A Comparison of Innate Immunity as Shown by Heterophil Function in Two Turkey Lines

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Introduction

The ability to resist a microbial infection is important not only to maximise poultry liveability and improve production efficiency, but it also means that there is an ability to also resist food borne pathogens. Resistance to important human pathogens could result in improved food safety of poultry for human consumption. Improving the poultry immune system and pathogen resistance has consequently been an area of research for many years, considering both improving liveability and also increasing resistance to specific disease agents. Due to its importance the primary breeding companies have selected within and between lines for improved liveability and disease resistance for many years (Flock et al. (2005)). Changes in the immune system as a result of selection depend on the breeding goals, genetic correlations with other traits, and whether the immune activities are selected upon directly or indirectly. Accordingly, the direction a selected population is taken can have unanticipated results; this is especially true of the immune system if it is not routinely measured in a breeding program. In the chicken it has been shown that selection based on growth and performance traits may have adversely affected immune competence and as a result left the bird more susceptible to both viral and bacterial diseases (Han and Smythe (1972; Janss and Bolder (2000)). Whether the change has a significant effect on liveability, health and food safety is breeding program specific.

Experimental selection for resistance to specific diseases has resulted in resistance to the disease but poor production traits have meant the selected lines have invariably been unsuitable for commercial use (Swaggerty et al. (2009)). Another issue with challenge based selection is the improved immune response is specific to that challenge but has poorer response to other diseases or vaccines. It would appear that selection based on specific challenges may not optimise generalised resistance and consequently another strategy that optimises overall resistance is required. What is clear is that any selection strategy that includes immune function or resistance in the breeding goal must be balanced with the economically important production traits so the end product can be used by the poultry industry. As a result, monitoring the immune system for differences within and between lines is important so that if not incorporated into a selection index at the very least it is monitored for any correlated change.

The immune system is composed of a number of components and the innate immune system provides the immediate, first line of defence against microbial infections. It does not provide

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the long lasting or protective immunity which is provided by the acquired (or humeral) immune system. The innate system is important as it directs the acquired immune response, and as a consequence an efficient innate immune system may improve the initial response and offer better overall immunity against infection. Turkeys that were selected for increased growth rate to 16 weeks of age showed differences in the acquired immune function compared with random bred controls (Li et al. (2001)). Changes included differences in some phagocytic abilities of the immune cells and also differences in mortality resulting from bacterial infection. These same lines were also shown to have a decreased disease resistance to viral infections (Tsai et al. (1992)). It would appear that selection for performance traits also affected the innate immune response but these differences were less well defined. A different study comparing commercial-type and wild-type Rio Grande turkeys showed that there were differences in heterophil function as an indicator of innate immune function (Genovese et al. (2006)). This showed a potential change in the innate immune response to selection for production and growth in the commercial turkeys. The aim of this study was to assess any differences in innate immune function in parental primary breeder lines.

Material and methods

Turkeys, Sample Source and Heterophil Isolation. This study used a similar method to that employed by Genovese, et al. ((2006)) to compare heterophil function between wild-type (Rio Grande) and commercial turkeys. Two pure lines (A,B) were used, each with a known genetic background and pedigree, with a mixture of breeding objectives from purely growth traits (A) to a more balanced breeding objective that includes both commercial and reproductive traits in the objective (B). In each of the two trials eggs were incubated under the same conditions and hatch was uneventful. Blood samples were obtained from 100 poults of each line at days 7 and 14 post hatch and the heterophils were isolated using an established isolation technique (He et al. (2003)). For each of trials 1 and 2 different poults were used.

Heterophil Degranulation. Heterophils were stimulated with *Salmonella enteritidis* (#97-11771). Degranulation was detected by quantifying the amount of β-glucuronidase activity in the medium following heterophil stimulation using a previously established technique (Genovese et al. (2006)).

Heterophil oxidative burst. Heterophil oxidative burst was measured by oxidation of 2'7'-dicichlorofluorescin acetate (DCFH-DA) after stimulation with phorbol A-myrisate-13-acetate using a previously described technique (He et al. (2003)). The oxidation product fluorescent DCF was measured for fluorescence 60 minutes after stimulation.

Statistical Analysis. T-tests assuming unequal variances were used to test for difference in means in response to heterophil stimulation.

Results and discussion

The results of the heterophil degranulation and oxidative burst for poults at 7 and 14 day are shown in Figures 1 and 2, respectively. The results are presented minus background control

levels and as two separate trials as the experimental data showed significantly different means and variances of both control and reaction response between trials. In both oxidative burst and degranulation Line B consistently showed a significantly greater response to stimulation than Line A.

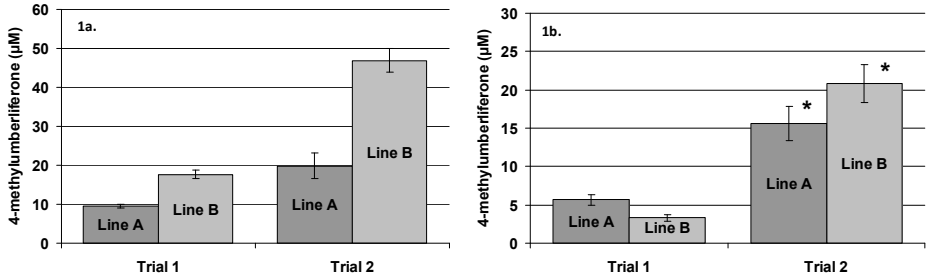

Figure 1 Mean and bars identifying standard deviation for heterophil degranulation for poults on day 7 (1a) and 14 (1b) from two separate trials on two lines (A, B) after stimulation with *Salmonella enteritidis*. Significant differences (P<0.001) are identified (*) with all other means non-significantly different (P<0.05).

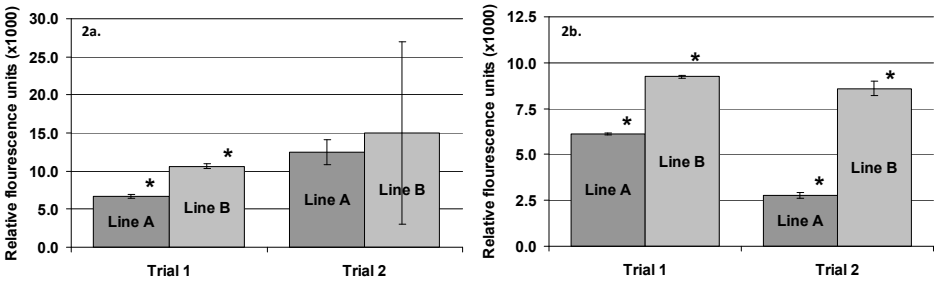

Figure 2 Mean and bars identifying standard deviation for heterophil oxidative burst for poults on day 7 (2a) and 14 (2b) from two separate trials on two lines (A, B) after stimulation with 2µ/ml phorbol A-myrisate-13-acetate. Significant differences (P<0.001) are identified (*) with all other means non-significantly different (P<0.05).

The two mechanisms measured represent different methods used by heterophils to neutralise a bacterial threat. Similar to Genovese, et al. ((2006)), there were identifiable differences between lines of turkeys but instead of the wild-type studied previously, in this case both lines were commercial lines that had undergone a significant number of generations of genetic improvement. The selection within both lines had a considerable emphasis on bodyweight and rate of gain but Line A had by far the greater amount selection pressure on bodyweight. This agrees with the previous finding in which the selection for commercial traits lead to a decrease in the innate immune response in the commercial line compared with the non-selected wild-type Rio Grande line.

Genetic differences in liveability and susceptibility to disease have been identified in both chickens and turkeys as a result of selection for commercial traits, but much of the mortality can be related to locomotion and leg strength later in life (Cheema et al. (2003; Quinton et al. (2010)). These traits are negatively correlated with bodyweight. Similarly, innate immune response also appears negatively correlated with growth and production traits. A genetic correlation between leg strength and immune function has not been studied. The ability to establish a correlation between innate immune response, resistance to infection and general liveability is difficult due to the fact that the causes of mortality are multi-factorial, such as breeder flock age, hatchery and brooder management. The effect of genetics and particularly genetic differences in immune response is thus difficult to quantify, but a better immune response could be assumed to improve the chances of survival under any management conditions. Swaggerty et al. ((2009)) have shown that selection for altered innate immune response is possible in the chicken and it is be expected that the same would also be possible in the turkey. The next step is to elucidate the economic advantages of either reducing mortality or enhancing poultry microbial food safety based on direct selection upon the innate immune system within a commercial selection index.

Conclusion

It is clear that there are measurable differences in the innate immune response between commercial and wild-type turkeys and also measureable differences between pure lines of commercially used turkeys. The difference in innate immune response was similar, with lines that had a greater selection emphasis placed on growth having a decreased response to immune stimulation. Oxidative burst and degranulation are directly related to the ability of the immune system to defend itself against microbial infection and as a consequence a decreased response may increase the susceptibility to infection. With further work it should be possible to define the genetic differences both within and between lines that result in these altered immune responses and also to define if this ultimately has an effect on morbidity and liveability.

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