Immunologic Function of Historic vs. Commercial Turkey Breeds

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Abstract

Historical turkey breeds are non-commercial lines of birds whose gene pool diversity is declining. As a result, conservation efforts are being made to optimize the biological health of these turkeys. A major component of animal/bird survival is a competent immune system. The cornerstone cells of the immune system are the lymphocytes. The primary immunologic function of lymphocytes is to mediate antibody production and coordinate cell-mediated response. Unfortunately, there are few documented studies involving the functional status of turkey lymphocytes across breeds as compared to the studies with chickens. The purpose of this study was to compare the immunologic function of historic and commercial turkey breeds based on cell recoveries, lymphocyte proliferation assays, and flow cytometric analysis. Blood was collected from six breeds of turkeys (five historic breeds and one commercial breed). Lymphocytes were isolated from the blood and stained to be identified and enumerated with a hemacytometer. A lymphocyte proliferation assay was done to measure the fluorescent changes. The lymphocytes were then analyzed with monoclonal antibodies by flow cytometry. In this preliminary study, differences among the breeds were observed. The majority of historic breeds had stronger immune indices than the commercial breed. This preliminary study possibly supports the potential use of historic turkey breeds as an additional gene pool for commercial flocks.

Introduction

The turkey is one of a few domesticated animals native to the Americas. Many breeds of turkeys (i.e. Black Spanish, Blue Slate, Royal Palm, Bourbon Red and Narragansett) are near extinction (Christman, 1999). Therefore, as a result, their potential to be utilized as genetic resources for commercial birds is diminishing. Thus, efforts to conserve these rare turkeys and to maintain their genetic diversity are important. Maintaining genetic diversity is beneficial to optimize the biological health of the turkey. One advantage of conservation breeding is that it maintains individual breed flocks and thus preserve that breed's genetic potential. Domestication of turkeys occurred about 2,000 years ago. Selection of turkeys by humans has gradually changed their conformation from the slim and long-legged appearance of a wild turkey to a heavier and short-legged domestic bird. Commercial turkeys, due to the intense selection, have lost the ability to mate naturally (Christman 1999). Further, these commercial breeds lack the genetic diversity needed to thrive in other production systems because of the health problems presented due to their restricted gene pool. Commercial turkeys are more productive than historic breeds and are primarily selected for production qualities (i.e. body size and growth rate). Although, commercial turkeys have strong production parameters, they are more susceptible to diseases. This is a significant contrast to historic breeds in that they retain their adaptability and survival characteristics in production parameters.

A functional immune system is necessary for survival. The immune system provides a defense against potential pathogens. The two branches of the immune system are cell-mediated immunity and antibody-mediated immunity. There are five types of white blood cells: neutrophils, monocytes, eosinophils, basophils and lymphocytes. Lymphocytes are one of the most important white blood cells. Mature lymphocytes are small, round cells present in the blood, spleen, lymph nodes, and the thymus. Lymphocytes have a specific function in which it defends against specific pathogens. The two subsets of lymphocytes are B cells and T cells. B cells are responsible for the production of antibodies and T cells produce cell-mediated immunity.

The benefits of understanding the turkey immune status will lead to identifying breeds with the potential of a compromised immune system and evaluating therapeutic treatments for immune-mediated diseases. The immune status examined in turkeys can lead to improvement of vaccines used to prevent diseases in both commercial and historic breeds. This improvement can deduce the effects of immunosuppression or boost the functions of their immune system. Immunologic competence in animals can be analyzed by a lymphocyte transformation test. One aspect of measuring the functionality of lymphocytes is the lymphocyte proliferation assay. It identifies major types of lymphocytes such as T cells and B cells. In this study, we examined six breeds (i.e. Blue Slate, Black Spanish, Narragansett, Bourbon Red, Royal Palm, and BUT). We conducted a pilot study to evaluate certain immune parameters such as cell recoveries, lymphocyte proliferation and flow cytometeric analysis.

Materials & Methods

Birds

- Six breeds of turkeys were used in this study.
  Historic breeds: Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Black Spanish
  Commercial breed: British United Turkey

Blood collection/lymphocyte isolation

- Peripheral blood (8-ml) collected from jugular vein with a 23-g needle attached to a pre-heparinzed 10-ml syringe
- Blood transferred to vacuum tubes were slow centrifuged 3x at 25ºC, 50xg for 10 mins.
- Buffy coat layer (lymphocyte-rich) collected by gentle "swirl" technique with a 1-ml sterile glass pipette
- Buffy coat collection repeated after each centrifugation, all suspensions collected were combined and chilled on ice
- Washed buffy coat suspensions 3x with RPMI 1640 media
  1st wash – 12ºC, 1200 rpm, 10 mins.
  2nd & 3rd wash – 12ºC, 1200 rpm, 7 mins.
- Resuspended pellet in 3-ml RPMI 1640 media
- Stained lymphocytes and other cells with Natt-Herrick's Stain
- Lymphocytes were identified and enumerated with a hemacytometer and adjusted to 5 x 10^6 cells/ml in RPMI 1640 media with 10% FBS

Lymphocyte proliferation assay

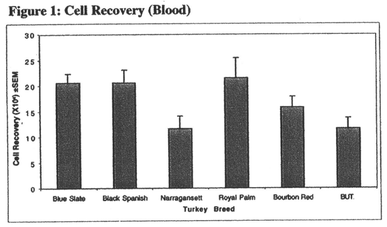
- To 96-well round bottom tissue culture plates, 100 ul of 5 x 10^6 lymphocytes/ml were added to quadruplicate wells containing 100 ul of medium alone and concanavalin A (Con A 5-100 ug/well)
- Incubated cells at 37ºC with 5% CO_2
- 20 ul of Alamar Blue dye added to each well at 24 hrs.
- 24 hrs. after dye added, fluorescent changes of plate were measured using a CytoFluorII Fluorescence Multi-well Microplate reader

Flow cytometric analysis

- Lymphocytes stained with monoclonal antibodies (PE-CD4, FITC–CD8)
- Analyzed by Epics XL flow cytometer and Immuno-4 software program

Results

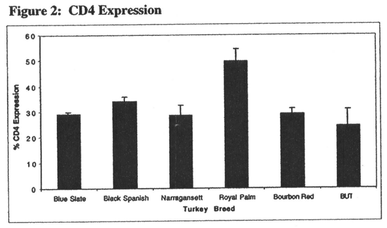
Figure 1: Cell Recovery (Blood)



Results: Among the breeds, the appearance of cell recovery differed in which the Royal Palms, Blue Slates and the Black Spanish had the highest cell recovery of lymphocytes.

Methods: Lymphocytes were isolated from heparinized blood by slow spin separation (3 x 10 min. x 50g). Stained with Natt-Herricks dye, lymphocytes were identified and enumerated on a hemacytometer.

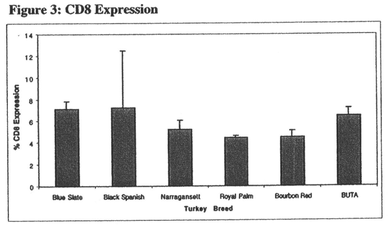
Figure 2: CD4 Expression



Results: The breed with the highest cell recovery, Royal Palm, also had a high percentage of CD4 expression. This indicates the large amount of lymphocytes in the peripheral circulation.

Methods: Stained a 100-ul aliquots of cells with PE-CD4 monoclonal antibodies and measured antibody fluorescence by flow cytometry.

Figure 3: CD8 Expression

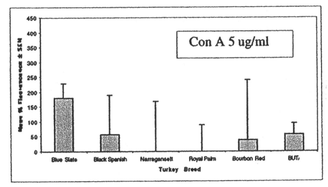


Results: There was no significant difference among the breeds in the percentage of CD8 expression.

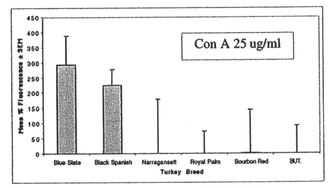
Methods: Stained 100-ul aliquots of cells with FITC-CD8 monoclonal antibodies and measured antibody fluorescence by flow cytometry.

Panel 1: Lymphocyte Proliferation (Concanavalin A)

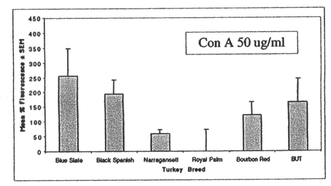
A



B



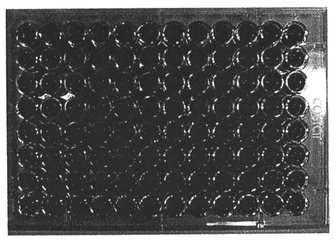
C



Results (A-C): With increasing concentrations of Con A (5-50 ug/ml), the Blue Slates and the Black Spanish responded the best. The Royal Palms had no reaction to any of the concentrations of Con A (5-50 ug/ml).

Methods: A 100-ul aliquot of cells and a 100-ul aliquot of each specified concentration of Con A was added to a tissue culture plate. The tissue culture plate was incubated for 24 hrs. at 37ºC with 5% CO_2. After 24 hrs. of incubation, 20-ul of Alamar blue dye was added and incubated for another 24 hrs. (48 hrs-Total incubation time)

Figure 4: Tissue Culture Plate – Lymphocyte Proliferation



Results: The blue color in the wells is the oxidized form of the Alamar blue dye which reduces (red/pink color) as the cells proliferate. The dye fluoresces when it is reduced. This is a sample plate with the colorimetric changes due to the Alamar blue dye.

Methods: At 24 hrs. of incubation, 20-ul of Alamar blue dye was added to each well of the plate. The colorimetric/fluorescent changes were measured 24 hrs. after the addition of Alamar blue.

Conclusions

The intensive selection of commercial breeds for efficient and consistent production has narrowed their genetic foundation and increased their susceptibility to health problems. Commercial breeds are a concern because they are a major contributor to the production market. In an environment with few or no changes, they thrive. However, when changes are made they are slow to adapt and are easily stressed. Some of the factors that lead to physiologic stress and immunosuppression are management and environmental changes i.e., changes in feed, temperature fluctuations, housing conditions etc. Historical breeds may prove genetically valuable because of their ability to adapt to changing conditions.

Some birds are easier to work with than others due to their behavioral characteristics. In our studies, the Blue Slates, Bourbon Reds, Black Spanish and BUTs were easier to work with than the Royal Palms and Narragansetts because they became less excitable as they were socialized. Conceivably, more excitable birds would exhibit "stress" related changes in their peripheral blood cell profiles e.g., heterophilia and lymphopenia.

As a research model, the historic breeds can be employed to further our understanding of turkey immunity. Using immune endpoints, we can assess immunological potential, identify breeds that are at immunologic risk, and evaluate response to disease challenge.

There were few differences among the breeds tested in terms of the cell recoveries, percentage of CD4 or CD8 expression and lymphocyte proliferation. The Royal Palms had a high cell recovery and percentage of lymphocytes expressing CD4 antigen. However, this breed did not exhibit good lymphocyte proliferation in response to Con A. The fact that "excitable" birds like the Royal Palms did not respond well to the T cell mitogen but did have high cell recoveries and CD4 expression seems contradictory in light of the current understanding of the effects of stress. A possible reason for this could be that the lymphocytes although increased in number, were immature and non-functional. As expected, those birds that were easier to work with and therefore less stressed i.e., the Black Spanish and Blue Slates, had the best T cell mitogen response out of all the breeds. In general, the historic breeds, especially the Blue Slates and the Black Spanish, seemed to have a higher level of immunocompetency than the commercial BUT strain.

Future studies to evaluate performance (feed conversion, daily/weekly body weight gain) as a function of immunological endpoint will be performed. This information will enable us to determine which breeds have the greatest potential for augmenting immunocompetency in the commercial turkey gene pool.

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