

A Method for the Integration of Histopathology with Gut Health Monitoring

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Introduction. Traditional applications of histopathology to intestinal health seek to define causes of morbidity and mortality. Although important, this is relatively insensitive and underutilizes the value of histopathology in identifying more subtle changes in gut health that have the potential to be linked to production parameters. A method is presented for histopathologic profiling of gut from commercial poultry that seeks a compromise between detailed measurements and routine diagnostics. The method seeks assess a defined panel of gut lesions over time, in order to provide insight and add value to decisions involving management, nutrition, and health programs.

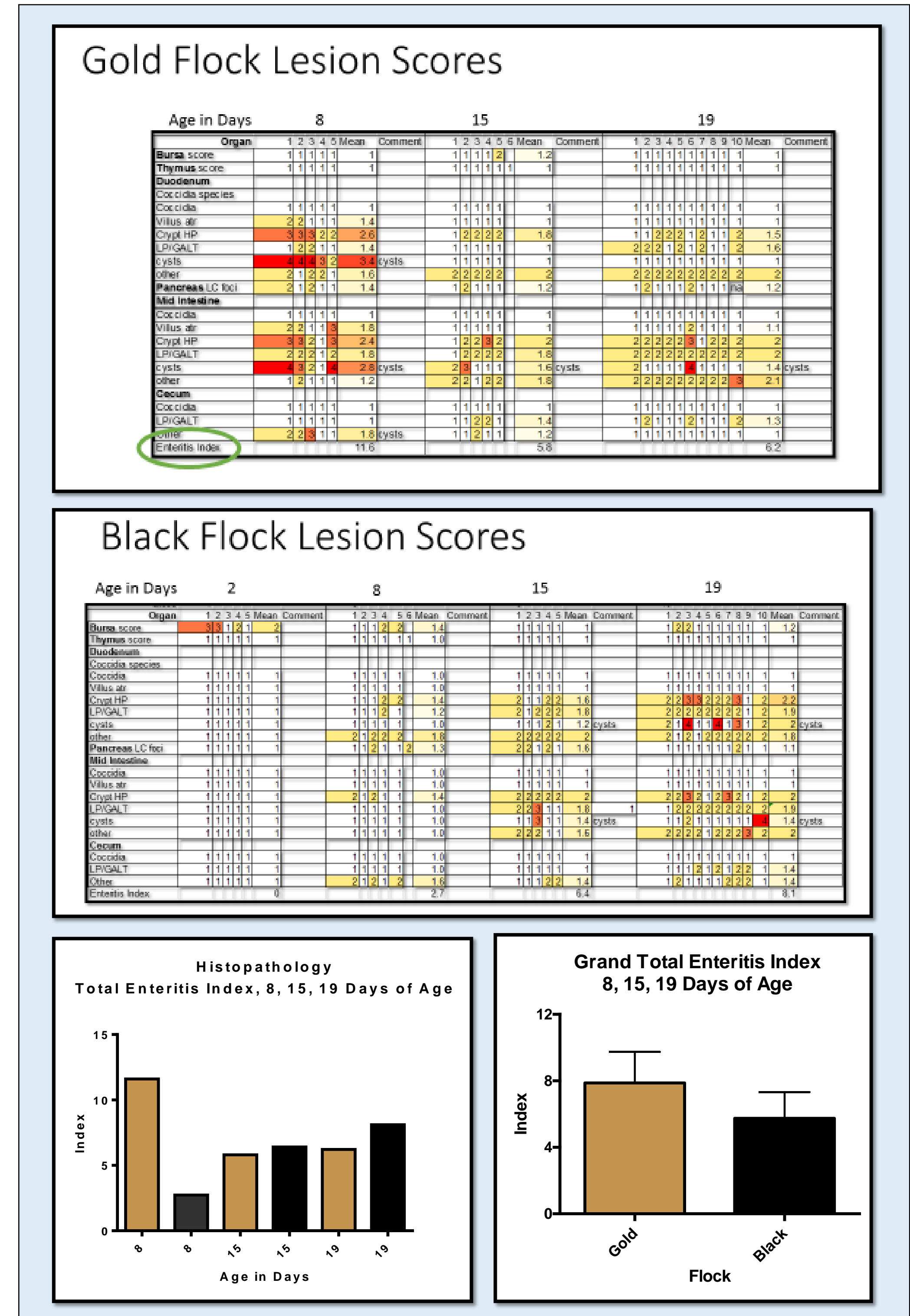
Method. Tissues are collected in neutral-buffered formalin from poultry in flocks at strategic ages, typically five birds per flock, and from specific locations in the gut. Studies can be designed with variable profiles of organs. For broilers and layers, key lesions for the small intestine are coccidiosis, villus shortening, crypt hyperplasia, lymphoid hyperplasia, bacteria, cystic crypts, and other, which includes intraepithelial leukocytes, excessive mucus, and heterophils. Parameters for cecum include coccidia, bacterial adherence, lymphoid hyperplasia, and other, which includes intraepithelial leukocytes, and dome epithelial changes. For turkeys, additional assessments are made for protozoa. Lesions are scored as 1, normal; 2, mild, 3, moderate, and 4, marked to severe, and each score is defined in writing to ensure reproducibility. Lesion indices for a single lesion or a cluster of lesions represent data transformed to remove normal scores for comparisons of lesion burden. Typical indices include coccidia, enteritis, and total gut lesion. Addition tissue can be added to the survey to reflect the needs of the program, including bursa of Fabricius, thymus, proventriculus, and gizzard.

Tissue Collection

- Tissue collected 2-5 minutes after euthanasia
- Neutral buffered formalin
- 10 formalin:1 tissue
- 1-2 cm sections, closed
- Consistent sites of collection
- Gently agitate formalin container to dislodge feed and allow formalin to enter gut

Study 1 Design. Bursa, thymus, duodenum, pancreas, jejunum (midgut), and cecum examined from flocks representing two different management systems for broilers. Tissues were collected from Gold flock at 2, 8, 15, and 19 days; and from Black flock at 8, 15, and 19 days.

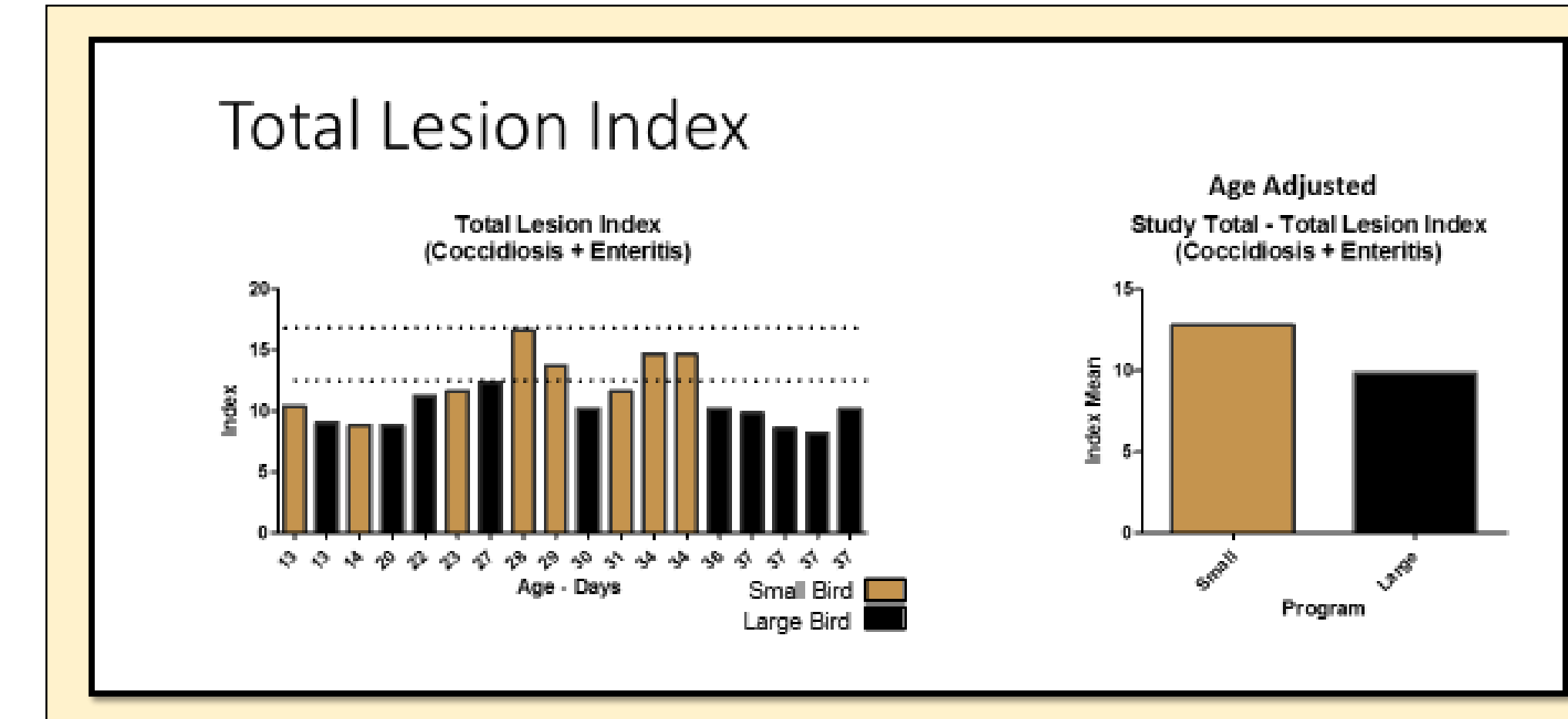
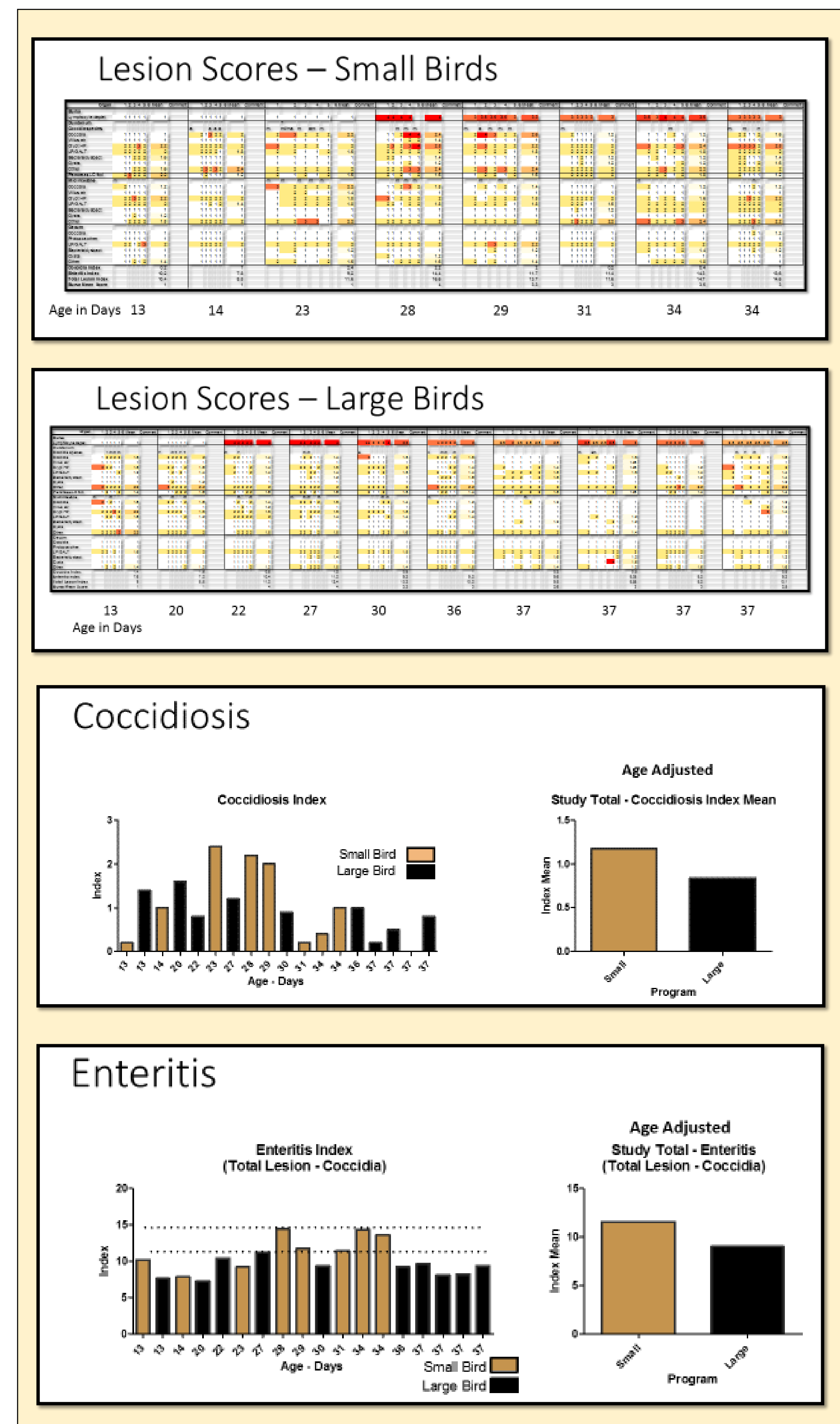
- **Total Gut Lesion Index** = (Sum of Intestine & Pancreas lesion means) – (total number of lesions scored)



Results Study 1. Coccidia were not observed in either flock. Both flocks developed enteritis consistent with viral enteritis: shortened villi, crypt hyperplasia, and cystic crypts. The lesions occurred at a younger age in the Gold flock but eventually occurred in the Black flock. The grand total enteritis lesion index for 19-day study was higher in the Gold flock, in agreement with lower production parameters (data not presented). The Black flock had nonspecific lymphocyte depletion from the bursa at 2 days age, but no differences occurred between flock at 8 to 19 days of age.

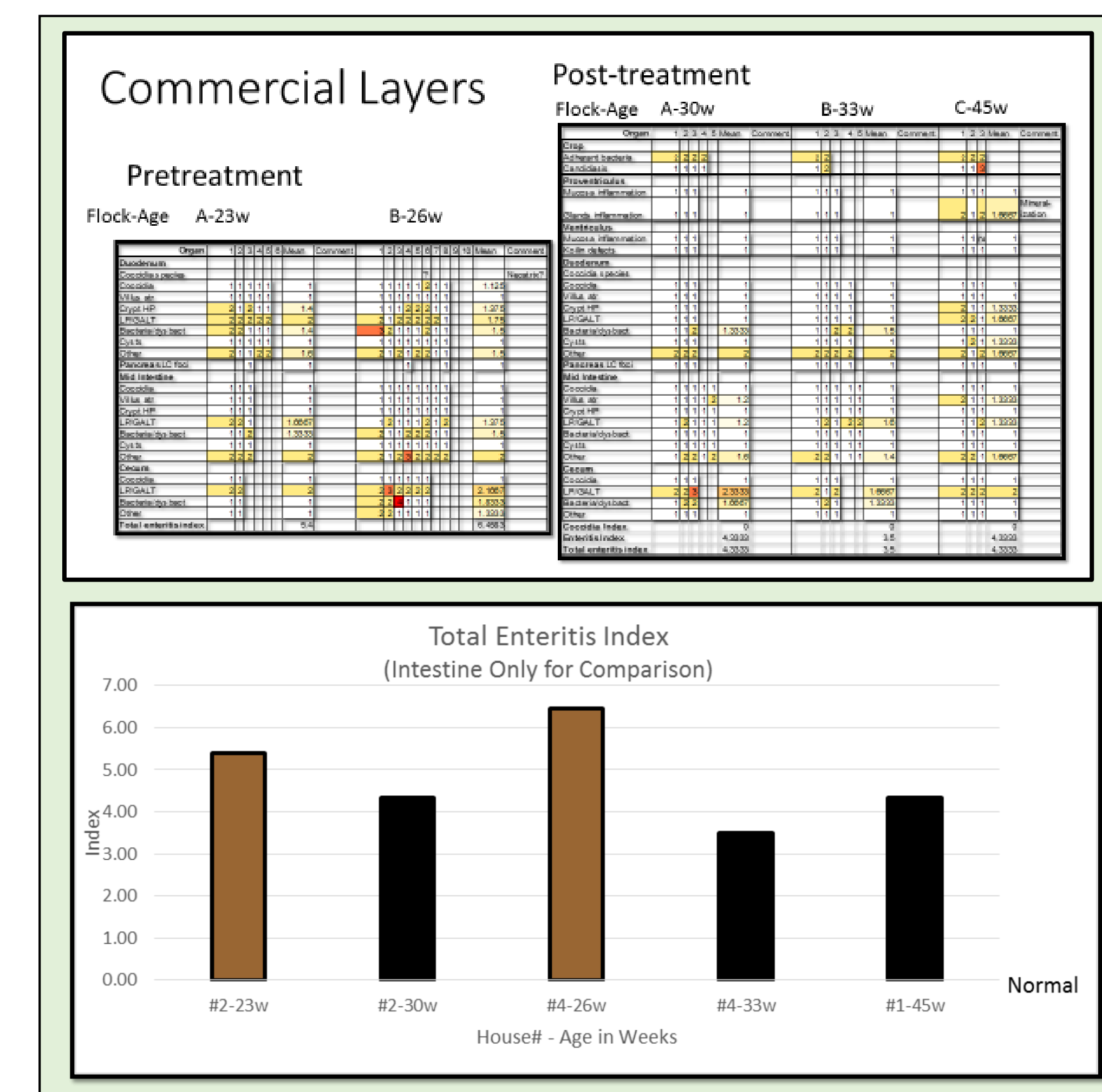
Study 2 Design. Bursa, duodenum, pancreas, jejunum, and cecum from broiler chickens were examined from flocks representing small bird and big bird programs, respectively. Tissues were collected from The Gold flock from 13 to 34 days of age, and the Black flock from 13 to 37 days of age. The study was read without knowledge of coccidia control programs.

- **Total Coccidia Index** = (Sum of Duodenum, Jejunum and Cecum Coccidia means, – 3 (levels scored)
- **Total Enteritis Index** = Total gut lesion index – total Coccidia Index
- **Total Gut Lesion Index** = (Sum of Intestine & Pancreas lesion means) – (total number of lesions scored)



Study 2 Results. The large birds developed acute necrotizing bursitis, consistent with infectious bursal disease at 22 days, and the small developed similar lesions at 28 days. The small birds had higher age-adjusted peaks in the coccidia index, enteritis index, and total gut lesion index. *Eimeria maxima* predominated in both flocks, with trace levels of *E. acervulina* and *E. tenella*. Enteritis was consistent with mild viral enteritis or the sequela to viral enteritis.

Study 3 Design. Commercial layers had lesions of enteritis with bacterial colonization of the tips and sides of villi, and mucosal folds in the cecum. Digestive tissues were examined during the problem and six weeks later following a mitigation procedure (specific information not provided).



Study 3 Results. Lesion the bacterial enteritis decreased in incidence and severity following the mitigation procedure. The histopathologic lesion index decreased in tandem with clinical signs of decreased severity.

Discussion. This histopathologic method is modeled on basic procedures in toxicological pathology. It involves sampling poultry of different ages and examining gut (and other tissues) with a moderately broad profile of lesions that can help to define the partitioning of nutrients away from growth and toward tissue damage, inflammation and repair. It can be done more rapidly than detailed measurements of gut parameters (cell counting, morphometric assessment and analysis) common to research environments. It has been used to assess differences in management, nutrition, and disease prevention and mitigation programs. Because each lesion and severity score is defined, it provides semiquantitative data that is reproducible and can be reanalyzed as the data collection expands over time. Although most applications to date have involved broiler chickens, it is applicable to breeders, commercial layers, turkeys, and other poultry.

Acknowledgements. The contributions of Marty Hoerr, Dr. John McCarty, Dr. Matilde Alphonso, Dr. Dan Wilson, and Dr. Joan Schrader are acknowledged and appreciated.

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