

# Effect of Dietary Protein Source and Litter Condition on Mitotically Active Cell and Macrophage Cell Density in the Duodenum of Broiler Chickens at Day 21

*Andrew J. Keel, Allan J. Calderon, Oscar J. Tejada, Jessica D. Starkey, Charles W. Starkey*

The small intestine (SI) serves as the site of nutrient absorption and defends against ingested pathogens. Proper nutrient absorption is dependent upon the constant proliferation of mitotically active cells to create new enterocytes, the absorptive cells of the SI, to replace those damaged by the harsh environment within the lumen. Proliferative cells in the small intestine are primarily located at the base of intestinal crypts. As these cells divide, the new cells migrate toward the tip of the villi before being shed. Immune cells, especially macrophages, are necessary to prevent pathogens from causing illness. Macrophages have multiple functions, such as warning other immune cells of pathogen invasion, destroying potential pathogens, and aiding in antibody production. It has been shown that certain dietary protein sources and litter conditions can affect the immune response and microbial population of the SI. Based on this information, it is of interest to determine if different dietary protein sources and litter conditions influence mitotically active cell and macrophage density in the SI of young broilers.

To explore this idea, an experiment with a 3 x 2 factorial treatment arrangement in a randomized complete block design was conducted. The three dietary protein sources used were porcine meat and bone meal, a 50:50 mix of poultry byproduct and poultry feather meal, and soybean meal. The two litter conditions used were new litter, which was fresh pine shavings, and used litter, which was pine shavings used to rear three previous broiler flocks. On day 0, 1,500 female Yield Plus x Ross 708 broiler chicks were placed in an environmentally controlled, raised floor pen facility. Broilers received *ad libitum* access to feed and water. On day 21, six broilers per treatment (total n = 36) were randomly selected for duodenal sample collection. Broilers were injected intraperitoneally with 5'-bromo-2'-deoxyuridine

(BrdU) to label mitotically active cells. Duodenal samples were collected approximately 5 cm distal to the duodenal loop. Samples were flushed with phosphate buffered saline, flash frozen in liquid nitrogen, and stored at -80°C for later analysis. Sample analysis began by collecting 5- $\mu$ m-thick cryosections from each sample on a glass slide. Cryosections were stained using indirect immunofluorescence staining to label BrdU+ cells and macrophages. Taxonomic data were collected by enumerating BrdU+ cells and macrophages to determine the number of mitotically active cells and macrophages per square millimeter of tissue using digital fluorescence microscopy.

Data collected for the study were analyzed using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, Cary, NC). Dietary protein source and litter condition were the fixed effects, and treatment means were separated using the PDIFF option of SAS. Means were declared significantly different when  $P \leq 0.05$ . Neither dietary protein source ( $P \geq 0.5946$ ) or litter condition ( $P \geq 0.3039$ ) significantly altered mitotically active cell or macrophage cell density. In conclusion, no treatment effects were observed in the duodenum on day 21. Although no significant differences were detected at this location and time point, further research will be conducted to study potential treatment effects in other locations of the SI at earlier time points.

## Statement of Research Advisor

Jake Keel's work is an initiation of a multi-systems approach to evaluate and improve upon overall health and performance of animals raised for food. The major contribution of this research is to advance our knowledge of producing healthy, nutritious, and safe food for the consumer.

– Charles Starkey, Poultry Science