

Baseline Immune Cell Composition in Broiler Spleen is Altered by Probiotic Supplementation

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Summary and Implications

Probiotics are supplemented in poultry diets to support beneficial intestinal microbial community members. The alteration of the microbiota due to probiotic supplementation is shown to improve host health, but the underlying changes to immune cell populations have not been well-described in poultry. The objective of this study was to examine the effects of probiotics at two different dietary inclusion levels on the immune cell populations in spleens of unchallenged broiler chickens. Probiotic supplementation did not impact overall percentages of leukocytes, but did alter the composition of cells making up leukocyte subpopulations ($P \leq 0.05$). Additional research is needed to evaluate how these changes to immune cell populations translate to a modulated immune response under challenge conditions.

Introduction

Commensal bacteria within the intestinal microbiota communicate with the host immune system to mediate inflammatory responses in the gut. Probiotics are often used to alter the intestinal microbiota of animals with the goal of improving animal health by increasing the proportion of beneficial microbes. The use of probiotics in poultry has been described and overall health benefits have been observed; however, changes to immune cell populations as a result of probiotic supplementation have not been extensively studied in this model. Additionally, the beneficial effects of probiotics vary due to inclusion level, bacterial composition, challenge, and individual variation in housing conditions. The objective of this study was to examine changes to the baseline immune cell populations in the spleen of broiler chickens fed two different probiotics at 0.05 and 0.1% of the diet.

Materials and Methods

All animal protocols were approved by the Iowa State University Institutional Animal Care and Use Committee. A total of 480 Ross 708 broilers were raised in 40 floor pens (12 birds/pen) for a total of 42d. Birds were randomly

assigned to one of five dietary treatments consisting of a corn-soybean meal basal diet without probiotic supplementation (control) and probiotic diets (PD) 1 and 2 with 0.05% or 0.1% inclusion of their respective probiotic. On d42, four birds/treatment were euthanized and the spleens were collected. Spleens were homogenized in phosphate buffered saline (PBS) and passed through a sterile 70 μ m cell strainer to isolate splenocytes. Cells were then counted with a hemocytometer using the trypan blue dye exclusion method and frozen in chicken serum supplemented with 15% DMSO at -80°C until further analysis by multicolor flow cytometry.

Cells were thawed, counted, and stained for extracellular markers that distinguish between cluster of differentiation (CD) and other proteins unique to different immune cell populations. Staining panels were divided between two mixes and included fluorescence minus one (FMO) controls. Mix 1 contained markers for CD45, TCR $\gamma\delta$, Bu-1, CD4, CD8 α , and CD28, whereas mix 2 contained CD3, CD4, CD8 α , TCR $\alpha\beta$ /V β 2, CD1.1, and monocyte/macrophage markers (Southern Biotech, Birmingham, AL). After staining, cells were washed, resuspended in PBS and analyzed by a FACSCanto™ cytometer (BD Biosciences, San Jose, CA). Further analysis of flow cytometry data was performed using FlowJo 10.5.0 software (FlowJo LLC., Ashland, OR). Statistics were completed using the MIXED procedure of SAS as repeated measures with bird as the subject and an autoregressive covariance structure. Contrast statements were used to analyze the difference between the control group and the different type of probiotic and dose used with significance reported at $P \leq 0.05$.

Results and Discussion

The main effect of PD type did not have an effect on overall populations of splenic leukocytes, but different subpopulations were affected. Regardless of inclusion level, birds fed PD2 had 17.0% fewer B cells (CD45⁺Bu-1⁺) than birds fed the control diet ($P = 0.02$; Figure 1A). Birds fed PD1 had 9.9 and 13.0% more helper T cells (CD3⁺CD4⁺) than birds fed the control diet and PD2, respectively ($P = 0.03$ and 0.0005 ; Figure 2). Feeding PD1 and PD2 resulted in 5.4 and 8.0% fewer activated cytotoxic T cells (CD8⁺CD28⁺) than birds fed the control diet ($P = 0.05$ and 0.005 ; Figure 3A). Other subpopulations of leukocytes were unaffected by the type of probiotic incorporated into the diet.

Similarly, the main effect of inclusion level did not alter overall leukocyte populations but caused shifts within underlying subpopulations. Supplementing probiotics at 0.1% of the diet resulted in a 12.5% reduction in B cells compared to birds fed the control diet, regardless of probiotic type ($P = 0.04$; figure 1B). Probiotic diets with inclusion levels at 0.1% of the diet increased the percentage of activated helper T cells ($CD4^+CD28^+$) by 15.2% compared to the control diet ($P = 0.04$). Feeding probiotics at 0.05 and 0.1% of the diet resulted in 7.3 and 6.1% reductions in the percentage of activated cytotoxic T cells, respectively, compared to control ($P = 0.01$ and 0.02 ; Figure 3B). Inclusion level did not alter the percentages of other analyzed immune cell subpopulations.

In all the immune cell populations analyzed, the interaction of probiotic type \times inclusion level did not alter overall leukocyte percentages but impacted one subpopulation of cells. The observed interaction between probiotic type and dosage was driven by 12.6, 14.8, and 16.5% increases in the T helper cell population in birds fed PD1 at 0.05% compared to birds fed the control diet and PD2, respectively ($P = 0.007$; Figure 2). No changes to overall percentages of $CD45^+$, $CD3^+$, $CD28^+$, and innate immune cells (monocyte/macrophage $^+$, $CD1.1^+$, or $CD1.1^+CD8^+$) were observed as a result of probiotic supplementation. Other tested subpopulations of immune cells were also not affected by dietary probiotic supplementation (Figures 1-4).

These results suggest that probiotic supplementation alters the composition of leukocyte and T cell populations without changing the overall percentage of these cell types within the spleen of unchallenged 6wk-old broiler chickens. Feeding PD1 increased the percentage of T helper cells, with increased activation observed at the higher inclusion level. Splenic B cell populations were reduced by PD2 at higher doses, but it is important to note that changes to B cells and activated helper T cells were accomplished at higher probiotic inclusion levels regardless of type. Reduction in B cell populations could be indicative of increases in the percentages of this cell type leaving the spleen as a concomitant to probiotic supplementation.

Both types of probiotic reduced the percentage of activated cytotoxic T cells compared to control, and reductions were seen at both inclusion levels regardless of probiotic type. In future work, the physiological impacts of these changes to immune cell populations could be examined using a pathogen challenge or isolating T cell populations for cellular assays. This work would better describe the biological impact of altering immune cell populations by probiotic supplementation in broiler chickens.

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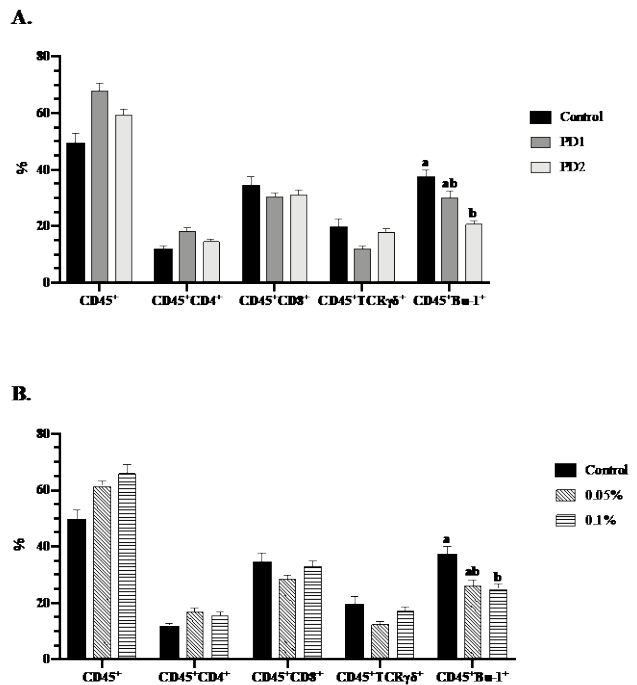


Figure 1: Percent of $CD45^+$ cells and underlying cell populations within the $CD45^+$ population in the spleen of Ross 708 broiler chickens fed different (A) probiotic diets and (B) inclusion levels. Data represent the mean and SEM of the percentage of cell types identified by flow cytometry in 4 spleens/treatment.

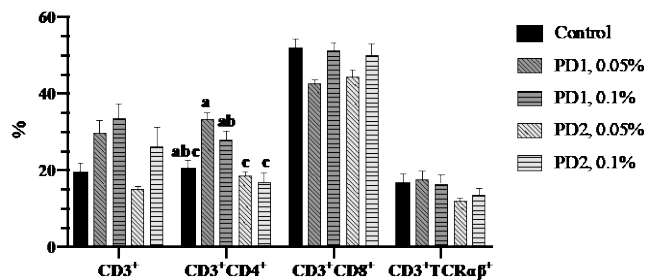


Figure 2: Percent of $CD3^+$ T cells in the spleen of Ross 708 broiler chickens and underlying cell populations within the $CD3^+$ population. Data representing the mean and SEM of the percentage of cell types identified by flow cytometry in 4 spleens/treatment. Columns with different letter superscripts are significantly different ($P \leq 0.05$).

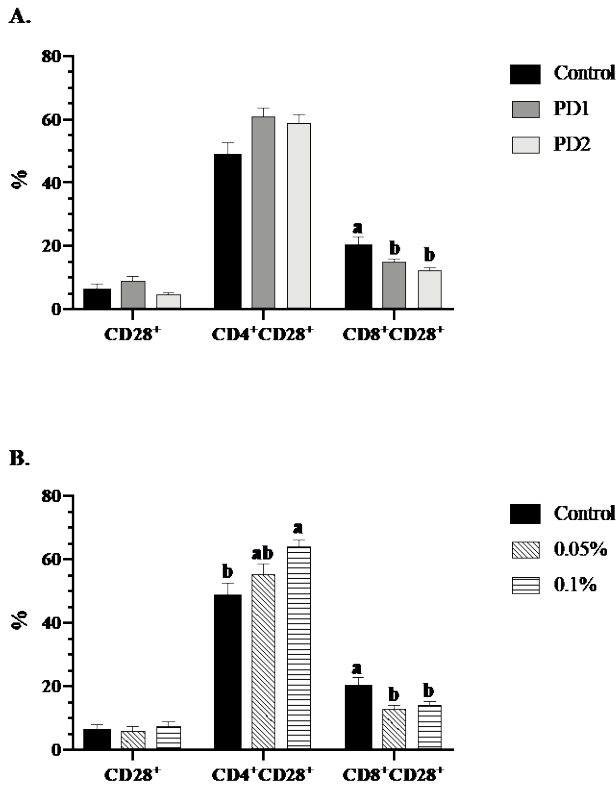


Figure 3: Percent of CD28⁺ T cells underlying cell populations within the CD28⁺ population in the spleen of Ross 708 broiler chickens fed different (A) probiotic diets and (B) inclusion levels. Data represent the mean and SEM of the percentage of cell types identified by flow cytometry in 4 spleens/treatment.

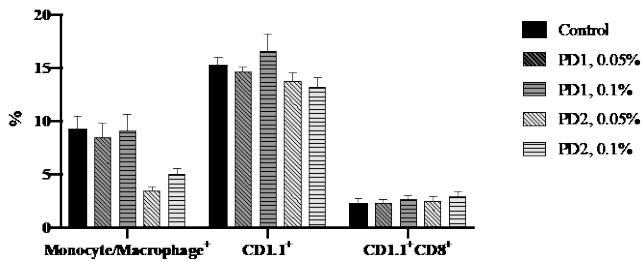


Figure 4: Percent of (A) monocyte/macrophage⁺, (B) CD1.1⁺, and (C) CD1.1⁺CD8⁺ cells in the spleen of Ross 708 broiler chickens. Data represent the mean and SEM of the percentage of cell types identified by flow cytometry in 4 spleens/treatment.