 2.3$ and $18.5 \pm 2.3 \mu M$ for HAS and LAS, respectively; $P = 0.30$). No genetic sensitivity was manifest in terms of plasma NO concentrations in these selection lines.

As anticipated, plasma carotenoid concentrations decreased with the presence of infection ($P < 0.001$), and there was a suggestion of a line by treatment interaction ($P = 0.05$). The mean plasma carotenoid concentrations were
8.71 ± 0.51 (uninfected control), 1.34 ± 0.51 (5,000 oocysts) and 1.79 ± 0.49 (7,500 oocysts) μM lutein equivalents. Although mean carotenoid concentrations differed between lines (3.04 ± 0.41 and 4.85 ± 0.41 μM in HAS and LAS lines, respectively; \( P = 0.005 \)), it was due to differences between lines in the control (uninfected) chicks. As with NO, although infection with *E. maxima* affected plasma carotenoid concentrations, it did not do so in a dose or genetic line-dependent fashion.

**Body weight and Daily Feed Intake.** Patterns of BW gain and DFI for chicks dispatched on d 14 and for those retained throughout the experiment were essentially the same. Only results for the latter will be presented.

Log transformed BW plotted against age post-hatch is shown in Figure 1. The HAS line chicks were heavier than LAS chicks throughout the experiment (\( P < 0.001 \)). Although an interaction among line, treatment and day was equivocal (\( P = 0.10 \)), *E. maxima* challenge clearly reduced BW gain across lines (\( P = 0.01 \)). The BW accumulation in inoculated HAS and LAS chicks slowed starting 6 and 5 d, respectively, post-challenge. Recovery began in both lines on d 8 post-challenge, with the rate of BW recovery slightly higher in HAS chicks.

![Figure 1. Natural log body weight (Ln BW; g) plotted against age (d) for high (HAS) and low (LAS) antibody selection lines, and dose of *E. maxima* inoculation on d 8 (arrow). The average and maximum standard error were 0.032 g and 0.041 g, respectively.](image)

In Figure 2, log transformed DFI is plotted against age post-hatch. Consistent with their heavier BW, DFI was higher in HAS than LAS chicks (\( P < 0.001 \)). Again, an interaction among line, treatment and day was equivocal (\( P = 0.06 \)), although pathogenesis substantially impacted DFI across lines (\( P < 0.001 \)). In HAS chicks, DFI fell appreciably 5 d post-inoculation, with recovery beginning 2 d thereafter. In LAS chicks, the reduction in DFI began sooner (4 d post-inoculation), with the start and pace of recovery seemingly slower than in HAS chicks. Although the extent of anorexia was similar across lines, its onset was sooner, and its duration longer, in LAS as compared to the HAS line.

![Figure 2. Natural log daily food intake (Ln DFI; g/d) plotted against age (d) for high (HAS) and low (LAS) antibody selection lines and dose of *E. maxima* inoculation on d 8 (arrow). The average and maximum standard error were 0.069 g/d and 0.17 g/d, respectively.](image)

Although the antibody lines differed in duration of anorexia, they were not distinguished by the other measures of resistance to infection. In previous studies (Dunnington et al. (1992); Gross et al. (2002)), the HAS line was found to cope better with a plethora of pathogens, including other *Eimeria* species, than the LAS line. Yet, that was not universally the case (Gross et al. (1980)). As mentioned by Gross et al. (2002), different parts of a population may devolve genetically high levels of resources against different types of diseases in order to conserve resources.

**Conclusion**

The antibody lines differed in their response to infection. As hypothesized, anorexia was of a shorter duration in HAS chicks. However, its magnitude was similar across lines. Line sensitivities to infection also were not evident from our metabolic measures, although intestinal damage was more severe in the presumably more resistant line. There was no dose response, suggesting a threshold for acute levels of infection. Our supposition of heightened immune-responsiveness to a novel pathogen resulting from selection for high antibody titer appears doubtful.

**Literature Cited**


