Light Turkey Syndrome: Field Study and Inoculation Trial

A THESIS
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

Alamanda Joy Calvert

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Sally L. Noll, Advisor

July 2012

© Alamanda Joy Calvert 2012

Acknowledgements

This thesis demonstrates the efforts of an interdisciplinary team composed of members from both animal science and veterinary medicine backgrounds. This project would not have been possible without Dr. Sally Noll's preparation and further development. Dr. Noll was a wonderful advisor whom I learned a great number of things from and was always there to guide me in the correct direction. In the turkey nutrition laboratory, employees including Jeanine Brannon, Wanda Weber and Igor Raodovic and student workers especially Nancy Warfield and Kathryn Meloche were directly involved with planning, sample collection and analysis. They made the numerous amounts of samples collected possible and made the process so much more delightful.

Outside of our laboratory the following people made the completion of this research possible. Yogesh Chander carried out the RT-PCR in Dr. Sagar Goyal's laboratory and fecal flotation methods used for the detection of astrovirus, rotavirus, reovirus and coccidia. Bacteria culturing was completed in Dr. Catherine Logue's laboratory at North Dakota State University by Julie Sherwood. Samples for the two North Dakota flocks were collected by Dr. Logue's laboratory staff. Tissues for histopathology were scored by Dr. Andre F. Ziegler. Dr. Ziegler and Dr Samuel Baidoo in addition to Dr. Noll served as my committee and I heartily thank them for their guidance. The Statistical Consulting Service at the University of Minnesota and in particular Dr. Aaron Rendahl, Patrick Zimmerman and Michael Soma collaborated on the statistical models for this thesis.

Dedication

First, I dedicate this thesis to my husband who has been there with me throughout this entire process.

Second, I dedicate this thesis to my godfather Lee Milligan (1954-2010) who witnessed the beginning of my journey and I am certain never doubted the completion of my degree. I will always remember him as the wonderful caring man in both his job as an agriculture extension agent and in his personal life.

Abstract

Light Turkey Syndrome (LTS) is characterized by lower than expected body weights of tom turkey flocks at market. Turkey producers have taken notice of LTS over the last five years. During brooding from two to three weeks of age is when weight gains begin to fall below what had previously been achieved. Speculation suggests that lighter poults are experiencing a different set of factors than the heavier weight poults in commercial flocks. The hypothesis for the field study was that poults from two weight groups (heavy and light) would have different histopathology scores for the intestine and immune tissue, different pathogens present and different xylose absorption. The objective of the inoculation trial was to determine if inoculated poults raised in research settings would exhibit similar attributes as the poults from the field study. In addition to the factors that had been looked at for the field study; weight gain, feed intake and feed conversion data were also determined. In both studies gut contents was collected for analysis by multiplex RT-PCR for astrovirus, rotavirus, reovirus and by culture methods for *Salmonella*, *Campylobacter*, *E. coli* and total plate counts for aerobic, anaerobic, lactobacilli and heterofermentative lactobacilli. Intestinal tissue was collected for scoring of heterophilic and lymphocytic infiltrates and select immune tissue was also scored. Xylose absorption was measured in plasma samples at zero and 60 minutes post gavage. Samples were collected at one, two and three weeks of age in the field study from four MN commercial flocks, two ND commercial flocks and two MN research flocks. Samples were collected at 14 days of age (seven days post inoculation) in the inoculation trial. For both studies more differences were seen between the different flocks than between the heavy and light weight groups. *Salmonella* and astrovirus were found in all flocks in the field study but reovirus was only found in two of the MN commercial flocks. In the field study histological differences were seen between weight groups with two flocks having an increased acute immune response in the light weight and heavy poults in all flocks showing increased lymphocytes in the intestinal tissue. For immune tissue lymphocytic necrosis and atrophy of the bursa were present in more light weight

poults than heavy weight poults. Xylose absorption was increased in heavy weight poults in three of the commercial flocks when compared to light weight poults. In the inoculation trial control poults had the best weight gain and feed conversion with poults gavaged with the inoculums from commercial flocks having the worst. Heterophilic infiltrates were the highest in the control poults and lymphocytic infiltrates were highest in the light weight poults. Lymphocytic necrosis was found in more of the light weight poults. No differences were seen in xylose absorption between the heavy, light and control groups in the inoculation trial. Light turkey syndrome cannot be easily defined by a specific pathogen's presence though a few different pathogens may likely play a role in the reduced weight gain seen in LTS poults. Histologically the gut and immune tissue indicate active immune responses that are decreasing the amount of nutrients available for growth of the bird. Nutrient absorption only appears to be negatively affected if the poult is actively showing signs of disease.

TABLE OF CONTENTS

TABLE OF CONTENTS v
LIST OF TABLES ix
LIST OF FIGURES xii
LIST OF APPENDIX TABLES xv

CHAPTER 1 1
INTRODUCTION 1

CHAPTER 2 2
LITERATURE REVIEW 2
Enteritis Syndromes 2
Poult Enteritis Syndrome. 2
Poult Enteritis and Mortality Syndrome. 4
Pathogens 5
Bacteria. 5
Viruses. 6
Protozoa. 8
Histopathology 9
Immune System. 9
Bursa. 10
Spleen. 11
Intestine. 11
D-Xylose Absorption 12
Chemical Analyses 12
Absorption and Metabolism. 12
Xylose Absorption Test In Poultry. 13

CHAPTER 3: FIELD TRIAL 18

INTRODUCTION 18

MATERIALS AND METHODS 18

Study Design 18

Sample Collection 19

Placement 19

Heavy or Light Weight Group Determination. 20

Gut Contents and Tissue Samples. 20

Plasma Collection. 21

Laboratory Procedures 23

Bacteria. 23

Virus and Protozoa (Eimeria sp). 25

Histopathology. 26

Xylose Assay. 26

Statistics 28

General Data Handling. 28

Ordinal Data. 29

Binomial and Quantitative Data. 30

Quantitative Data. 31

RESULTS 33

Weights 33

Virus 33

Bacteria 35

Protozoa 37

Histopathology of Intestinal Tissue 38

Density of Heterophilic Infiltrates. 38

Distribution of Heterophilic Infiltrates. 39

Density of Lymphocytic Infiltrates. 42

Distribution of Lymphocytic Infiltrates. 43

Histopathology of Immune Tissue 45
Bursa of Fabricius. 45
Spleen. 48
Xylose 49
DISCUSSION **52**
TABLES **58**
FIGURES **74**

CHAPTER 4: INOCULATION TRIAL **101**

INTRODUCTION **101**
MATERIALS AND METHODS **101**
Study Design 101
Sample Collection 102
Laboratory Procedures 103
Statistical Analysis 103
RESULTS **107**
Body Weights 107
Feed Intake and Conversion 107
Virus 108
Bacteria 108
Histopathology 109
Heterophilic Infiltrates in the Gut Tissue. 109
Lymphocytic Infiltrates in the Gut Tissue. 110
Immune Tissue. 111
Xylose 111
DISCUSSION **113**
TABLES **119**
FIGURES **122**

CHAPTER 5 .... **139**

SUMMARY **139**

REFERENCES **140**

APPENDIX **144**

LIST OF TABLES

CHAPTER 2

Table 2.1 Xylose absorption in PEMS and control poults (Doerfler et al., 2000)......... 14
Table 2.2 Xylose absorption for astrovirus inoculated and control poults, Experiment 1 (Reynolds and Saif, 1986)... 15
Table 2.3 Xylose absorption for astrovirus inoculated and control poults, Experiment 2 (Reynolds and Saif, 1986)... 15
Table 2.4 Peak Xylose levels (Goodwin et al., 1984)... 16
Table 2.5 Absorption of xylose from 30 to 180 minutes after administration (Goodwin et al., 1984) 17

CHAPTER 3: FIELD TRIAL

Table 3.1 Flock information for commercial and research poults 58
Table 3.2 Average sample flock weights 58
Table 3.3 Average body weight of poults used in field study broken apart by age and weight group 59
Table 3.4 Statistical results for virus presence... 59
Table 3.5 Percent of pools positive for astrovirus 60
Table 3.6 Percent of pools positive for rotavirus.. 60
Table 3.7 Percent of pools positive for reovirus 60
Table 3.8 Statistical results for bacteria presence... 61
Table 3.9 Percent of pools positive for *Salmonella spp*... 61
Table 3.10 Percent of pools positive for *Campylobacter spp*....................................... 61
Table 3.11 Statistical results for plate counts for bacteria 62
Table 3.12 Statistical results for aerobic and anaerobic bacteria.................................. 62
Table 3.13 Statistical results for lactobacilli and heterofermentative lactobacilli 62
Table 3.14 Average counts for aerobic bacteria 63

Table 3.15 Average counts for anaerobic bacteria 64
Table 3.16 Average counts for lactobacilli 65
Table 3.17 Average counts for heterofermentative lactobacilli 66
Table 3.18 Direction of change for bacteria counts from one to three weeks of age 67
Table 3.19 Bacteria plate counts from samples at placement 67
Table 3.20 Flocks listed had significantly higher counts than those that are not listed. 67
Table 3.21 Statistical results for presence of coccidia from histopathology 68
Table 3.22 Score levels for heterophilic and lymphocytic infiltrates 68
Table 3.23 Heterophilic density statistical results 68
Table 3.24 Heterophilic distribution statistical results 69
Table 3.25 Lymphocytic density statistical results 69
Table 3.26 Lymphocytic distribution statistical results 69
Table 3.27 Bursa statistical results 70
Table 3.28 Spleen statistical results 70
Table 3.29 Average body weights for poults used for xylose analysis 70
Table 3.30 Average xylose absorption at 60 minutes 71
Table 3.31 Statistical results for xylose absorption at 60 minutes 71
Table 3.32 Average xylose absorption prior to gavage 71
Table 3.33 Differences between heavy and light weight groups for D-xylose concentrations found in plasma collected from poults 60 minutes after being gavaged with a 5 % D-xylose solution 72
Table 3.34 Differences between two and three week old poults for D-xylose concentrations found in plasma collected from poults 60 minutes after being gavaged with a 5 % D-xylose solution 72
Table 3.35 Differences between heavy and light weight groups for D-xylose concentrations found in plasma collected from poults before being gavaged with a 5% D-xylose solution 72

Table 3.36 Differences between two and three week old poults for D-xylose concentrations found in plasma collected from poults before being gavaged with a 5% D-xylose solution 73

CHAPTER 4: INOCULATION TRIAL

Table 4.1 Body weight, gain, feed intake and feed conversion for 12 treatment groups and control group 119
Table 4.2 Statistical results for body weight, gain, feed intake and feed conversion .. 119
Table 4.3 Individual poult body weights per pen at 13 days of age 120
Table 4.4 Average daily gain per poult per day from 7 to 13 days of age 120
Table 4.5 Average feed intake per poult per day from 7 to 13 days of age 120
Table 4.6 Feed conversion per poult per day from 7 to 13 days of age 121
Table 4.7 Baseline values for xylose concentration from 15 day old poults prior to gavage 121
Table 4.8 Xylose concentration in plasma from 15 day old poults 60 minutes after gavaging with 5 % D-xylose solutions 121

LIST OF FIGURES

CHAPTER 3: FIELD TRIAL

Figure 3.1 Picture of gut sections collected for contents and histopathology 74
Figure 3.2 Duodenum from three week old light weight poults 75
Figure 3.3 Average poult weights over age 76
Figure 3.4 Virus presence among flocks.. 77
Figure 3.5 Astrovirus presence among flocks... 77
Figure 3.6 Astrovirus presence over age... 78
Figure 3.7 Bacteria presence among flocks 78
Figure 3.8 *Salmonella spp.* presence among flocks 79
Figure 3.9 Total plate counts for bacteria 79
Figure 3.10 Aerobic bacteria by flock and age 80
Figure 3.11 Anaerobic bacteria by flock and age 80
Figure 3.12 Lactobacilli by flock and age 81
Figure 3.13 Heterofermentative lactobacilli by flock and age...................................... 81
Figure 3.14 Lactobacilli in Flock 4 by weight group.. 82
Figure 3.15 Coccidia present in gut tissue samples by flock.. 82
Figure 3.16 Coccidia present in gut tissue samples over age 83
Figure 3.17 Distribution of heterophilic infiltrates in the jejunum in Flocks 3 and 8 by weight group 83
Figure 3.18 Distribution of heterophilic infiltrates in the jejunum in Flocks 3, 5, 6 and 8 over age 84
Figure 3.19 Distribution of heterophilic infiltrates in the ileum and ceca over age 84
Figure 3.20 Distribution of heterophilic infiltrates in the duodenum and ileum by flock 85
Figure 3.21 Distribution of heterophilic infiltrates in the jejunum by flocks 86
Figure 3.22 Density of lymphocytic infiltrates in the ileum and ceca by weight group 87

Figure 3.23 Density of lymphocytic infiltrates in the jejunum and ceca over age 87
Figure 3.24 Density of lymphocytic infiltrates in the jejunum by flock........................ 88
Figure 3.25 Distribution of lymphocytic infiltrates in the jejunum at 2 weeks and ileum by weight group 88
Figure 3.26 Distribution of lymphocytic infiltrates over age 89
Figure 3.27 Distribution of lymphocytic infiltrates over flock..................................... 90
Figure 3.28 Density and distribution of heterophilic infiltrates in the bursa over age .. 91
Figure 3.29 Density and distribution of heterophilic infiltrates in the bursa by flock... 91
Figure 3.30 Distribution of lymphocellular depletion in the bursa by weight group 92
Figure 3.31 Distribution of lymphocellular depletion in the bursa over age 92
Figure 3.32 Density and distribution of lymphocellular depletion in the bursa by flock 93
Figure 3.33 Atrophy of the bursa at three weeks of age by weight group 93
Figure 3.34 Atrophy of the bursa for light weight poults over age............................... 94
Figure 3.35 Atrophy of the bursa by flock... 94
Figure 3.36 Lymphocellular depletion in the spleen for Flocks 4 and 7 95
Figure 3.37 Germinal center hyperplasia and red pulp abnormalities in the spleen over age 96
Figure 3.38 Lymphocellular depletion, red pulp abnormalities and ellipsoid necrosis in the spleen by flock 97
Figure 3.39 Body weights of poults used for D-xylose absorption 97
Figure 3.40 Xylose in plasma from poults prior to gavaging 98
Figure 3.41 Xylose in plasma from poults 60 minutes after gavaging by flock............ 98
Figure 3.43 Xylose in plasma from poults 60 minutes after gavaging in flocks 1and 6 by weight group 99
Figure 3.44 Xylose in plasma from poults 60 minutes after gavaging in Flocks 1, 3, 5 and 6 by age 100
Figure 3.45 Xylose in plasma from poults 60 minutes after gavaging in Flock 4 by age 100

CHAPTER 4: INOCULATION TRIAL

Figure 4.1 Body weights from seven and 13 day old poults 122
Figure 4.2 Body weights at 13 days of age (6 days post inoculation) 122
Figure 4.3 Average daily gain per poult from seven to 13 days of age 123
Figure 4.4 Feed intake per poult per day from seven to 13 days of age 123
Figure 4.5 Feed conversion from seven to 13 days of age 124
Figure 4.6 Astrovirus presence 125
Figure 4.7 Rotavirus presence 126
Figure 4.8 Reovirus presence 127
Figure 4.9 *E. coli* presence 128
Figure 4.10 *Campylobacter* presence 129
Figure 4.11 *Salmonella* presence 129
Figure 4.12 Bacteria total plate counts 130
Figure 4.13 Lactobacilli total plate counts by weight group 131
Figure 4.14 Anaerobic bacteria by flock 131
Figure 4.15 Heterophilic density in the jejunum by weight groups 132
Figure 4.16 Heterophilic distribution in the ileum by weight groups 132
Figure 4.17 Heterophilic density and distribution in the ileum and ceca by flock 133
Figure 4.18 Lymphocytic density and distribution in the duodenum by weight group 134
Figure 4.19 Lymphocytic distribution in the duodenum and ceca by flock 135
Figure 4.20 Distribution of lymphocytic necrosis in the bursa by weight group 135
Figure 4.21 Distribution and density of lymphocytic necrosis in the bursa by flock .. 136
Figure 4.22 Xylose concentrations from 15 day old poults 137
Figure 4.23 Xylose concentrations prior to gavaging by weight group 138
Figure 4.24 Xylose concentrations 60 minutes after gavaging by flock 138

LIST OF APPENDIX TABLES

Table 1 Presence of coccidia in duodenum from field study 144
Table 2 Presence of coccidia in jejunum from field study 145
Table 3 Presence of coccidia in ileum from field study 146
Table 4 Presence of coccidia in ceca from field study 147
Table 5 Heterophilic density in the duodenum from field study 148
Table 6 Heterophilic density in the jejunum from field study 149
Table 7 Heterophilic density in the ileum from field study 150
Table 8 Heterophilic density in the ceca from field study 151
Table 9 Heterophilic distribution in the duodenum from field study 152
Table 10 Heterophilic distribution in the jejunum from field study 153
Table 11 Heterophilic distribution in the ileum from field study 154
Table 12 Heterophilic distribution in the ceca from field study 155
Table 13 Lymphocytic density in the duodenum from field study 156
Table 14 Lymphocytic density in the jejunum from field study 157
Table 15 Lymphocytic density in the ileum from field study 158
Table 16 Lymphocytic density in the ceca from field study 159
Table 17 Lymphocytic distribution in the duodenum from field study 160
Table 18 Lymphocytic distribution in the jejunum from field study 161
Table 19 Lymphocytic distribution in the ileum from field study 162
Table 20 Heterophilic density in the bursa from the field study 163
Table 21 Heterophilic distribution in the bursa from the field study 164
Table 22 Lymphocytic necrosis, density in the bursa from the field study 165
Table 23 Lymphocytic necrosis, distribution in the bursa from the field study 166
Table 24 Atrophy of the bursa from field study 167
Table 25 Lymphocellular necrosis of the spleen from the field study 168
Table 26 Germinal center hyperplasia in the spleen from field study 169
Table 27 Ellipsoid necrosis in the spleen from field study 170

Table 28 Abnormal red pulp in the spleen from field study 171
Table 29 Percent of pools positive for rotavirus and astrovirus in the inoculation trial 172
Table 29 Percent of pools positive for *E. coli* 173
Table 30 Average counts for bacteria (log_{10} cfu/g) 173
Table 31 Heterophilic density in the inoculation trial 174
Table 32 Heterophilic distribution in the inoculation trial.. 175
Table 33 Lymphocytic density in the inoculation trial 175
Table 34 Lymphocytic distribution in the inoculation trial 176
Table 35 Bursa density and distribution scores in the inoculation trial 176
Table 36 Bursa atrophy scores in the inoculation trial 177
Table 37 Spleen scores in the inoculation trial 177

Chapter 1

INTRODUCTION

Light weight turkey syndrome (LTS) is characterized by flocks with lower market weights than expected. It is seen primarily in heavy tom flocks partially because toms are raised to an older age (18-22 weeks) than hens (12 weeks of age). The lower market weights are possibly a sign of the uneven growth that is apparent over the bird's life time. In the previous five years turkey producers have noticed and named this syndrome in Minnesota flocks. Retrospective examination of a producer's records indicate that the differences between flock body weight and genetic potential for body weight starts in brooding and thereafter the birds never seem to recover from the lost weight gain (S. Noll, communicated). Speculation exists that the less than desired performance is related to factors occurring during brooding but this syndrome is not well understood and the causes are unknown. Possible factors of LTS range from producer practices, pathogen presence, microbial communities, nutrient absorption, gut health and immune function of poults. The possible factors that this research is interested in are primarily related to the gut of young poults and secondarily to the immune response. These include nutrients absorbed, microbes present and immune tissue in the intestine and immune organs. Previous studies have hypothesized that poult enteritis syndrome may predispose birds to developing LTS (Kumar et al, 2011). Our preliminary studies include the analysis for enteric virus presence but also look at LTS from several broad points of view to see if more general knowledge of this syndrome can be determined relative to differences between heavy and light weight poults in the same flocks. This study hopes to set the ground work for future in depth studies that could look more closely at the individual factors.

Chapter 2

LITERATURE REVIEW

Poult enteritis complex (PEC) is a group of syndromes that affect turkeys and poults. Some symptoms of PEC appear to be similar to poults with light turkey syndrome (LTS). Poult enteritis complex is described as infectious intestinal enteritis in which poults have decreased growth, feed usage and possible increased mortality (Jindal et al., 2009b). Poult enteritis complex decreases the poultry producers profit primarily because of the decreased gains. A decreased weight gain of only 10-15% could cost the US turkey industry 300 to 400 million dollars annually (Barnes, et al., 2000). Regardless of the cause of LTS, the possible economic losses of poor gain as indicated above makes the understanding of LTS a very important challenge for the MN turkey industry.

Enteritis Syndromes

Poult Enteritis Syndrome. Poult enteritis syndrome (PES) is believed by some researchers to be a contributing factor to LTS (Jindal et al., 2009a). PES has been described in MN by Jindal et al. (2009a; 2009b; 2009c; 2010) and primarily affects poults under 3 weeks of age with symptoms including decreased growth, enteritis, pale watery intestinal tissue, dullness, and clinical signs of diarrhea, lethargy and depression. A similarity with LTS is that high levels of mortality are not normally associated with PES.

A retrospective study was conducted in MN to look at poults with PES symptoms to determine the pathogens present (Jindal et al., 2009b). The pathogens detected in the greatest number of samples were rotavirus and *Salmonella* with others including *Enterococcus*, *E. coli*, reovirus, small round virus (possibly Astrovirus) and *Eimeria*. Flocks with PES were more likely to have two or more pathogens present (68.2%) and be under three weeks of age (73.5%). Poults less than three weeks of age were more likely to have more types of pathogens present than poults from four to nine weeks of age. Pathological changes noticed in the PES affected poults included thin intestines and distended ceca with watery contents in both. The histological changes in the intestine

included inflammatory infiltrates of either heterophilic, plasmacytic or lymphocytic cells; necrosis and clumping of the distal villi tips. Lymphocellular depletion was present in the bursa, thymus and spleen. Over the six years of this retrospective study, the authors speculated that there was a higher occurrence of PES in the fall because of the warm temperatures during summer that lead to a proliferation of pathogens.

Viruses were detected from 44 PES positive flocks using reverse transcription-PCR (RT-PCR) and electron microscopy (EM) (Jindal et al., 2010; Jindal et al., 2011). Electron microscopy was found to under report the presence of viruses; therefore, RT-PCR was relied upon for results. The percent of samples positive by PCR for rotavirus, astrovirus and reovirus were 93, 84 and 40 % respectively. Combinations of two or three different viruses were present in 81% of flocks. No coronavirus was detected in any of the samples. There were 37 positive flocks for rotavirus, 32 of them were similar to previously described rotavirus from MN and WI with the other five being similar to strains from Missouri. Of the 33 flocks positive for astrovirus, all were turkey astrovirus type 2 (TAstV-2), with 30 of them in one group and three in a separate group based on differences in the genetic material. Only ten TAstV-2 have been previously published with half of them genetically similar to each other and the remaining half genetically similar to themselves. For reovirus, 13 flocks were positive with nine similar to strains from Missouri and the other four similar to strains from Wisconsin.

In another study conducted by Jindal et al. (2009c) three week old poults were inoculated with intestinal contents from poults affected with PES to see if the syndrome could be experimentally reproduced. Average growth depression over the five trials after one week post inoculation was 32% and was most severe for poults inoculated with sediment from the intestinal contents in four of the five trials. Astrovirus, rotavirus and *salmonella* were identified in all inoculants from PES positive poults. Enterovirus was detected in two of the inoculums but only in one trial of inoculated poults. Astrovirus and rotavirus were detected in all of the treated poults but each trial had different sampling times. *Salmonella* was found in some of the treated groups but not in all of them. Coronavirus was not found in any of the inoculums or trial poults and *Eimeria sp.* was

only found in one of the experiments at five days post inoculation with unfiltered supernatant. Inoculum from two week old poults expressed the same PES symptoms in poults as inoculum from poults up to six weeks of age. No mortality was observed in any of the trials.

In another research study similar to the above, poults were inoculated at 14 days of age and samples were taken every 10 to 50 days post inoculation (Jindal et al., 2009a). This was completed to look at the affect on growth reduction over a longer time period. Rotavirus, astrovirus, and *salmonella* were present in the inoculums used. Astrovirus was only detected at 10 days post inoculation for all three groups whereas rotavirus was detected in poults of each group for at least two time points between 10 and 40 days post inoculation. No *salmonella* was isolated from any poults. The weight depression was not as severe in this study, ranging from 8-18%. However, the intestines were pale and distended with watery contents.

Poult Enteritis and Mortality Syndrome. Poult enteritis and mortality syndrome is more severe than PES as it is characterized by higher mortality rates. PEMS is usually caused by coronavirus and is identified by high mortality and decreased growth and immune function (Saif, 2003). PEMS affects poults of one to four weeks of age and mortality is higher than 2% in one form called Excess Mortality of Turkeys (EMT) and higher than 9% in a more severe form called Spiking Mortality of Turkeys (SMT). PEMS was first discovered in North Carolina in 1991 where it caused financial losses and decreases in turkey production of 13 to 63%. This syndrome is similar to PES in that it is multifactorial and there seems to be an additive affect of coronavirus with either astrovirus or *E.coli*. Multiple studies found that one agent by itself only produces mild diarrhea with no mortality whereas adding an additional agent or inoculums from PEMS positive poults induces a high mortality. The above information along with the observation that not one of these pathogens has been found in every flock affected with PEMS, seems to indicate that it is an additive disease with possibly other agents that have not been fully identified. Histological changes of infected poults include lesions found in the small intestine and bursa. The epithelial cells seem to be the center of attack. The

cells display atrophy, hyperplasia and sloughing of cells with necrotic macrophages in the lamina propria and heterophils in the lumen of the tissue. Bursal cores made up of caseous exudates are present in some poults affected with the coronavirus version of PEMS. The one thing that defines all cases of PEMS is the presence of high mortality from an unknown cause.

Pathogens

The agents associated with poult enteritis complex include astrovirus, reovirus, rotavirus, *Salmonella*, *Campylobacter*, *E. coli* as well as many others. (Barnes et al., 2000; Jindal et al., 2009a) . These pathogens can damage the gut tissue and have a negative impact on growth in addition to causing disease symptoms in poults.

Bacteria. *Salmonella* can cause disease in young poults and is also of interest because of food safety concerns. The motile *Salmonella* are referred to as paratyphoid salmonellae and are from the family Enterobacteriaceae which can be divided into five biochemically distinct subgenera (Saif et al., 2008). However, because of similarities between the subgenera some argue that there are only two species. The bacteria are straight rods, non-spore forming and can grow in both aerobic and anaerobic conditions. Their viability can be maintained for several years. The route of transmission can be either vertical or horizontal. Once they have infected an animal, salmonellae must adhere to the epithelium of the intestine and then invade the mucosa (Saif et al., 2008). The toxins produced from the bacteria can cause fluid buildup in the epithelium of the intestine causing edema. Young poults are highly susceptible to salmonellae but as the bird grows and more microflora are present in the maturing gut the competition between organisms decreases the poults susceptibility to infection. *Salmonella* first invades the intestinal tract but extensive bacteremia can occur if the *Salmonella* crosses over the intestinal barrier using the macrophage-phagocyte process and then infects internal tissues including the liver and spleen. If this occurs, the morbidity and mortality can be very high. Some *Salmonella* can be vertically transmitted into the egg if the hen's infection has moved to the ovary and oviduct (Gast and Beard, 1990). Coccidia can increase the ability of *salmonella* to infect the poult. Below optimal brooding

temperatures and decreased water access have been associated with an increase of disease symptoms. The clinical signs of *salmonella* infection include drooping wings, ruffled feathers, closed eyes and anorexia.

Campylobacter spp. specifically *Campylobacter jejuni* are associated with the highest number of human enteritis cases and can also cause diseases in poults (Saif et al., 2008). These bacteria are thermophilic, do not survive well below 0°C and need microaerophilic conditions to grow in. Infections of *Campylobacter* are widespread throughout the poultry industry being found in chickens and turkeys all over the world. Horizontal transmission, from bird to bird is seen but vertical transmission, from hen to poult has not been proven. Diarrhea and watery fluid in distended intestines are general signs of an infection.

Colibacillosis is caused by *Escherichia coli* and is a localized or systemic disease in poultry. *E. coli* is part of the Enterobacteriaceae family and can grow in both aerobic and anaerobic conditions using simple carbon and nitrogen sources for food (Saif et al., 2008). *E. coli* is a gram negative bacterium, rod shaped and non-spore forming. The infection is most likely transmitted on egg shells from passing through the coprodeum of the hen and has a high rate of transmission between poults after hatch. In localized infections it can cause yolk sac infection. *E. coli* only rarely causes enteritis in poultry and has not been studied much. In cases where enteritis is present a primary infection or coinfection with another pathogen is common. In these cases *E. coli* is a secondary infection that would not have occurred if the bird had not first been infected with another pathogen.

Viruses. Astrovirus has been associated with acute gastroenteritis in turkeys and is normally found in poults younger than five weeks of age. Flocks undergoing an enteric disease are more likely to have astrovirus present than healthy flocks but astrovirus has been found in apparently healthy flocks (Saif et al., 2008). Astrovirus is small, only 25-35 microns and is named after its star-like shape. The virus is nonenveloped and contains 3 open RNA reading frames. Astrovirus is very stable even to acids, chloroforms, heat, lipid solvents and most alcohol solutions. Formaldehyde and 90% methanol will disinfect

a surface (Saif, 2003). Infection normally appears between one to three weeks of age and lasts up to two weeks with symptoms of decreased growth and diarrhea. Cecal and intestinal contents can be frothy and watery; gut tissue appears thin. The diarrhea is related to undigested and unabsorbed disaccharides that attract water into the lumen. Astrovirus may leave the system before the clinical signs and pathologic changes have gone away. The transmission is assumed to be fecal to oral and it is unknown if maternal antibodies can provide protection. If poults are given both astrovirus and type D rotavirus, astrovirus will be shed before the rotavirus.

Rotavirus is known as a common cause of enteritis and is mostly associated with diarrhea and some mortality (Metz, 1986). Rotavirus is in the family Reoviridae as their own genus. Rotavirus virions have a core surrounded by a capsid made up of two shells. If the outer shell of the virus capsid is lost then the virus will lose much of its virulence (Saif, 2003). It possesses a double stranded RNA genome consisting of 11 segments. Pathogenicity is mainly from the inner and outer layers of the capsid. Rotavirus types A, D, F and G have been found in birds but only types A and D have been seen in turkeys along with an additional strain that has not been classified. Electrophoresis classifies the rotavirus found in turkeys into groups one, two, three and five. Rotavirus has not been shown to differ in virulence between different types. Naturally occurring infections usually begin before six weeks of age. Horizontal transmission occurs but vertical transmission has not been demonstrated. The most likely route for three day old poults that have tested positive for rotavirus is the eggshell. The incubation period is short between 1-5 days and the symptoms include abnormal amounts of gas and fluid found in the intestines and ceca with pale and thin intestinal walls. Rotavirus replicates in the cytoplasm of mature epithelial cells of the villi in the distal third of the intestine as seen in SPF turkey poults (Saif , 2003). Infected poults absorptive columnar cells are mostly composed of immature cells during an infection. The cells they are replacing are being destroyed by the virus and there is not enough time for the cells to fully differentiate and mature. Undigested carbohydrates and sugars remaining in the intestines may cause the presence of frothy fluids. The undigested substances are fermented by cecal bacteria

which then draw water into the ceca by osmosis which creates the symptoms of diarrhea. Maternal antibodies do not appear to protect poults from infection unless the hens were hyper immunized and then the protection will only last for the first week of life.

Avian reovirus is in the *orthoreovirus* genus which is in the Reoviridae family, the same family as rotavirus. It similarly has a double shelled capsid and is nonenveloped with 10 double stranded RNA segments (Joklik, 1981). It ranges in size from 50 to 75 nm. The virus replicates in the cytoplasm of infected cells and has been associated with uneven growth and mortality. Reovirus has been found in healthy poultry with about 80% of the cases having viruses left over from vaccinations and not pathogenic. Reovirus has been found in poultry with enteric symptoms and there is a possible increased infection when other pathogens are present. High mortality has been seen in turkeys (Yersin, 1990). Non pathogenic and enteritis inducing strains of reovirus have been found in turkeys. Immunization of hens can protect poults as well as vaccinating poults at one day of age. The transmission can be horizontal with the virus being shed from intestinal or respiratory tracts and possibly vertically (Saif, 2003). It has also been shown that poults infected with rotavirus absorb less D-xylose than normal poults up to eight days of age (Hayhow and Saif, 1993).

Protozoa. Coccidiosis is a disease that can be present in all young animals including poultry. It is caused by the protozoa *Eimeria* with four different species that are important because of the economic cost to producers (Saif et al., 2008). The different species are classified based on their morphology and location in the gastrointestinal tract where they tend to infect the host. They can cause weight loss and diarrhea in poultry but symptoms are less severe in turkeys compared to chickens. As the bird ages they recover faster from the disease and their susceptibility to infection is reduced. Prevention of poults from *Eimeria sp.* includes the use of coccidiostats in the feed up to eight weeks of age or the application of an immunization of poults during the first week of brooding. The life cycle of *Eimeria sp.* begins with the ingestion of oocyst by the bird. The oocysts are crushed in the gizzard which releases sporozoites. Two to four cycles of asexual reproduction occur after the sporozoites invade the cells of the intestine. This then leads

to the sexual phase where microgametes and macrogametes form zygotes inside the cells of the intestine. Once the zygote has matured into an oocyst it is released from the intestinal cells to be shed in the feces and restart the cycle. The time of most damage to the tissue is usually during the last asexual phase because of the rupture of the intestinal cells. The gametocytes do not cause as much direct cell destruction but they can elicit an immune response which will lead to inflamed tissue.

Histopathology

Immune System. In poultry the immune system includes primary immune organs, secondary immune organs and secondary immune tissue located throughout the body. Our research focused on the bursa, spleen, small intestine and cecal tissues. The bursa is a primary and the spleen a secondary organ of the poult's immune system. In addition, secondary immune tissue is found in the intestine and ceca and is referred to as gut associated lymphoid tissue (GALT), Peyer's patches and cecal tonsils (Whittow, 2000). These lymphoid aggregates are very important to the bird's immune system as they do not have specific lymph nodes as seen in other mammals.

The bird's immune response is composed of two different systems; innate and acquired. The development of the innate immune system does not require high levels of energy or nutrients as it is nonspecific. The use of the acute disease response is very costly for the bird and requires high amounts of energy and nutrients when implemented. When an injury or infection occurs the innate system is the first to respond with heterophilic infiltrates, macrophages and primitive non specific lymphocytes (Klasing, 2007). An active immune system will require six times more lysine then if the poult was not undergoing an immune challenge. Additionally, bursal cells have a higher priority for nutrients than other processes. Therefore if the poult is undergoing a challenge, nutrients available for growth and development will most likely not be adequate as the poult will be directing the nutrients to deal with the immune challenge. The acute inflammatory system is the most costly part of this system because it is unfocused. Although it can effectively kill bacteria it will also cause damage to the surrounding tissue because of the release of granulocytes. Heterophils play a primary role in this acute response and are

active within the first 6 to 24 hours after an infection is detected. These heterophils also are part of the innate immune response that is so crucial to young poults since they have not had time to develop their acquired immune system (Harmon, 1998).

The acquired immune response uses a lot of energy and nutrient resources to develop but once the body has refined the defensive mechanisms against a disease it requires only a small amount of energy to put it into use. The acquired response is not effective for first time infections but with secondary infections or those that last longer than a week, it is very successful in responding and protecting the body against further insult (Klasing, 2007). The primary mechanism of the acquired immune response is through the use of lymphocytes that target specific antigens. The specificity is developed in the thymus (T-cells) and bursa (B-cells) when antigens are provided to a large number of differently coded lymphocytes. The lymphocyte that binds to the antigen are then changed into longer living B cells similar to memory B-cells in mammals.

Bursa. The bursa of Fabricius is made up of 8,000 to 12,000 follicles (Oláh and Glick, 1978) . Each follicle has a cortex and a medulla which are similar to a lymph node's germinal center (Malewitz, 1958; Ackerman and Knouff, 1959). The bursa is the site of B lymphocyte maturation. After hatch, intestinal content comes in contact with the lumen of the bursa providing antigens that enter each individual follicle through the follicular associated epithelium. Once in the medulla the antigen helps stimulate the production of B lymphocytes. Once the chosen B-lymphocytes are mature they move into the cortex of the follicle to proliferate before being sent out to the secondary immune tissue (Whittow, 2000; Arakawa et al., 2002; Davison et al., 2008; Hodges, 1974). Poults affected with poult enteritis syndrome (PES) show lymphocellular depletion of the bursa follicles (Jindal et al., 2009b). It is believed that depletion of lymphocellular organs decreases the poults immune response capabilities and predisposes them to other infectious agents. The functioning of the bursa declines within the first few months of age based on the maximum weight of the bursa being achieved by eight weeks of age followed by a decline in weight thereafter (Glick, 1956).

Spleen. The spleen plays an important role in B cell rearrangement before the lymphocytes migrate to the bursa during incubation (Masteller and Thompson, 1994). The spleen is composed of white pulp which contains lymphocytes and red pulp which primarily includes eosinophils and macrophages (Malewitz, 1958). Within the white pulp there are regions that are thymic and bursal dependent. The bursal dependent areas of the spleen include both the periellipsoid white pulp and the germinal centers (Oláh and Glick, 1982; Whittow, 2000). After hatch and as the bursa and thymus begin involution B lymphocytes migrate from the bursa to the spleen. These post bursal lymphocytes in the spleen are the beginning of the production of a population of B cells that are produced at a steady rate and become the primary supply of B-lymphocytes after bursa degeneration (Paramithiotis and Ratcliffe, 1994). The maximum size of the spleen compared to body weight is reached by 10 weeks of age with the greatest rate of growth in the first six weeks (Norton and Wolfe, 1949).

Intestine. The small intestine in a young poult goes through rapid development and change in the first few weeks of life. Gut associated lymphoid tissue is important in supplying the immune protection to the intestinal tract (Malewitz and Calhoun, 1958). Maturation of the GALT occurs in two stages, the first stage occurs during the first week after hatch and the second during the second week of age (Bar-Shira, et al, 2002). Both stages are characterized by an increase of cytokine mRNA. The second stage also begins with the increase of CD3+ cells. It is unclear if the immune tissue development begins in the distal or proximal intestine. The same study with chickens found an increase of B cells in the small intestinal GALT at four days of age which was equal to the level seen in the cecal tonsils and hind gut at six days of age (Bar-Shira, et al, 2002). In contrast, in this same study, higher levels of chicken Interleukin 2 was seen in the distal parts of the intestine in chicks at hatch but was similar in all parts of the intestine by four days of age. The small intestinal epithelium consists of several different layers of tissue including the mucosa, submucosal, and muscular layers. The immune tissues are located in the lamina propria throughout the intestine (Sturkie and Benzo, 1986). The small intestine is lined by simple columnar epithelium and goblet cells. Villi are present from the duodenum to the

ceca and decrease in height as you move distally. The small intestine is the primary location of digestion and absorption. If the intestinal cells are damaged by pathogens new cells must replace them. These new cells are usually immature cells that have not fully developed into the specific cells that the gut requires for the release of mucous for the protection of the gut lining and enzymes for the digestion of feed.

D-Xylose Absorption

Chemical Analyses. Eberts et al. (1979) describe a simplified micromethod using phloroglucinal as the color reagent instead of *p*-bromaniline (Roe and Rice, 1947). The phloroglucinal method requires only 4 minutes in a hot water bath where as the previous method required up to 60 minutes. The phloroglucinal reacts with the furfural that is released when xylose is heated with hydrocholoric and acetic acid (Trinder, 1975). Under these conditions glucose in the sample is not converted well enough to conflict with the xylose. The procedures that we followed were developed by Goodwin et al (1983) which he called the modified micromethod. This used the same color reagent and 4 minute hot water bath but the amounts of samples were decreased to only need 20 µl of plasma so that the test could be carried out on young birds.

Absorption and Metabolism. D-Xylose has been used as a measure of absorption capacity in horses, dogs, humans and rats (Butterworth et al., 1959; Levitt et al., 1969) and more recently in turkeys and broilers (Goodwin et al., 1984; Goodwin et al., 1985). D-Xylose and D-glucose are absorbed in the intestine the same way via diffusion, convection and active transport. The active transport of glucose is about five times as fast as xylose (Fowweather, 1953; Levitt et al., 1969)). The presence of glucose interferes with the absorption of xylose indicating a similar transport pathway. The active transport is a phlorizin-sensitive, Na+-dependent mobile carrier mechanism that is partially energy dependent (Francisco, 1967). D-Xylose has also been stated to be absorbed through the small intestine into the blood stream by a similar process to amino acid active transport that is sodium-dependent (Atkinson, 1954; Goodwin et al., 1984; Sturkie and Benzo, 1986). Xylose is therefore a good measure of the absorption capacity of the intestines for glucose because of the similar mechanisms of absorption. Xylose is also a good predictor

of malabsorption as it has been shown that over 90% of the administered dose is absorbed within one hour in humans and that patients suffering from malabsorption have lower xylose values and peaked later than the normal patients within the first few hours (Butterworth et al., 1959). In rats, xylose does not increase liver glycogen therefore showing that xylose is not metabolized to any great extent. D-xylose has also been shown to inhibit growth when it is fed at high amounts and lead to depletion of glycogen from the liver in chickens (Wagh and Waibel, 1966). The decreased growth is most likely from the interference of xylose with glucose absorption as they use similar pathways but the birds cannot use xylose to synthesis glycogen.

Xylose Absorption Test In Poultry. Doerfler et al. (2000) used the D-xylose absorption to compare absorption between poults inoculated with fresh feces from PEMS affected poults. The study poults were British United turkey male poults from commercial hatcheries in NC and were raised in battery cages with infected poults in separate isolation rooms from uninfected poults. Poults were inoculated at 6 days post hatch and samples were taken at 1, 4, 7, 11 and 15 days post inoculation (or 7, 10, 13, 17 and 21 days post hatch). After a 12 hour fast on sampling days blood samples were collected at 30, 60, 90, 120, 150 and 180 minutes post gavage with a 5% solution of xylose given at 0.5 g D-xylose per kg body weight. High plasma levels of xylose reflect higher absorption. For general analysis an average value for each group was calculated over the 6 time periods for each day of sampling. Control poults absorbed significantly more xylose than PEMS inoculated ones at 4-11 days post inoculation (10-17 days post hatch). At 15 days post inoculation (PI), inoculated poults absorbed more xylose but this difference was not statistically significant (Table 2.1).

Table 2.1 Xylose absorption in PEMS and control poults (Doerfler et al., 2000)

Days PI	1	4	7	11	15
	Xylose concentration (mg/dL)[1]				
Control	36.6	30.4	37.6	29	41.8
PEMS	36.1	20.1	17.3	18.5	46.4
Difference	0.5	10.3*	20.3*	10.5*	-4.6

*Denotes a significant difference

Doerfler also looked at the differences among the xylose response curves over time (30, 60, 90, 120, 150 and 180 minutes post gavage) between controls and infected poults. From 4 to 11 days PI peak xylose absorption was seen at either 30 or 60 minutes and controls had significantly higher xylose levels than inoculated poults. At 90 minutes 4 and 7 day PI control poults still had significantly higher xylose levels which were seen out to 150 minutes in the 7 days PI birds. At 11 days post inoculation xylose levels of controls poults were similar to PEMS poults from 90 -150 minutes with plasma xylose from control poults numerically lower than PEMS poults at 90 minutes.

Reynolds and Saif (1986) looked at the absorption of D-xylose from poults inoculated with intestinal contents that only contained astrovirus and had been filtered to remove bacteria. Specific pathogen free poults were poults raised in sterile isolators with controls and inoculated poults in separate rooms. Two experiments were conducted with poults inoculated at one day of age. For the first experiment, blood samples were taken at 5, 6, and 7 days post inoculation (6, 7 and 8 days of age). Poults were fasted for 14 hours and pre-dose and 90 minute post xylose administration blood samples were collected. Xylose was given at 0.5grams per kg body weight. Xylose levels in plasma from pre-dose blood samples for Experiment 1 ranged from 1.5 to 5.8 mg D-Xyloses /dL for all poults (Table 2.2). Significant differences between inoculated and control poults were seen in every trial.

Table 2.2 Xylose absorption for astrovirus inoculated and control poults, Experiment 1 (Reynolds and Saif, 1986)

Days PI	5	6	7	7	7
	Xylose concentration (mg/dL)[1,2]				
Control	43.9	24.2	48.3	44.7	29.7
Inoculated	22.8	14.7	31.8	18.6	14.8
Difference	21.2*	9.5*	16.5*	26.1*	14.9*

[1] Average of 8 to 25 poults
[2] Samples collected 90 minutes post gavage
*Denotes a significant difference

The second experiment had two trials with samples collected at 1, 3, 5, 7, 9, 11 and 13 days post inoculation or 2, 4, 6, 8, 10, 12 and 14 days of age. For this experiment, the difference between control and inoculated poults for pre-dose plasma was within 0.7 mg xylose /dL for all fourteen sampling times except for trial two at 5 days PI where controls were 3 mg/dL higher than inoculated poults. Significant differences were not determined for pre-dose plasma samples. Significant differences were seen for the 90 minute plasma samples at 3, 5, 7, 9 and 12 days PI for at least one of the two trials in experiment two (Table 2.3). Xylose levels were only significantly different between controls and inoculated poults in both trials at 5 days PI. Inoculated poults had lower levels of xylose than control poults at all sampling times.

Table 2.3 Xylose absorption for astrovirus inoculated and control poults, Experiment 2 (Reynolds and Saif, 1986)

Days PI	1	3	5	7	9	11	13
	Controls – Inoculated[1,2]						
Trial 1	3.1	9.0*	15.0*	7.8	16.9*	4.3	4.4
Trial 2	0.8	6.2	47.2*	46.2*	8.4	15.2	12.3*

[1]D-xylose (mg/dL)
[2] Samples collected 90 minutes post gavage
*Denotes a significant difference

Goodwin et al. (1984) developed the micromethod for the determination of xylose in plasma that is used in small birds which is able to use smaller amounts of plasma samples than previously used. Poults from a breeder flock that were negative for reovirus antigen and antibody were inoculated with cloned reovirus at two days of age. At 24, 72 and 120 hours post inoculation (1, 3, and 5 days PI or 3, 5 and 7 days of age) poults were fasted for 12 hours and a pre-gavage blood sample collected. After being administered 0.5 g D-xylose per kg body weight, blood samples were collected at 30, 60, 90, 120 and 180 minutes post gavage. For comparison of groups, peak xylose levels were used. Control poults always had higher xylose than reovirus inoculated poults except at 120 hours PIfrom the 120, 150 and 180 minute sampling times (Table 2.4). The peak response to xylose bolus varied with sample time. At 24 and 72 days PI peak xylose levels were significantly different with control poults peak at 60 minutes and inoculated poults at 90 minutes. At 120 hours PI, groups were not significantly different and peaks occurred 30 minutes earlier. Xylose levels of control birds after the peak stayed high for another 60 minutes. For the inoculated poults xylose levels within 30 minutes of the peak did not change but after 30 minutes levels decreased.

Table 2.4 Peak Xylose levels reovirus inoculated and control poults (Goodwin et al., 1984).

Days PI	1	3	5
	Xylose concentration (mg/dL)		
Control	39.9	41.2	46.8
Inoculated	25.9	29.7	40.1
Difference	14.0*	11.5*	6.7

*Denotes a significant difference

In a similar subsequent study D-xylose absorption was measured at 30, 60, 90, 120 and 180 minutes post gavage of 0.5 g D-xylose/kg bw from normal turkeys at 8 weeks of age and poults at 3 days of age (Goodwin et al., 1984). For pre-gavage blood samples (collected after a 12 hour fast) the micromethod did not detect any xylose though

the older method developed by Roe and Rice (Roe and Rice, 1948) which uses larger amounts of blood did detect 2-3 mg xylose/dL in pre gavage samples. The micromethod determined average levels of xylose between 17 and 39 mg/dL between 30 and 180 minutes after xylose gavage. Xylose levels peaked at 60 minutes for both 8 week old and 3 day old poults (Table 2.5). Three day old poults had higher levels of xylose than 8 week old poults.

Table 2.5 Absorption of xylose from 30 to 180 minutes after administration (Goodwin et al., 1984)

Minutes	30	60	90	120	180
	Xylose concentration (mg/dL)				
8 week olds	25.5	29.5	28.7	25.5	17.3
3 days old	34.8	39.9	35.3	30.8	23.5

Chapter 3: Field Trial

INTRODUCTION

The objective of the field trial was to conduct a preliminary study about what is occurring with poults experiencing light turkey syndrome (LTS). Research and commercially grown flocks were compared on performance, pathogen presence, histopathology and other characteristics. The goal was to determine if there were significant differences between poults that weighed more than the average flock weight and poults that weighed less than the average flock weight. The hypothesis was that differences existed between the heavy and light weight poults. These possible differences included the presence/absence of certain viruses and bacteria; and, quantity of different types of bacteria in the gut contents. In addition histopathology differences in gut and immune tissue were compared for inflammatory changes and coccidia presence. Differences between light and heavy weight poults were also expected between absorption levels of D-xylose as an indirect measure of nutrient absorptive capacity.

MATERIALS AND METHODS

Study Design

Samples were collected from eight turkey brood flocks at zero, one, two and three weeks of age (+/- 3 days). For all flocks days of age were rounded to the nearest age in weeks to compare the samples collected from each flock. Table 3.1 outlines the general characteristics of the eight flocks. Six of the turkey flocks were raised in Minnesota and two in North Dakota. Four of the MN flocks were commercially raised toms with Flocks 1 and 2 reared on a different brooder farm than Flocks 3 and 4. All MN commercial flocks were from larger brooder barns having on average 16,000 poults per barn. Samples for Flocks 1 and 2 were collected at placement, nine, 16 and 23 days of age. Flocks 3 and 4 were sampled at placement, six, 13 and 20 days of age. All four MN commercial flocks (Flocks 1-4) were sampled in the fall. Farm locations were geographically diverse with

Flocks 1 and 2 being raised in northern MN and Flocks 3 and 4 being raised in Western MN. The remaining two MN flocks were research flocks raised in battery brooder cages at the University of MN. The research flocks were sampled at placement, six, 13 and 20 days of age. Both flocks were raised during the winter at separate times. The two flocks from ND were commercially raised turkeys reared on separate farms at different times. Flock 7 was sampled in the spring and Flock 8 was sampled in the summer. Flock 8 had 12,000 poults in the brooder barn and was indicated to be hens but it is not known what size and gender was for Flock 7. Flock 7 was sampled at placement, six, 13 and 20 days of age. Flock 8 was reported to be sampled at 3, 10, 17 and 24 days of age.

At each sampling point flocks had weights, gut contents and tissue samples collected. MN flocks additionally had plasma samples collected for xylose testing at two and three weeks of age. Light weight turkey syndrome is expected to develop in the first few weeks of brooding and samples collected were focused on that time period. A major part of our study design was comparing the heavy weight poults to the light weight poults in each flock. Each sampling period at one, two and three weeks of age had separate groups of poults defined as heavy or light weight poults as compared to the average flock weight. All animal work was previously approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

Sample Collection

Placement. Limited samples were collected at day of placement from some flocks. Body weights were measured at placement (zero days of age) for all flocks except Flock 8. MN commercial flocks had 10% of poults weighed to determine average body weight and distribution. All of the MN research poults were weighed at placement. The ND flocks had average weight information for Flock 7 at placement and for Flock 8 at three days of age. All flocks had pools of gut contents collected as was done for 1 to 3 week samples. Samples were analyzed for bacteria present and total plate counts for zero day of age samples from Flocks 1 through 7, and three days of age samples for Flock 8. Flocks 1 and 2 did not have gut contents analyzed for virus presence at placement but the remaining Flocks 3 through 7 did. Flock 8 had samples at three days of age. For

histopathology there were no tissue samples collected at zero days of age from Flocks 1 and 2 but Flocks 3, 4, 5, 6 and 7 had all tissues (duodenum, jejunum, ileum, ceca, spleen and bursa) collected. Flock 8 had histopathology tissue collected at three days of age.

Heavy or Light Weight Group Determination. For MN flocks, poults were grouped into heavy or light weight groups based on the average sample body weight of each flock. To calculate the average sample flock weight for the MN commercial flocks 40 birds from each flock were weighed or an average weight from the barn scale readings was used. The weight groups were determined as 10-15% above (heavy) or below (light) the average sample flock weight. Therefore each flock had a different range of weights for the weight groups depending on what the flock's estimated average weight was. The MN research flocks had all the poults weighed to obtain an average weight. For ND flocks, birds were chosen based on the farmer's decision of which poults looked under weight (light) and which poults appeared at normal weight (heavy). The light weight groups for the ND flocks were most likely more than 15% below the average flock weight since the light poults would have normally been culled by the farmer if they had not been used in this study.

Gut Contents and Tissue Samples. The same procedure was used to sample poults in all the MN commercial and research flocks while some changes were needed for sampling the ND flocks based on producer and ND staff constraints. On day of placement two pools of five poults were euthanized for gut contents and tissue samples. At one, two and three weeks of age, four pools of five poults from MN flocks or two pools of five poults from ND flocks were euthanized, per weight group and sampling day, for gut contents and tissue sampling.

On the MN farms poults were sacrificed by cervical dislocation by the farm veterinarian or a trained professional. Tissue samples collected for histopathology were collected as shown in Figure 3.1. Gut tissues included a five centimeter section of the duodenal loop, three centimeters from the ileum (five to eight centimeters above Meckel's diverticulum (MD), five centimeters of the jejunum (five to eight centimeters below MD) and three centimeters up from the distal tip of one cecum. For immune tissue

the entire bursa and spleen were collected. The intestinal sections were flushed with 10 % neutral buffered formalin (NBF) using a 3 ml syringe without a needle. All tissue samples were placed in 10 % NBF within 15 to 30 minutes of euthanasia. Two sections of tissues were collected for gut contents using floss to tie the ends closed before cutting to prevent the loss of contents (Figure 3.1). The section of tissue collected for gut contents that was used for bacteria culturing was the 13 cm section that including MD between the jejunum and ileum samples taken for histopathology. These gut sections were kept on ice and shipped overnight with frozen cold packs to NDSU. The second section collected for gut contents was the remaining section of the ileum up toan inch before the ileal-cecal junction. This section was collected for bacterial, virus and coccidia presence determination and for inoculum contents for the inoculation trial. This section was frozen for storage between sampling time, analysis and inoculation preparation.

For ND flocks, poults were euthanized on the farm and transported back to NDSU where they were weighed and samples collected. This additional transportation time decreased the sample quality especially for histopathology results and likely changed the microflora, especially the bacteria. Samples were collected as for MN flocks except the cecum section was collected near the ileal-cecal junction instead of the distal tip and only 13 centimeters of the ileum was sent for virus and coccidia presence determination. Not enough content was therefore available from the ileal section to include the ND flocks in our subsequent inoculation trial.

Plasma Collection. For the MN flocks blood samples were collected for determination of absorption of D-xylose from ten poults per weight group at two and three weeks of age after a 12 hour fast. No blood samples were collected from the ND flocks. Two blood samples were collected per poult with one at time zero before the poult was gavaged with D-xylose and the second at 60 minutes after the poult was gavaged with D-xylose. Poults were gavaged with a solution of xylose to give 0.5 grams of D-xylose per kg of average weight for each heavy or light weight group. A 5% solution of (98%) D-(+)-xylose (Alfa Aeser, Ward Hill, MA) in sterile water was given to the birds using a three to six milliliter syringe (depending on amount needed) and plastic Norton®

Tygon® tubing R3603 (Saint-Gobain Performance Plastics, Akron, OH). The tube was passed down the esophagus into the crop where the syringe was slowly depressed to release the fluid into the crop.

The equation used to determine the amount of 5% D-xylose solution to gavage poults was:

[(0.5g xylose) (kg BW average)] /(0.05g xylose/1mL solution) = mL xylose solution

The poults used for plasma collection were different from poults used for gut contents and tissue sampling as they had to be fasted (no food or water) for 12 hours prior to blood collection and would therefore not have had any gut contents in their system to collect. Fasting of the birds would also affect the gut tissue appearance and microbial content. At commercial locations, a sample of poults roughly three times the number needed was caged in crates with no feed or water about 12 hours before sampling started. Forty of the poults were weighed to determine the average sample fasted poult weight as was done for the gut contents poults. Ten poults were then chosen that were 10 to15% above the average fasted poult weight for the heavy weight group and ten poults that were 10 to 15% below for the light weight group. Fasted poults had a blood sample collected from the superficial medial wing vein after an alcohol prep pad was used to clean the location at time zero. The poults were then gavaged with D-xylose and placed into a holding crate for 60 minutes when a second blood sample was collected from the other wing.

Several different methods of blood collection were used because of the difficulty of sampling small poults. The first method used was outlined by Goodwin et al. (1984) and consisted of lancing the wing vein with a sterile 5/8 inch 25 gauge needle and collecting blood into glass or plastic heparinized microhematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA) and sealing with Fisherbrand Hemato-Seal™ tube sealing compound (Fisher Scientific, Pittsburgh, PA). This method was later adapted to replace the microhematocrit tubes with safe lock 0.5 ml tubes and 1.5ml Eppendorf tubes

(Eppendorf North America, Hauppauge, NY) that were coated with heparin (Elkins-Sinn Inc., Cheryy Hill, NJ). The second method included the use of a 23 gauge needle and three cc syringe to draw the blood out of the vein and deposit it into heparinized Eppendorf tubes. Different gauges and sizes of syringes were tried to determine the best method of collection based on ease of sampling and sample quality. Larger gauge needles (18 to 20) which would prevent hemolysis of the blood cells increased blood loss and percentage of poults that developed hematomas. Smaller needles, 25 gauge and 1ml syringes were too small to collect enough blood before coagulation would start and were more difficult to handle. Therefore the best method for collection of blood was the three cc syringe with a 23 gauge needle.

The 0.5 ml and 1.5ml Eppendorf tubes were coated with 10 and 20 international units of heparin. This was to give two IU of heparin per 100 µl blood collected. Heparin concentrated at 10,000 IU per ml was added at 0.2 ml to 19.8 ml of deionized water. Each tube 0.5 or 1.5 was filled with 100 µl or 200 µl of diluted heparin and placed in an oven to evaporate the water at 70°C for four to 12 hours.

After blood samples were collected into either the capillary or Eppendorf tubes the containers were placed into their corresponding centrifuges and spun for at least 2 minutes with up to an additional 3 minutes until the plasma and the red and white blood cells were separated. Plasma was then collected using a Hamilton syringe for the capillary tubes and disposable glass pipettes for the Eppendorf tubes. Plasma was placed into new Eppendorf tubes, transported on ice and frozen for later analysis of D-Xylose.

Laboratory Procedures

Bacteria. At North Dakota State University Dr. Catherine Logue and Julie Sherwood cultured bacteria and determined total plate counts from the gut contents. The presence of *Campylobacter spp., Clostridium spp., E.coli*, and S*almonella spp.* were determined by enrichment protocols. Total plate counts were determined for aerobic bacteria, anaerobic bacteria, lactobacilli and heterofermentative lactobacilli. Samples sent from MN and those collected by ND staff were opened aseptically and stomached for 90 seconds in a IUL Masticator (Lennox Laboratory Supplies Ltd., Dublin, Ireland) with 10

ml of buffered peptone water (BPW) (Voigt Global Distribution Inc., Lawrence, KS). Serial dilutions using up to nine milliliters of maximum recovery diluents were made using one milliliter of the stomached homogenate. Dilutions were plated in 0.1 ml amounts.

Plate Count Agar (PCA) was used for both aerobic and anaerobic growth both being incubated at 37°C for 48 hours. The anaerobic conditions for one set of the plates were provided by a gas generating kit (Mitsubishi, New York, NY). For the detections of lactobacilli both De Mann Rogosa Sharp (MRS) and all purpose tween agar (APT) was used. These were held at 30°C for 48 hours with the MRS plate being held in gas jars under microaerophilic conditions using kits from Mitsubishi and the APT plate being held under anaerobic conditions as was done for the anaerobic PCA plate. Plate counts for these four methods were determined in colony forming units (cfu) per gram of material.

For the enrichment of *Clostridium spp.* one ml of the homogenate was added to cooked meat medium and held at 37°C for 48 hours. Then a loopful of medium from the bottom of the tube was struck on sheep blood agar plates and anaerobically incubated at 37°C for 48 more hours. The plates were then examined for clostridium strains that would cause hemolysis of the blood. *Campylobacter spp.* was detected by the addition of two milliliters of homogenate to two milliliters of 2X Preston broth and incubated at 42°C for 48 hours. This was then struck on blood free charcoal agar and held at 42°C for 48 hours under microaerophilic conditions. Species with suspect campylobacter morphology were transferred to blood agar and identified by PCR as necessary.

The remainder of the BPW homogenate was incubated at 37°C for 48 hours. For *E. coli* a loopful was then struck to MacConkey and Eosin Methylene Blue agars. Both types of plates were held at 37°C for 18-24 hours. The remainder of the incubated homogenate was used for determination of salmonella presence with 0.5 ml and 0.1 ml added to Tetrathionate broth (Tet) and Rappaport Vassiladis broth (RV) respectively. Tet and RV treated plates were incubated at 37°C and 42°C, respectively for 24 hours. Both *E. coli* and *Salmonella spp.* enriched broths were then struck to mannitol lysine crystal

violet agar (MLCB) (Oxoid Limited, Hampshire, United Kingdom) and modified brilliant green agar (mBGA) and incubated for 49 hours at 37°C. Suspect *E. coli* and *Salmonella spp.* colonies on their respective plates were identified using the TREK diagnostic GNID panel.

Virus and Protozoa (Eimeria sp). Ileal sections containing gut contents were thawed and 10 ml of sterile PBS (phosphate buffered saline) were added to each whirl top bag that contained a pool of five samples. The material was prepared for analysis in a Stomacher® 80 Biomaster (Seward Laboratory Systems Inc., Port Saint Lucie, FL) on high for 180 seconds. The gut contents and PBS were separated from the ileal tissue by pouring the liquid into a new container and holding the intestinal tissue in the bag. This mixture of contents and PBS was frozen until analyzed for presence of Astrovirus, Rotavirus, Reovirus and *Eimeria sp.*

The presence of astrovirus, rotavirus, and reovirus from gut contents in PBS was determined using a multiplex reverse transcriptase PCR (mRT-PCR) (Jindal et al. unpublished data). Gut contents, 0.5 ml was briefly centrifuged (Hermle® Microcentrifuge 223 M-2, LabNet, Edison, NJ) for 3 min at 8000 rpm. The supernatant was than collected and RNA extracted from 140 µl of this supernatant. The extraction was completed using the QIAamp Viral RNA kit (Qiagen, Valencia, CA) and following the manufacturer's directions. Virus RNA amplification was carried out using the OneStep RT-PCR kit (Qiagen, Valencia, CA). The reaction mix for the amplification consisted of: 1× RT-PCR reaction buffer, 2 µl of dNTP mix (320 µM of each dNTP), 0.6 µM of forward and reverse primer of each of the three viruses, 2 µL of enzyme blend, 1.5 mM of $MgCl_2$, 3 µL of extracted RNA and water to make a total volume of 50 µL. The amplification steps included reverse transcription at 50°C for 30 min and an initial denaturation at 94°C for 15 min. This was then followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 2 min, extension at 72°C for 2 min and a final extension at 72°C for 10 min. The amplification was carried out in the Eppendorf Vap.Protect device. The PCR products were separated by electrophoresis on 1.2% agarose gel in Tris–acetate–EDTA buffer. A 100 bp DNA ladder was used as a molecular

weight marker and bands at 630, 802, and 1120 bp positions on the agarose gel confirmed the presence of rotavirus, astrovirus and reovirus respectively.

Coccidia presence in gut contents in PBS was determined by fecal flotation using the modified McMasters test (Sloss et al., 1994). Gut contents left over from the virus analysis was mixed with a floatation material. After mixing, material was passed through cheesecloth and then chambers of the McMasters slide were filled with the filtered fluid. The slide was then allowed to sit for a few minutes and then presence of protozoa (*Eimeria sp*.) was determined.

Histopathology. Tissue samples for histopathology were allowed to set from one to 30 days in 10% NBF until tissues were properly fixed but did not start to deteriorate. During this time period fixed tissue samples were sliced to 3mm thickness and placed into cassettes. These were embedded in paraffin to permanently preserve the tissue and a five to six micron slice was placed on a slide. The slides were then stained with Hematoxylin and Eosin and a coverslip applied (Luna, 1968).

For the gut tissues, heterophilic and lymphocytic or plasmacytic infiltrates into the lamina propria or villus crypts were scored for distribution and density. Distribution of infiltrates was scored as absent, focal, multifocal or generalized (Table 3.22). Density of infiltrates was scored as absent, low, moderate or high (Figure 3.2). Cecal tissue was not scored for distribution of lymphocytic cells. All intestinal tissues were also scored for the presence of coccidia organisms at levels of absent, low, moderate or high.

The immune tissue, bursa and spleen were both scored on distribution and density of lymphocellular necrosis or depletion. The bursa was additionally scored for heterophilic infiltrates (distribution and density) and atrophy severity (absent, low, moderate, high). The spleen was also scored for germinal center hyperplasia (density and distribution), red pulp abnormalities and ellipsoid necrosis. Distribution of infiltrates was scored as absent, focal, multifocal or generalized. Density of infiltrates was scored as absent, low, moderate or high (Figure 3.2).

Xylose Assay. The xylose assay followed the micro-method for the determination of D-xylose in turkey poults designed by Goodwin et al. (1984). The original method

called for the addition of two milliliters of color reagent to 20 µl of the plasma sample. This was vortexed and then incubated in a 100°C hot water bath for four minutes. Each sample was then cooled to room temperature and the absorption of light at 554 nm wavelength read in a spectrophotometer to determine the amount of xylose present. Xylose reacts with phloroglucinol causing a pink color to form and the amount of light absorbed would then indicate how much xylose had reacted with the color reagent. The color reagent for each assay was made up of 0.5 g Phloroglucinol, 100 mL of glacial acetic acid and 10 mL of concentrated hydrochloric acid and was stable for four days if protected from light.

The micromethod by Goodwin et al. (1984) was altered in our laboratory to use 10 µL of plasma samples instead of 20 µL and one milliliter of color reagent versus two. For each assay, 22 plasma samples were run with additional blanks, control pools and standards. These additional samples went through the entire assay with the plasma samples. The blank consisted of deionized water. The control pools included previously collected plasma samples from turkeys that had been gavaged with xylose. The first control pool was a low pool to represent poults with a lower absorption capacity and the second control pool was a higher pool to represent poults that had good absorption of D-xylose at 60 minutes post gavage. These control samples were included at the beginning and end of each assay so that the assays could be compared to each other and so that early readings of an assay could be compared to its final readings. The standards were present for the calculation of a standard curve relating the absorption of light for each sample with the amount of xylose present. The standards consisted of increasing amounts of D-xylose: 0, 10, 20, 30, 40, 50, 60, 80 and 100 mg D-xylose in one deciliter of saturated benzoic acid.

The amount of light absorbed by the sample was determined with a Beckman DU-70 Spectrophotometer set at 554 nm in the visible spectrum. A cuvette filled with DI water was used to zero the spectrophotometer and account for any variance due to the cuvette or water. Next the blank, that had DI water and the phloroglucinal color reagent which had gone through the hot water bath was read to determine if any remaining

variance was present due to the color reagent. Then the standards, first controls, samples and final controls were read.

The blank value was subtracted from all standards, samples and controls to remove any variation due to the color reagent in the absence of xylose. After this for each assay, the known amounts of D-xylose in the standards and the corresponding absorbance given by the spectrometer were used to develop a standard curve, regression equation and fit statistics (Microsoft Excel 2007). The regression equation from the standard curve was then used to convert the samples and control absorbency (nm) into concentration of D-xylose (mg/dL) for each of the collected samples.

Statistics

General Data Handling. SAS version 9.2 was used to run all statistical analyses. Procedures Mixed, Glimmix and Logistic were used. Samples from placement (day zero in Flocks 1-7 and three days of age in Flock 8) were not included in the statistical analysis unless indicated. The model and data included or excluded will be referenced for each sample in the results section because of the numerous different models that were used for each of the different results as explained in the following paragraphs.

For an examination of fit a progression of statistical models was used. The starting model included the three factors; flock (eight flocks), age (three sampling times), weight group (heavy or light) and the possible interactions (flock*age, flock*weight group, age*weight group and age*flock*weight group). If any interactions were not significant they were removed from the model. If interactions were significant they remained in the first model and then secondary models were run for individual groups based on which factors were in the significant interaction.

For all models there was also the possibility of a separation of data points which would lead to a nonconverging statistical model. This happened if all the data points were the same value in one of the factor’s levels. For example, several flocks had no coccidia present, these specific flocks were removed from the data and the statistics would then only be determined for flocks that had at least one sample positive for coccidia. This also could happen if no virus presence was found at a certain age. The data for that age group,

for example, three weeks, would be removed and differences would only be determined between samples from one and two week old poults. In cases where data was removed from the statistical data set it was also not included in the figures. Each figure is the arithmetic mean of the statistical data and will have marked what groups of data were removed from the data set.

In a few cases quasi-separation of data was present which is caused if only a few positive data points are present in each group. The removal of a group of data (Flock 1 or week one) was not done since this would be removing positive outcomes from the data set. When models would not work in this case, factors were removed from the model (flock or age) since our primary goal was to determine if there were differences between the heavy and light weight groups. The factor causing the quasi-separation of data points would have a coefficient of infinity and the p-values would be unreliable for that factor.

Ordinal Data. The Logistic procedure was used to analyze the scores for the tissue observations. This was used because the tissues scores are ordered data (least to most). The data was translated from four scores into binomial data for the statistical analysis. This was done because only a few of the middle scores were present in most of the data which made it difficult to fit a model and decipher the meaning of the statistical results. Observations of absent, low or focal were translated to a score of zero while the observations of moderate, severe, multifocal or generalized were considered a score of one for heterophilic or lymphocytic infiltrates. For the bursa, any amount of atrophy - mild, moderate or severe was considered a one and score of zero was given to tissue without any atrophy present. The presence of coccidia was considered a score of one if it was present at low, moderate or high levels and a score of zero if it was not present. For the spleen, any presence of germinal center hyperplasia, necrosis of the ellipsoids or red pulp abnormalities was a score of one. The following is an example program with all variables included in the model. The first model is without any interaction terms and the second model is with all interactions. The “&dat” and “&var” would be replaced with the data set and variable being analyzed using the SAS macro program.

```
Proc Logistic data=&dat; Class AGE WTGP FLOCK &var;
Model &var = AGE WTGP FLOCK;
```

```
Proc Logistic data=&dat; Class AGE WTGP FLOCK &var;
Model &var = AGE WTGP FLOCK
AGE*WTGP FLOCK*AGE WTGP*FLOCK AGE*WTGP*FLOCK;
```

Binomial and Quantitative Data. The Glimmix procedure which fits generalized linear mixed models was used to analyze for the incidence of various viruses and bacteria. The Glimmix procedure is a simpler program than the logistic program and is meant to be used with data that is binomial or quantitative but not ordinal. The Glimmix procedure also has more additional options than the Mixed procedure. One of these options is the ability to include a random statement that helps the model fit the data better. For the virus and bacteria, the procedure included a random statement. The random statement tells the program to treat a group of scores as the random variable instead of individual scores. For virus and bacteria presence the individual weight groups at each age (ID) were used as the random component in the model. This increases the model's accuracy giving it a more precise fit to the data. Also included in the virus and bacteria presence model was a statement that the data was binary. Least square means were determined for the difference between variable levels for each factor. The following is a model for *campylobacter spp.* presence without flocks 6 and 8 as they did not have any *campylobacter spp.* present in any pools.

```
Proc Glimmix data=bacteria157; Class ID AGE WTGP FLOCK;
Model CAMPY = AGE WTGP FLOCK / dist = bin solution;
LSmeans AGE WTGP FLOCK / e; Random ID / solution;
```

For xylose analysis a similar model to the above was used without the binary distribution since the xylose concentrations were quantitative. The procedure also

included a random statement that told the program to treat the individual weight groups at each age (ID) as the random component in the model. Separate models were run for plasma samples collected pre-gavage (T0) and post-gavage (T60). The following model is for the data from T0 samples which did not have any significant interactions.

```
Proc Glimmix data=xyloseT0; Class ID AGE WTGP FLOCK;
Model XYLOSE = AGE WTGP FLOCK / solution; Random ID / solution;
```

For the xylose data at T60 significant interactions were found between flock, age and weight groups. Secondary models were then run for each individual flock. This model worked for Flocks 2 and 4.

```
Proc Glimmix data=xyloseT60; Class ID AGE WTGP FLOCK;
Model XYLOSE = AGE WTGP/ solution; Random ID / solution; By FLOCK;
```

For Flocks 1, 3, 5 and 6 a significant interaction was found between age and weight groups. For each of these flocks two different models were used. The first to look at differences between weight groups at two weeks of age and separately at three weeks of age. The second model was used to look at differences between age of poults for light weight poults and separately for heavy weight poults.

```
Proc Glimmix data=xyloseT60Flock1; Class ID AGE WTGP;
Model XYLOSE = WTGP / solution; Random ID; By AGE;
```

```
Proc Glimmix data=xyloseT60Flock1; Class ID AGE WTGP;
Model XYLOSE = AGE / solution; Random ID; By WTGP;
```

Quantitative Data. For assessing the differences in total plate counts for aerobic, anaerobic, lactobacilli and heterofermentative lactobacilli the Mixed procedure was used.

Significant interactions between flock and age were found for all total plate counts so the following two models were used.

```
Proc Mixed data=baclog; Class AGE WTGP FLOCK;
Model AEROBIC = WTGP FLOCK; LSmeans WTGP FLOCK / pdiff;
BY AGE;
```

```
Proc Mixed data=baclog; Class AGE WTGP FLOCK;
Model AEROBIC = AGE WTGP; LSmeans AGE WTGP / pdiff;
BY FLOCK;
```

The following program could be used after either Mixed or Glimmix procedures. The previous program must include an LSmeans statement. This program will then take the least squares means and use them to determine the superscripts for differences among flock, age and weight groups.

```
Ods output diffs=ppp lsmeans=mmm; Ods listing exclude diffs lsmeans; Run;
%include 'C:pdmix800_03.sas'; %pdmix800(ppp,mmm,alpha=.05,sort=no);
```

After any model, contrast statements were used to determine the odds ratio for difference between two different inoculum groups (heavy vs light or Flock 1 vs Flock 2).

```
Contrast 'wtgp h l' wtgp 1 -1 / estimate=exp;
Contrast age '1 2' wtgp 1 -1 / estimate=exp;
Contrast age '2 3' wtgp 0  1 / estimate=exp;
Contrast age '1 3' wtgp 1 0  / estimate=exp;
Contrast 'flock 1 2' flock 1 -1 0 0 0 0 / estimate=exp;
Contrast 'flock 1 3' flock 1 0 -1 0 0 0 / estimate=exp;
Contrast 'flock 1 4' flock 1 0 0 -1 0 0 / estimate=exp; Etc.
```

RESULTS

Weights

At placement poult weights ranged from 51 to 58 grams for the MN commercial Flocks 1, 2, 3 and 4. The research Flocks 5 and 6 ranged from 62 and 56 grams at placement. The poults from Flock 7 a ND commercial flock averaged 60 grams. Flock 8, the other ND commercial flock, was sampled at three days of age instead of day of placement and the average weight was 88 grams. Table 3.2 lists the average weights with standard error thatis based on a sample of random poults from each farm and age. Mean weights in kilograms with standard errors are listed for each flock and sampling week. Only mean weights are available for Flocks 7 and 8 at all time periods and for Flocks 1 and 2 at two and three weeks of age. The standard error among weights increased over age from 0.2 to 14.3 grams for the MN commercial flocks and from 0.3 to 6.5 grams for the MN research flocks.

For weights from one to three weeks of age flocks grouped themselves into two similar patterns. The first included the MN commercial flocks and one of the ND flocks, (Flocks 1, 2, 3, 4 and 7) these flocks' body weights averaged 124, 262 and 471g at one, two and three weeks of age. The research flocks and Flock 8 made up the second grouping with body weight averages of 160, 359 and 669 g at one, two and three weeks of age. This is illustrated in Figure 3.3 with the average weights at each day of age graphed for each flock. The average weights with standard error and correlation of variation for heavy and light weight groups for each flock are shown in Table 3.3. The heavy and light weight groups follow the same trend that was shown with the average weights with Flocks 5, 6 and 8 having the highest average weight for both heavy and light weight groups compared to Flocks 1, 2, 3, 4 and 7.

Add market data for half of flocks 3/4 and 1/2

Virus

No differences were found between heavy and light weight groups for the presence of Astrovirus (Table 3.4). Astrovirus was present in all MN and ND commercial

and research flocks in at least one pool of samples (Table 3.5). Astrovirus differences in number of pools positive were observed among flocks (*P* < 0.0059) see Figure 3.5. Flocks 2 and 5 had only one pool positive each for astrovirus while the other flocks had at least seven pools positive out of a possible 24 pools for MN flocks and 12 pools for ND flocks. Astrovirus was the only virus to show significant differences over age incidence, decreasing from 53.6% to 14.3% from one to three weeks of age (*P* < 0.0043) see Figure 3.6. One week old poults have 2.74 times the odds as three week old poults of being positive for astrovirus (*P* < 0.0011). Two week old poults have 1.98 times the odds as three week old poults of being positive for astrovirus (*P* < 0.0167).

Rotavirus was found in five flocks all from MN including both research flocks and three of the commercial flocks (Table 3.6). Heavy and light weight groups did not differ for presence of rotavirus. No rotavirus was found in ND commercial flocks or Flock 2 (MN commercial flock). Rotavirus was only detected at one and two weeks of age. The statistical model compared poults in Flocks 1, 3, 4, 5 and 6 at one and two weeks of age. Rotavirus trended differently among Flocks 1, 3, 4, 5 and 6 (*P* < 0.0914) see Figure 3.4. Only one pool (6.3%) was positive for Flock 5 while the remaining positive flocks had three to nine pools positive (18.8 to 56.3%). Differences among one and two week old poults were not significantly different.

The percent of pools positive for reovirus from MN flocks and ND flocks is shown in Table 3.7. Reovirus was found in two of the four Minnesota commercial flocks. No reovirus was found in any of the research or ND flocks. Reovirus was found in seven of the 24 pools in Flock 3 and four of the 24 pools collected from Flock 4. The statistical model included Flocks 3 and 4 with data from one and two weeks of age since no pools were positive at three weeks of age. Differences in reovirus present were not seen between Flocks 3 and 4. Also significant differences between one and two week old poults were not found. No differences were found between heavy and light weight groups for the presence of reovirus.

Bacteria

Escherichia coli was found in 99% of pooled samples which including all pools except one from the heavy weight poults at one week of age in Flock 6 (Figure 3.7). No differences were found between heavy and light weight groups for the presence of *E. coli*. Differences were also not seen among flocks or age groups (Table 3.8).

Salmonella spp. was found in 68% of pooled samples (Table 3.9). No differences were found between heavy and light weight groups for the presence of *Salmonella spp*. *Salmonella spp.* was not found in Flock 6 so the statistical data only included Flocks 1, 2, 3, 4, 5, 7 and 8. A trend for differences among these flocks was observed for *salmonella* (P<0.0617). Research Flock 5 had 48% of pools positive for *salmonella* while the remaining commercial flocks had more than 79% positive (Figure 3.8). No differences were seen over age for *salmonella* presence.

Campylobacter spp. was found in 25% of pooled samples but was not found in research Flock 6 or ND commercial Flock 8 (Table 3.10). The MN commercial flocks and research Flock 5 had between four to twelve pools (16.7% to 50%) positive for *Campylobacter* but no statistical differences were seen in these flocks for weight group, age or flock differences. *Clostridium spp.* was not found to be present in any pools.

Average concentration of bacteria present was 7.25, 7.36, 7.56, and 7.59 $\log_{10}$ colony forming units per gram (cfu/g) of material for aerobic bacteria, anaerobic bacteria, lactobacilli and heterofermentative lactobacilli, respectively (Figure 3.9). Because of the significant interaction between age and flock the model was run separately for each flock or age group (Table 3.11). Flocks 1, 2, 5, 6, 7 and 8 had some plate counts that were too numerous to count or did not show any growth and were treated as missing values. Flocks 3 and 4 did not have missing data. Minimum values included plates with 3.32 log10 cfu/g for aerobic bacteria. Maximum values included plates with 9.94 log10 cfu/g for lactobacilli. For general plate counts for aerobic bacteria, anaerobic bacteria, lactobacilli and heterofermentative lactobacilli no differences were seen between heavy and light weight groups. Differences were seen over age for each of the different bacterial plate

counts. The most common trend was a decrease in cfu's from one week of age to three weeks of age (Table 3.18).

In all MN flocks (run separately because of the previous interaction between age and flock), aerobic bacteria decreased from one to three weeks of age ($P < 0.05$) (Table 3.12 and 3.14). In the ND flocks, aerobic bacteria decreased from one to two weeks of age ($P < 0.05$). Aerobic bacteria levels at three weeks of age were not significantly different from one week of age in ND flocks. In three of the MN commercial flocks anaerobic bacteria plate counts were not significantly different over age (Table 3.12 and 3.15). Flock 3, the two research flocks and Flock 8 had anaerobic counts significantly decrease from one to three weeks of age ($P < 0.05$). Flock 7, the remaining ND flock actually had increasing anaerobic counts from one to three weeks of age ($P < 0.05$). For lactobacilli, counts decreased from one to three weeks of age in Flocks 1, 2, 4, 5, 6 and 8 ($P < 0.05$) (Table 3.13 and 3.16). No differences were seen over age for lactobacilli in Flock 3. In Flock 7 lactobacilli decreased from one to two weeks of age ($P < 0.05$). The levels at three weeks of age in Flock 7 were statistically similar to one week of age for lactobacilli. Heterofermentative lactobacilli plate counts significantly decreased from one to three weeks of age in all flocks (Table 3.13 and 3.17).

The only differences between heavy and light weight groups were found in Flock 4 for lactobacilli ($P < 0.0664$). Light weight poults had higher plate counts for lactobacilli than heavy weight poults (Figure 3.14). No other differences were seen between weight groups.

In most flocks, samples from placement had significantly lower bacterial counts than samples collected from weeks one, two or three ($P < 0.05$). For aerobic, anaerobic bacteria and lactobacilli samples collected at placement were statistically equal to samples collected at either one, three or both weeks of age in Flocks 1 and 8. For heterofermentative lactobacilli samples collected at placement were statistically equal to samples collected at three weeks of age in Flocks 1, 5 and 8. For Flock 6, growth of all bacteria plate counts at zero days of age were too numerous to count (TNTC) and were

therefore unable to be compared to samples from one, two or three weeks of age (Table 3.19).

Flock differences were seen for all counts in at least three of the four age periods ($P < 0.05$). Flocks with the highest counts tended to change from week to week but overall Flock 8 had the highest numbers of bacteria present. (Flock 8 samples were also collected from heavy hens that were older by 3 days than the remaining poults). Flock 8 had the highest plate counts in ten out of a possible 16 times. Two of these 16 times, aerobic bacteria at one week of age and anaerobic bacteria at two weeks of age showed no significant differences among flocks. The four times that Flock 8 did not have the highest bacterial counts were at two weeks of age for aerobic bacteria and both lactobacilli and at three weeks of age for lactobacilli. (Table 3.20)

Protozoa

None of the pooled samples were positive for coccidia presence from the fecal floatation procedure. Intestinal tissue slides did show presence of coccidial organisms. Intestinal tissue from Flocks 3, 4 and both ND flocks had varying life stages of coccidia organisms present. Tissues with coccidia ranged from 3% in the jejunum from Flock 7 to 63% in the ileum from poults in Flock 4. The statistical data included only Flocks 3, 4, 7 and 8. Differences between heavy and light weight groups were not seen but differences between the four flocks and over age were seen (Table 3.21).

Differences over age were seen in all four gut tissue samples (Figure 3.10). Coccidia presence increased from 8% at one and two weeks of age to 42% of samples at three weeks of age in the duodenum ($P < 0.0078$). In the jejunum, coccidia were found at two and three weeks of age but not at one week of age. Coccidia presence increased from 17% of samples at two weeks to 61% at three weeks of age in the jejunum ($P < 0.0001$). Three week old poults had 4.2 times the odds as two week old poults of having coccidia in the jejunum. Presence of coccidia in the ileum was significantly different among weeks increasing from 3% at one week of age to 72% at three weeks ($P < 0.0001$). Three week old poults had 8.2 times the odds as one week old poults of having coccidia in the ileum ($P < 0.0028$). Presence of coccidia in the ceca was only found at one and three weeks.

One week old poults had coccidia present in 3% of the ceca and three week old poults had it in 38% of ceca samples ($P < 0.0042$). Three week old poults had 5.0 times the odds as one week old poults of having coccidia in the ceca. Cecal tissue was not collected at weeks one and two from Flocks 1 and 2 and no life stages of coccidia were seen in any of the other tissues at any time point.

In the duodenum, no differences were seen among flocks for number of samples with coccidia present. Significant differences for coccidia presence in the jejunum were seen among flocks (Figure 3.10). MN commercial Flocks 3 and 4 have more tissues with coccidia than the ND flocks ($P < 0.0001$). Flock 8 also had significantly more coccidia present in the jejunum than Flock 7. Presence of coccidia in the ileum for each flock ranged from 8% to 54%. The factor flock was removed from the model as the limited amount of data kept the model from working. Presence of coccidia in the ceca in each flock ranged from 3% to 25% with MN Flocks 3 and 4 trending to have more coccidia present than ND flocks ($P < 0.1138$).

Histopathology of Intestinal Tissue

Density of Heterophilic Infiltrates. Tissue score differences were primarily found among different ages and flocks with a few differences between weight groups. On average 3% of poults had moderate or high heterophilic density and 97% of poults had low density or no heterophilic infiltrates present in the duodenum and jejunum. On average 5% of poults had moderate or high heterophilic density and 95% of poults had low density or no heterophilic infiltrates present in the ileum and ceca. In the duodenum, moderate or high density of heterophilic infiltrates were only present in light weight poults at one and three weeks of age in Flocks 1, 2, 3 and 8. The statistical data for the duodenum therefore included the data from just these flocks at one and three weeks of age. The statistical model for the duodenum included flock and age factors, but not weight group. In the jejunum, moderate or high density of heterophilic infiltrates were only present in poults in Flocks 3, 4, 5 and 6. The statistical data for the jejunum only included the data from these flocks and the model for the jejunum included all three factors; flock, age, and weight group. In the ileum, moderate or high density of

heterophilic infiltrates were only present in poults in Flocks 2, 4, 5, 6 and 8. The statistical data for the ileum only included the data from these flocks and the model for the ileum included all three factors; flock, age, and weight group. In the ceca, moderate or high density of heterophilic infiltrates were only present in poults in Flocks 1, 5 and 8. Ceca tissue was only collected in Flocks 1 and 2 at three weeks of age, but it was collected at all three time points for Flocks 3 through 8. Two different data sets were used to look at the ceca differences for heterophilic density. The first compared the scores taken at three weeks from Flocks 1, 5 and 8 but this model did not converge. The second model used data from Flocks 1, 5, and 8 and treated the scores from Flock 1 at one and two weeks of age as missing variables.

For the jejunum, ileum and ceca significant differences in number of poults with moderate or high heterophilic infiltrate density was not seen between heavy and light weight groups (Table 3.23). In the duodenum, moderate or high density of heterophilic infiltrates were seen in 16 % of light weight poults. Infiltrates were not seen in any heavy weight poults. Statistical significance was not able to be determined because there were no moderate or high scores in the heavy weight poults. For the small intestine and ceca differences in poults with moderate or high heterophilic infiltrate density was not seen over age. Moderate or high heterophilic infiltrate density was not present at two weeks of age in the duodenum. For all three sections of the small intestine and the ceca differences among flocks for number of poults with moderate or high density of heterophilic infiltrates was not seen.

Distribution of Heterophilic Infiltrates. Tissue score differences were primarily found among weeks and flocks with a few differences between weight groups. In the duodenum and ceca 20 and 24 % of poults had multifocal or generalized distribution of heterophilic infiltrates and 80 and 76 % of poults had focal distribution or no heterophilic infiltrates present. In the jejunum and ileum 39 and 46 % of poults had multifocal or generalized distribution of heterophilic infiltrates and 61 and 54 % of poults had focal distribution or no heterophilic infiltrates present. In the duodenum multifocal or generalized distribution of heterophilic infiltrates was present in all flocks. No data or

factors were removed from the statistical model for the duodenum. In the jejunum multifocal or generalized distribution of heterophilic infiltrates was present in all flocks. In the first statistical model for the jejunum a significant interaction was found between all three factors: age, weight group and flock. The secondary statistical analysis for the jejunum was then determined individually for each flock. In the ileum multifocal or generalized distribution of heterophilic infiltrates was present in all flocks. No data or factors were removed from the statistical model for the ileum. In the ceca multifocal or generalized distribution of heterophilic infiltrates was present in all flocks except Flock 1. Ceca tissue was only collected in Flocks 1 and 2 at three weeks of age but it was collected at all three time points for Flocks 3 through 8. Two different data sets were used to look at the ceca differences for heterophilic density. The first compared the scores taken at three weeks from Flocks 2 through 8 but this model did not converge. The second model used data from Flocks 2 through 8 and treated the scores from Flock 2 at one and two weeks of age as missing variables.

For the duodenum, ileum and ceca differences in number of poults with multifocal or generalized heterophilic infiltrate distribution were not seen between heavy and light weight groups (Table 3.24). In the jejunum, differences for heterophilic distribution for weight groups were examined for individual flocks because of the significant interaction between flock, age and weight group. Differences between heavy and light weight groups were observed for Flocks 3 and 8 (Figure 3.12). For Flock 8 significantly more light weight poults had multifocal or generalized heterophilic infiltrates present than heavy weight poults ($P < 0.0474$). Light weight poults in Flock 8 have 2.09 times the odds as heavy weight poults for having multifocal or generalized heterophilic infiltrates. Flock 3 showed a trend for more light weight poults having multifocal or generalized heterophilic infiltrate distribution than heavy poults ($P < 0.1098$). The remaining Flocks 1, 2, 4, 5, 6 and 7 did not have significant differences between weight groups.

For the duodenum, differences in number of poults with multifocal or generalized heterophilic infiltrate distribution were not seen over age. For the jejunum, differences

among age groups for multifocal or generalized heterophilic infiltrate distribution were examined for individual flocks because of a significant interaction between flock, age and weight group. Flocks 5 and 6 showed a significant increase in number of poults with multifocal or generalized heterophilic infiltrates over age ($P < 0.0024$, $P < 0.0349$) (Figure 3.13). Poults with multifocal or generalized heterophils increased from 13 % of poults at one week of age to 81 % at two weeks of age in Flock 5 ($P < 0.0010$). Poults with multifocal or generalized heterophils increased from 44 % of poults at one week of age to 94 % at three weeks of age in Flock 6 ($P < 0.0152$). Flock 3 showed a significant decrease over age and Flock 8 showed a trend of a decrease for number of poults with multifocal or generalized heterophilic infiltrates ($P < 0.0470$, $P < 0.0789$). Poults with multifocal or generalized heterophils in the jejunum decreased from 56 % and 44 % of poults at one week of age to 13 % and 6 % at three weeks of age in Flocks 3 and 8 respectively ($P < 0.0269$, $P < 0.0680$). Differences in poults with multifocal or generalized heterophilic infiltrate distribution were seen over age in the ceca and trended differently in the ileum (Figure 3.14). Multifocal or generalized heterophils in the ceca increased over age ($P < 0.0054$). Poults at one week of age had heterophilic infiltrates present in the ceca in 8 % of poults which increased to 29 % at two weeks of age ($P < 0.0114$). Poults at one week of age had heterophilic infiltrates present in 8 % of poults in the ceca which increased to 36 % at three weeks of age ($P < 0.0020$). Multifocal or generalized heterophils in the ileum were different over age ($P < 0.0679$).

Differences among flocks for numbers of poults with multifocal or generalized distribution of heterophilic infiltrates were seen in the duodenum and ileum ($P < 0.0081$, $P < 0.0007$) (Figure 3.15). In the duodenum more poults in Flock 5 had increased heterophilic distribution than poults in Flocks 1, 3, 4, 6, 7 and 8. Also Flock 2 had more than Flocks 3, 7 and 8. In the ileum the number of poults with increased heterophilic distribution was found in the most poults in Flock 6 and the least poults in Flock 1.
In the jejunum a significant three way interaction made it necessary to look at flock differences by individual weight and age group (Figure 3.16). Significant differences among flocks were present for heavy weight poults ($P < 0.0009$). Flocks 5 and 6 had

more poults with increased heterophilic distribution than Flocks 1, 2, 3 and 8. Flock 6 also had more than Flock 7. Differences among flocks for light weight poults were also seen ($P < 0.0552$). Flocks 4 and 6 had more poults with increased distribution of heterophils than Flock 1. Flock 6 also had more than Flocks 2 and 8. Significant differences were seen for poults at one and three weeks of age among flocks ($P < 0.0277$, $P < 0.0012$). At one week of age poults in Flocks 3, 4, 6 and 7 all had increased distribution of heterophils compared to Flocks 1 and 5. Flocks 3 and 4 also had more poults than Flocks 2 and 8 at one week of age. For three week old poults Flock 6 had more poults than all other flocks. Flock 5 had more poults with heterophilic distribution than Flocks 1, 2 and 3. A trending difference was seen among flocks for two week old poults ($P < 0.0568$). At two weeks of age more poults in Flock 5 had increased distribution of heterophils than Flocks 1, 2, 3, 7 and 8. Flock 6 also had an increased distribution of heterophils than Flock 1 at two weeks. No differences were seen among flocks for multifocal or generalized distribution of heterophilic infiltrates in the ceca.

Density of Lymphocytic Infiltrates. In the duodenum, ileum and ceca an average of 12 % poults had moderate or high density of lymphocytic or plasmacytic infiltrates and 88% of poults had low density or no lymphocytic or plasmacytic infiltrates present. In the jejunum 6 % of poults had moderate or high density of lymphocytic or plasmacytic infiltrates and 94 % of poults had low density or no lymphocytic or plasmacytic infiltrates present. In the duodenum moderate or high density of lymphocytic infiltrates was present in all flocks. No data or factors were removed from the statistical model for the duodenum. In the jejunum moderate or high density of lymphocytic infiltrates was present in all flocks except Flock 4. The statistical data for the jejunum included Flocks 1, 2, 3, 5, 6, 7 and 8 and no factors were removed from the statistical model. In the ileum moderate or high density of lymphocytic infiltrates was present in all flocks. No data or factors were removed from the statistical model. In the ceca moderate or high density of lymphocytic infiltrates were not present in poults in Flocks 4 and 5. Also, cecal tissue was only collected in Flocks 1 and 2 at three weeks of age but it was collected at all three time points for Flocks 3 through 8. Two different data sets were used to look at the ceca

differences for lymphocytic density. The first compared the scores taken at three weeks from Flocks 1, 2, 3, 5, 6 and 8 and included flock and weight group as factors in the model. The second model used data from Flocks 1, 2, 3, 5, 6 and 8 and treated the scores from Flock 1 and 2 at one and two weeks of age as missing variables. These two models both showed the same statistical results for possible differences between weight groups and flocks. Since the second model was also able to look at differences among age groups, this one will be reported.

For the duodenum and jejunum, a difference in number of poults with moderate or high density of lymphocytic infiltrates was not seen between heavy and light weight poults (Table 3.25). However, differences were observed in the ileum and ceca (Figure 3.17). In the ileum, a trend for more heavy poults with moderate or high density of lymphocytic infiltrates was seen compared to light weight poults ($P < 0.1044$). In the ceca, significantly more heavy weight poults had moderate or high density of lymphocytic infiltrate than light weight poults ($P < 0.0048$). Heavy weight poults had 2.5 times the odds as light weight poults for having a moderate or high density of lymphocytic infiltrates in the ceca.

For the duodenum and ileum, significant differences in number of poults with a moderate or high density of lymphocytic infiltrate was not seen over age. In the jejunum and ceca, a significant increase was seen over age (Figure 3.18). Poults with moderate or high density of lymphocytic infiltrates increased from 1 % and 6 % of poults at one week of age to 14 % and 35 % at three weeks of age in the jejunum and ceca, respectively ($P < 0.0017$, $P < 0.0013$).

In the jejunum differences in the number of poults with a moderate or high density of lymphocytic infiltrate were seen among flocks ($P < 0.0356$). Flock 7 had significantly more poults with an increased density of lymphocytes than Flocks 2, 3, 5 and 7 (Figure 3.19). Differences among flocks were not seen for lymphocytic density in the duodenum, ileum and ceca.

Distribution of Lymphocytic Infiltrates. In the duodenum, jejunum and ileum, on average 54% of poults had multifocal or generalized distribution of lymphocytic or

plasmacytic infiltrates and 46% of poults had focal distribution or no lymphocytic or plasmacytic infiltrates present. Ceca tissue was not scored for distribution of lymphocytic infiltrates. In the duodenum multifocal or generalized distribution of lymphocytic infiltrates was present in all flocks. No data or factors were removed from the statistical model for the duodenum. In the jejunum multifocal or generalized distribution of lymphocytic infiltrates was present in all flocks. In the first statistical model for the jejunum a significant interaction was found between age and weight group. The secondary statistical analysis for the jejunum was then determined individually for each flock. In the ileum multifocal or generalized distribution of lymphocytic infiltrates was present in all flocks. No data or factors were removed from the statistical model.

For the duodenum, a difference in number of poults with multifocal or generalized lymphocytic infiltrate distribution was not seen between heavy and light weight groups (Table 3.26). In the ileum, a trend for more heavy poults with multifocal or generalized distribution of lymphocytes was seen compared to light weight poults ($P < 0.0914$) (Figure 3.20). For the jejunum, differences in poults with multifocal or generalized lymphocytic infiltrate distribution were determined for specific sample age because of a significant interaction between age and weight group. Only weight group was included in this model (if flock was included the model did not converge). At two weeks of age, significantly more heavy weight poults had multifocal or generalized distribution of lymphocytic infiltrates in the jejunum than light weight poults (Figure 3.20). For two week old poults, heavy weight poults had 1.58 times the odds as light weight poults of having multifocal or generalized lymphocytes in the jejunum ($P < 0.0131$). No differences were seen between heavy and light weight groups at one or three weeks of age for lymphocytic distribution in the jejunum.

For the duodenum and ileum a significant increase in number of poults with multifocal or generalized distribution of lymphocytic infiltrate was seen over age (Figure 3.21a). Poults with multifocal or generalized distribution of lymphocytic infiltrate increased from 14 % of poults at one week of age to 75 % at three weeks of age in the duodenum ($P < 0.0001$). Poults with multifocal or generalized distribution of

lymphocytic infiltrate increased from 25 % of poults at one week of age to 81 % at three weeks of age in the ileum ($P < 0.0001$). In the jejunum differences in poults with multifocal or generalized lymphocytic infiltrate distribution were determined for individual weight groups because of a significant interaction between age and weight group (Figure 3.21b). For heavy and light weight poults the number of poults with multifocal or generalized distribution of lymphocytic infiltrates increased over age ($P < 0.0001$, $P < 0.0001$). At two weeks of age 69 % of heavy weight poults had multifocal or generalized lymphocytes. At two weeks of age 47 % of light weight poults had multifocal or generalized lymphocytes. At one and three weeks both light and heavy poults had an average of 16 % and 87% of poults with multifocal or generalized lymphocytes.

For the duodenum and ileum a significant difference in the number of poults with multifocal or generalized distribution of lymphocytic infiltrate was seen among flocks (Figure 3.22a). In the duodenum Flock 7 had significantly more poults with higher distributions of lymphocytic infiltrates than all other Flocks ($P < 0.0031$). In the ileum Flocks 6, 7 and 8 had more poults with higher distributions of lymphocytic infiltrates than Flocks 1, 2 and 5 ($P < 0.0001$). In the jejunum the number of poults with multifocal or generalized lymphocytic infiltrate distribution was different among flocks (Figure 3.22b). More poults in Flocks 7 and 8 had increased lymphocytic distribution than poults in all other Flocks ($P < 0.0001$).

Histopathology of Immune Tissue

Bursa of Fabricius. Moderate or high density of heterophilic infiltrates in the bursa was seen in 9% of poults while 91% of poults had low density or no heterophilic infiltrates present. No Moderate or high density of heterophilic infiltrates in the bursa were found in Flock 7 and it was removed from the statistical data. Multifocal or generalized distribution of heterophilic infiltrates was seen in 23% of poults and 77% of poults had focal distribution or no heterophilic infiltrates present in the bursa. All data was used for statistical data for distribution of heterophils in the bursa.

Heterophilic infiltrates in the bursa were not significantly different between weight groups for either distribution or density (Table 3.27). Moderate or high density of

heterophilic infiltrates in the bursa was significantly different in poults over age (Figure 3.23). Moderate or high density of heterophilic infiltrates were found in the bursa of 17 % of poults at one week of age and increased to 31 % at three weeks of age ($P < 0.0577$). Multifocal or generalized distribution of heterophilic infiltrates was different in poults over age (Figure 3.23). Multifocal or generalized distribution of heterophilic infiltrates were found in the bursa of 6 % of poults at one week of age and increased to 16 % at three weeks of age ($P < 0.1035$).

Moderate or high density of heterophilic infiltrates in the bursa was significantly different among flocks (Figure 3.24). Flock 6 had significantly more poults with moderate or high density of heterophils than Flocks 2, 3, 4 and 8 ($P < 0.0593$). Multifocal or generalized distribution of heterophilic infiltrates in the bursa was significantly different among flocks (Figure 3.24). Flock 6 had significantly more poults with multifocal or generalized distribution of heterophils than Flocks 1, 2, 3, 4, 7 and 8 ($P < 0.0015$). Flock 5 had significantly more than Flocks 1 and 2. Flock 3 had significantly more than Flock 1.

Moderate or high density of lymphocellular depletion was seen in 21% of poults and 79% of poults had low density or no lymphocellular depletion present. Flocks 1 and 8 did not have any moderate or high density of lymphocellular depletion and were removed from the statistical data. Multifocal or generalized distribution of lymphocellular depletion was seen in 30% of poults and 70% of poults had focal distribution or no lymphocellular depletion present. Flock 8 did not have any multifocal or generalized distribution of lymphocellular depletion and was removed from the statistical data.

Moderate or high density of lymphocellular depletion in the bursa was not different between heavy and light weight poults. Multifocal or generalized distribution of lymphocellular depletion in the bursa was significantly different between weight groups (Figure 3.25). Light weight poults have 1.5 times the odds as heavy weight poults for multifocal or generalized distribution of lymphocellular depletion in the bursa ($P < 0.0304$).

Moderate or high density of lymphocellular depletion in the bursa was not significantly different in poults over age. Multifocal or generalized distribution of lymphocellular depletion was significantly differently in poults over age (Figure 3.26). Multifocal or generalized distribution of lymphocellular depletion was found in the bursa of 44 % of poults at one week of age and decreased to 13 % at three weeks of age ($P < 0.0002$).

Moderate or high density of lymphocellular depletion in the bursa trended differently among flocks (Figure 3.27). Flock 2 had significantly less poults with moderate or high density of lymphocellular depletion than Flocks 1, 3, 4, 6 and 7 ($P < 0.0717$). Multifocal or generalized distribution of lymphocellular depletion in the bursa was significantly different among flocks (Figure 3.27). Flock 2 had significantly less poults with moderate or high density of lymphocellular depletion than Flocks 3, 4, 6 and 7 ($P < 0.0526$).

Atrophy of the bursa was present in 28% of poults sampled and 72 % of poults did not have bursal atrophy. In the first statistical model an interaction between age and weight group was significant. Two secondary models were run; one comparing weight groups at each individual age and the second comparing different ages for heavy or light poults. At one and two weeks of age there were no differences between heavy and light weight poults. At three weeks of age more light weight poults had atrophy of the bursa than heavy weight poults (Figure 3.28). For three week old poults, light weight poults have 1.9 times the odds as heavy weight poults of having atrophy present in the bursa ($P < 0.0248$). Differences over age for atrophy of the bursa were not seen for heavy weight poults. Differences over age for atrophy of the bursa were seen for light weight poults (Figure 3.29). One week old light weight poults had atrophy present in 25 % which increased to 46 % of light weight poults at three weeks of age ($P < 0.0308$). Differences in number of poults with atrophy were seen among flocks (Figure 3.30). Flocks 4 and 6 had significantly more poults with bursal atrophy than the remaining flocks ($P < 0.0005$). Flock 8 also had significantly more poults with bursal atrophy than Flock 1.

Spleen. Moderate or high numbers of germinal center hyperplasia in the spleen was found in 9% of poults and 91% of poults had low or no germinal center hyperplasia present. Germinal center hyperplasia was not present in Flocks 3, 4, 7 and 8. The statistical data included Flocks 1, 2, 5 and 6. Moderate or high density of lymphocellular depletion was found in only 3% of poults and 97% of poults had low density or no lymphocellular depletion present. Lymphocellular depletion was only found in flocks 4 and 7 so all other flocks were removed from the statistical data. Red pulp abnormalities were seen in the spleen in 9% of poults and 91% of poults did not detect any abnormalities in the red pulp. Abnormalities in the red pulp were only seen in Flocks 3 through 8, so Flocks 1 and 2 were removed from the statistical data. Necrosis of ellipsoids was present in 12% of poults in the spleen and 88% of poults did not have necrosis of the ellipsoids. Necrosis was only present in Flocks 3, 5, 7 and 8. Flocks 1, 2, 4 and 6 were removed from the statistical data for ellipsoid necrosis.

No differences between heavy and light weight poults were seen for germinal center hyperplasia, red pulp abnormalities or ellipsoid necrosis (Table 3.28). A trend for less lymphocellular depletion in light weight poults was seen (Figure 3.31). Heavy weight poults were 2.7 times as likely as the light weight group to have lymphocellular depletion in the spleen ($P < 0.0951$).

Lymphocellular depletion and ellipsoid necrosis did not vary over age. Differences over age were seen for germinal center hyperplasia in the spleen (Figure 3.32a). Germinal center hyperplasia presence increased from 3% of poults at one week of age to 41% at three weeks of age ($P < 0.0021$). Red pulp abnormalities trended to increase over age ($P < 0.0857$) (Figure 3.32b).

Germinal center hyperplasia was not different among flocks. Moderate or high density of lymphocellular depletion in the spleen trended to be different among flocks (Figure 3.33). Moderate or high density of lymphocellular depletion in the spleen was found in more poults in Flock 7 than Flock 4 ($P < 0.0951$). Differences among flocks were seen in the numbers of poults with red pulp abnormalities and necrosis of ellipsoids (Figure 3.33). Flock 5 had more poults with red pulp abnormalities than Flocks 3, 4, 6

and 7 ($P < 0.0106$). Flock 8 had significantly more poults with ellipsoid necrosis than Flock 5 ($P < 0.0004$).

Xylose

Xylose absorption was determined for the Minnesota commercial and research flocks but not for the ND flocks. Average body weights are based on a sample size of 10 poults per weight group and can be seen in Figure 3.34 and Table 3.29. When comparing weight groups at two or three weeks of age poults from Flocks 5 and 6, the research flocks had the highest body weights at each age and weight group. For the commercial flocks sampled, Flocks 1 and 2 had higher weights than Flocks 3 and 4 for both weight groups at each sampling time. The weights from the light poults in Flocks 3 and 4 averaged 277 g at three weeks of age which was less than the heavy weight poults at two weeks of age which averaged 320 from the research Flocks, 5 and 6.

Xylose ranged from 16 to 39 mg D-xylose/dL for poults gavaged with D-xylose 60 minutes earlier (T60 samples) (Figure 3.36 and Table 3.30). A significant three way interaction between flock, weight group and age was found so statistics were determined for each individual flock (Table 3.31). Subsequent models looked at individual sample times (two or three weeks) for Flocks 1, 3, 5 and 6 because a significant interaction between weight group and age was found when the individual flock statistics were run. The interaction term for weight group and age was not significant for Flocks 2 and 4 so the model that was used included both sampling times for each individual flock.

A few differences were seen between heavy and light weight groups for absorption of D-xylose (Table 3.32). Heavy weight poults in Flocks 1 and 3 at three weeks of age had a 5.66 and 11.55 mg more D-xylose/dL than light poults in their plasma ($P < 0.0150$, $P < 0.1031$) (Figure 3.37). Flock 4 showed a trend with heavy weight poults absorbing 4.29 mg more D-xylose/dL plasma than light weight poults over both weeks ($P < 0.0883$). Light weight poults in Flock 1 at two weeks of age and Flock 6 at three weeks of age absorbed 10.08 and 10.1 mg more D-xylose per dL than heavy weight poults ($P < 0.0198$, $P < 0.0552$) (Figure 3.38). Flocks 2 and 5 did not show any significant differences between heavy and light weight groups at two or three weeks of age. Flock 6

at two weeks of age did not show any significant differences between heavy and light weight groups.

Xylose absorption differed in poults over age in Flock 1, 3, 4, 5 and 6 (Table 3.33). In Flocks 1 and 3 for light weight poults only two week old poults absorbed 12.67 and 8.16 mg more D-xylose than three week old poults ($P < 0.0009$, $P < 0.0515$) (Figure 3.39). In Flocks 5 and 6 for heavy weight poults only, two week old poults absorbed 14 and 5.93 mg more D-xylose than three week old poults ($P < 0.0249$, $P < 0.0737$). In Flock 4 three week old poults (both light and heavy weight groups) absorbed more D-xylose than two week old poults ($P < 0.0527$) (Figure 3.40). Flock 2 did not show any differences between sampling ages for heavy and light weight poults. Flocks 1 and 3 did not show any differences for heavy weight poults between two and 3 weeks. Flocks 5 and 6 did not show any differences between ages for light weight poults.

A baseline blood sample was collected from the wing vein after a 12 hour fast and before the poults were gavaged with D=Xylose (T0 samples). In these samples, small amounts of xylose or other reactive substances were found with an average concentration of 7.25 mg D-xylose/dL (Figure 3.35). A significant three way interaction between flock, weight group and age was found so statistics were determined for each individual flock. Subsequent models looked at individual sample times (two or three weeks) for Flocks 2, 3 and 5 because a significant interaction between weight group and age was found when the individual flock statistics were run. The interaction term for weight group and age was not significant for Flocks 1, 4 and 6 so the model that was used included both sampling times for each individual flock.

Some differences existed between weight groups for samples taken at T0 (Table 3.34). In Flock 2 at two weeks of age, heavy weight poults had 4.8 mg more D-xylose/dL than light weight poults ($P < 0.0181$). In Flock 3 at three weeks of age, light weight poults had 2.11 mg more D-xylose/dL than heavy weight poults ($P < 0.0011$). No differences in amount of D-xylose in samples collected prior to gavaging were found between weight groups for Flocks 1, 4, 5 and 6.

Differences between ages at sampling time were observed in two flocks (Table 3.35). In Flock 3 samples from three week old light weight poults had 1.32 mg more D-xylose/dL than two week old light weight poults ($P < 0.0278$). In Flock 5 samples collected at two weeks of age in heavy and light weight poults had 4.99 and 13.7 mg more D-xylose/dL than samples collected at three weeks of age ($P < 0.0061$, $P < 0.0025$). No differences in amount of D-xylose in samples collected prior to gavaging were found between weight groups for Flocks 1, 2, 4 and 6.

Commercial Flock Performance

The commercial flock performance is reported as an average of half the turkeys from Flocks 1 and 2 and an average of half the turkeys from Flocks 3 and 4. The poults from Flocks 1 and 2 were all raised in the same brooder barn and then divided into two separate grower barns. The same is true for Flocks 3 and 4. Flocks 1 and 2 were 146 days of age when processed and Flocks 3 and 4 were at 150 days of age. Live weight at processing averaged to 18.41 kg for Flocks 1 and 2 and 16.69 kg for Flocks 3 and 4. Flocks 1 and 2 gained an average 0.126 kg of body weight per day and Flocks 3 and 4 gained 0.111 kg of body weight per day. Flocks 1 and 2 feed conversion was 1.40 and Flocks 3 and 4 was 1.46. The percent mortality was 17.95% in Flocks 1 and 2, and 35.76% in Flocks 3 and 4.

DISCUSSION

The difference between heavy and light weight poults was anticipated to lead to a possible explanation of what is or is not happening in poults with light turkey syndrome. The majority of the results did not show any differences between heavy and light weight poults including the presence of pathogens. Some of the results including the histopathology and xylose absorption tests did show differences between the heavy and light weight poults. Most of the weight group differences were similar to previous findings but a few were contrary to what had been seen in the literature.

In a retrospective study on PES, researchers found inflammatory infiltrates (lymphocytes, plasmacytes and heterophils) in the lamina propria and villi in the intestine (Jindal et al., 2009b). Lymphocytic and heterophilic infiltrates were also seen in most of the poults in our study. The jejunum was the only tissue to show an increase of heterophilic infiltrates in the light weight poults. This indicates that these poults were undergoing an acute immune response. Though this was only seen in two of the flocks it did partially answer one of our main questions regarding whether the light weight poults are undergoing a different immune response than the heavy weight poults. In these two flocks the light weight poults appeared to be experiencing more of an acute immune challenge which could stem from multiple reasons. The light weight poults may be more susceptible to infection and therefore their immune system is activated whereas the heavy weight poults are not as susceptible. Another possibility is that the acute inflammatory phase in the light weight poults is still active because the chronic/acquired phase is not mature enough to respond to the infection. The acute phase requires a lot of energy which decreases the energy available for growth. The decreased energy would then result in lighter body weights.

The chronic/acquired immune response (presence of lymphocytic infiltrates) was seen in increasing amounts in the heavy weight poults in the jejunum (at two weeks), ileum and ceca. The two explanations for increased lymphocytic presence are an increased chronic immune response to a disease that had been present for more than a week and the normal maturation of lymphoid tissue in the gut (Bar-Shira et al, 2002).

Both of these indicate a functioning immune system. The first indicates a mature immune system that is producing specific lymphocytes and antibodies to fight off the disease or challenge. The second explanation indicates a prepared maturing immune system that is sending out mature lymphocytes to be sentinels in the gut tissue for possible infections. The decreased numbers of lymphocytes in light weight poults corresponds with the previous possibility that more heterophilic infiltrates are present in light weight poults because the acquired immune phase is not mature and is unable to produce enough lymphocytes. The decreased lymphocytes seen in the light weight poults may also indicate that the body is in the process of developing the chronic response (antigen specific lymphocytes in the bursa) and therefore the light weight poults are using a large amount of energy to prepare the acquired immune response which is decreasing resources available for growth. In comparison the heavy weight poults show more lymphocytes present because they have a well developed acquired immune response which is able to deal with pathogens quickly and with less cost to the bird's energy supplies.

In the same previously cited retrospective study researchers found (mild to moderated and generalized) lymphocellular depletion in the bursa of poults diagnosed with PES (Jindal et al., 2009b). In our study light weight poults had more lymphocellular depletion and atrophy of the bursa than heavy weight poults. Light weight poults were likely undergoing a disease challenge to the bursa that was causing the lymphocellular depletion. The challenge that is affecting the bursa could be the same challenge that is affecting the gut or it could be completely different. The parts of the bursa directly responsible for B-cell maturation are being compromised in the bursa which could be a possible factor why light weight poults have reduced lymphocytes in the gut tissue. The bursa could be involved in its own immune challenge that is hindering its ability to respond to other immune needs and the normal maturation and production of protective B lymphocytes. The general increased incidence of bursal atrophy as seen in light weight poults also indicates that it is being challenged in other ways besides just direct insult on the lymphocyte production. In a study conducted by Spackman et al. (2010) body weights

of poults inoculated with one to three different viruses (astrovirus, rotavirus and reovirus) had decreased weights and showed more bursal atrophy than the sham inoculated poults.

Previous studies have found that poults inoculated with astrovirus, reovirus and gut contents from poults with poult enteritis and mortality syndrome usually absorb significantly less D-xylose than control poults (Doerfler et al., 2000). Significant differences seem to last until 11 or 13 days post inoculation but not 15 days post inoculation (12 and 17 days of age but not 21 days of age). In our study we compared the light and heavy poults absorption levels to each instead of using the inoculated to control comparison. The expected results were that heavy weight poults would absorb more D-xylose than the light weight poults. In three of the flocks we did see this response (Flocks 1 and 3 at three weeks of age and Flock 4 over both weeks). These results seemed to indicate that light weight poults did absorb less D-xylose and this could possibly explain their lack of weight with the fact that they were not absorbing the same amount of nutrients in their intestine as the heavy weight poults. Flocks 3 and 4 were also undergoing a more apparent disease challenge.

Unexpected results were seen in two flocks (Flock 1 at two weeks and Flock 6 at three weeks) with light weight poults absorbing more D-xylose than heavy weight poults. These last two results and the remaining heavy verses light weight groups that did not show any differences seem to indicate that LTS is not primarily caused by the reduced ability of the intestine to absorb nutrients. Another possibility for the unexpected results is that all previous turkey studies dealt with comparing enteric virus inoculated poults with controls. In our study we did not have controls and we were not inoculating poults. We were simply seeing what a commercial flock's absorption of D-xylose was and comparing it between the weight groups. It appears from our study that using this assay to judge reduced absorption in commercial birds is highly variable and will not lead to easily decipherable results as most of the previous research has shown when using inoculated and control groups.

There were fewer pools positive for viruses in our study compared to the previous study by Jindal et al. (2010) that looked at presence of virus in poults with poult enteritis

syndrome (PES). A total of 93, 84 and 40 % of cases were found to be positive for astrovirus, rotavirus and reovirus in flocks from the PES study compared to our study that had 36, 14 and 7 % of pools positive for astrovirus, rotavirus and reovirus. The pools from the PES study are based on a sampling of poults from 43 flocks whereas our pools are from six individual flocks and individual time points. The same method (RT-PCR) was used in our study as this previous PES study for detection of the three different viruses. In a retrospective study on PES, researchers used electron microscopy methods to look at presence of enteric viruses (Jindal et al., 2009b). By electron microscopy (EM) 48 % of PES flocks less than three weeks of age were positive for rotavirus and 20 and 9 % for small round virus (SRV) and reovirus. Small round viruses include astrovirus but they can also include other viruses such as enterovirus. In the previous study by Jindal et al (2009b), EM was shown to detect viruses at a lower rate than RT-PCR. It would be expected that even though these amounts are similar to our amounts the true prevalence of viruses in poults affected with PES is higher than LTS poults (Jindal et al., 2010). Poults in the previous study would be expected to have more disease organisms present since they are showing enough symptoms to be submitted for analysis. As a result, this study may over report the number of viruses that are actually present in PES affected poults.

In a retrospective study on PES, researchers used enrichment procedures for the presence of *E. coli* and *Salmonella* that were similar to the methods used in our study (Jindal et al., 2009b). *Salmonella* and *E. coli* were detected in 64 and 32 % of samples from under three week old poults with PES. In our study 66 and 99 % of samples were positive for *Salmonella* and *E.coli*.

For *Emeria* oocysts 14% of PES flocks less than three weeks of age were positive by fecal flotation. None of our samples were positive for *Emeria* oocysts by the fecal flotation method but various coccidial life stages were seen in 13 % of the intestinal histopathology slides reviewed. The flocks in our study that showed evidence of coccidia did not represent the normal cycling seen in birds that have been vaccinated as these birds were on a coccidiostat and had not been vaccinated. Further research could look to see if

differences in the number of coccidial life stages present are different between light and heavy weight poults. In our study there were not enough samples positive for *Emeria* oocysts to be able to determine a statistical difference for load between heavy and light weight poults.

Different combinations of viruses and bacteria were found in all of the flocks so they cannot be ruled out as potential factors in LTS. A difference between weight groups was not seen but it now seems that if pathogens play a role, it is not just the presence that is important but the pathogenicity as well. Future research on pathogens present in LTS flocks could focus on the load the poult is experiencing and the pathogenicity of the microorganisms.

In the research Flocks 5 and 6 poults had higher body weights at all time periods than poults from the MN commercial Flocks 3, 4 and ND Flock 7. These flocks were all sampled at six, 13 and 20 days of age. Flocks 1 and 2 had similar body weights to the research flocks but they were sampled three days older than the research flocks at nine, 16 and 23 days of age. When comparing on an average week basis it appears Flocks 1 and 2 are close and possibly have higher body weights than the research flocks. In Figure 3.3 weights are graphed out by day of age. The projected weight of the flocks shows that the research flocks have higher body weights at all time points. Flock 8, the final ND flock, has weights above the research flocks when compared on a weekly basis and when looking at a projected weight. This flock was sampled four days later than the research flocks. The weights were expected to be higher for the research flocks since they were raised in brooder battery cages and therefore had a cleaner environment because of the wire floor which reduced the exposure to excreta. In the commercial flocks the poults were also raised at a higher density and on bedding which would increase the fecal oral spread of pathogens between poults. What was not completely expected was that the heavy and light weight groups of different flocks would be so different from each other. Heavy weight poults from Flocks 3 and 4 had average weights that were less than the light weight poults in the research flocks at the same age. The average weight of all poults sampled in Flocks 3 and 4 was lower than even the light weight poults in Flocks 5

and 6 which indicates that even though we are looking at differences between heavy and light weight groups the weight group that a poult belonged to is highly determined by the flock it was raised in not just on its perceived heavy or light weight (at least for MN flocks).

TABLES

Table 3.1 Flock information for commercial and research poults

Flock	Type	Size	State	Coccidia Prevention	Age (days)	Season
1	Commercial	Large	MN	Medication for coccidia	0, 9, 16, 23	October
2	Commercial	Large	MN	Medication for coccidia	0, 9, 16, 23	October
3	Commercial	Large	MN	Coccidia vaccine	0, 6, 13, 20	November
4	Commercial	Large	MN	Coccidia vaccine	0, 6, 13, 20	November
5	Research	--	MN	Coccidia vaccine	0, 6, 13, 20	December
6	Research	--	MN	None	0, 6, 13, 20	January
7	Commercial	Small	ND	Unknown	0, 6, 13, 20	April
8	Commercial	Small	ND	Medication for coccidia	3, 10, 17, 24	August

Table 3.2 Average sample flock weights

Age	Placement		1 week		2 weeks		3 weeks	
Flock	Weight (g)	Standard Error[1]	Weight (g)	Standard Error[1]	Weight (g)	Standard Error[1]	Weight (g)	Standard Error[1]
1	52.5	0.4	135.6	3.9	285.0	.	520.0	.
2	57.8	0.2	156.2	3.6	322.0	.	580.0	.
3	51.4	0.3	104.7	2.3	239.2	8.4	417.9	12.4
4	53.4	0.2	103.8	2.2	203.9	7.2	376.4	14.3
5	61.9	0.3	136.0	1.3	290.7	3.3	596.1	.
6	56.5	0.3	127.0	1.0	300.9	2.8	592.8	6.5
7	59.7	.	117.2	.	260.6	.	462.5	.
8	87.6[2]	.	216.3	.	485.2	.	817.2	.

[1] The missing standard errors are times when scale barn readings were used in Flocks 1 and 2 and only average data was available from ND for Flocks 7 and 8

[2] Flock 8 birds were three days of age

Table 3.3 Average body weight of poults used in field study broken apart by age and weight group

		Heavy Weight Group			Light Weight Group		
Age (weeks)	Flock	Weight (g)	Standard Error	CV	Weight (g)	Standard Error	CV
1	1	160.8	2.6	7.2	111.5	2.3	9.1
	2	171.6	1.6	4.0	127.4	2.8	9.8
	3	114.0	0.5	2.0	96.5	0.6	2.6
	4	112.0	0.6	2.2	96.5	0.7	3.1
	5	150.3	1.3	3.8	122.8	1.5	5.6
	6	140.2	1.1	3.4	112.4	0.9	3.8
	7	140.1	2.8	6.4	83.8	5.7	21.5
	8	245.9	4.7	6.1	142.6	10.6	23.5
2	1	331.7	5.9	7.9	240.9	7.0	12.9
	2	361.6	5.0	6.2	277.6	4.4	7.1
	3	229.1	2.0	3.9	178.8	2.2	5.6
	4	219.5	2.1	4.3	181.1	2.5	6.2
	5	332.7	1.9	2.6	278.8	3.5	5.6
	6	328.8	2.6	3.5	264.0	8.1	13.7
	7	313.6	10.2	10.3	168.5	4.8	9.1
	8	524.5	12.5	7.5	322.0	11.2	11.0
3	1	580.9	6.6	5.1	458.5	6.8	6.7
	2	629.7	5.1	3.6	544.8	6.2	5.1
	3	471.3	3.4	3.2	375.4	3.7	4.4
	4	418.1	2.0	2.2	342.3	3.0	3.9
	5	662.0	9.6	6.5	551.0	7.1	5.8
	6	661.0	4.8	3.3	531.6	5.8	4.8
	7	515.5	14.0	8.6	287.4	22.7	25.0
	8	896.3	19.2	6.8	475.7	49.7	33.0

N = 20 for MN Flocks 1 through 6 and N = 10 for ND Flocks 7 and 8

Table 3.4 Statistical results for virus presence

Organism	Flock	Age	Weight Group	Interactions
Astrovirus	P<0.0059	P<0.0043	NS[1]	NS
Rotavirus	P<0.0914	NS	NS	NS
Reovirus	NS	NS	NS	NS

[1] NS = not significant ($P > 0.05$)

Table 3.5 Percent of pools positive for astrovirus

Flock	1 week		2 weeks		3 weeks	
	Heavy	Light	Heavy	Light	Heavy	Light
1	100%	75%	0	0	0%	0
2	0	0	0	0	25%	0
3	50%	50%	75%	50%	0	0
4	50%	50%	50%	100%	50%	50%
5	0	0	0	0	25%	0
6	75%	100%	25%	75%	0	0
7	100%	100%	100%	100%	0	100%
8	100%	100%	100%	100%	0	0

Astrovirus was significantly different over flock and age ($P<0.0059$, $P<0.0043$)
N = 4 for MN Flocks 1 through 6 and N = 2 for ND Flocks 7 and 8

Table 3.6 Percent of pools positive for rotavirus

Flock	1 week		2 weeks		3 weeks	
	Heavy	Light	Heavy	Light	Heavy	Light
1	50%	0%	25%	25%	0	0
2	0	0	0	0	0	0
3	75%	50%	75%	25%	0	0
4	0	50%	50%	50%	0	0
5	25%	0	0	0	0	0
6	50%	0	0	25%	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0

Rotavirus was significantly different over flock ($P<0.0914$)
N = 4 for MN Flocks 1 through 6 and N = 2 for ND Flocks 7 and 8
Flocks 2, 7 and 8 and data from week three were removed from statistical data set

Table 3.7 Percent of pools positive for reovirus

Flock	1 week		2 weeks		3 weeks	
	Heavy	Light	Heavy	Light	Heavy	Light
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	50%	50%	25%	50%	0	0
4	0	25%	25%	50%	0	0
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0

Reovirus was not significantly different over flock, age or weight group
N = 4 for MN Flocks 1 through 6 and N = 2 for ND Flocks 7 and 8
Flocks 1, 2, 5, 6, 7 and 8 and data from week three were removed from statistical data set

Table 3.8 Statistical results for bacteria presence

Organism	Flock	Age	Weight Group	Interactions
Salmonella	*P*<0.0617	NS[1]	NS	none
Campylobacter	NS	NS	NS	none
E. Coli[2]	NS	NS	NS	none

[1] NS = not significant (*P* > 0.05)
[2] *E. Coli* was found in all pools but one

Table 3.9 Percent of pools positive for *Salmonella spp.*

Flock	1 week		2 weeks		3 weeks	
	Heavy	Light	Heavy	Light	Heavy	Light
1	100%	75%	100%	75%	75%	50%
2	75%	100%	75%	100%	50%	100%
3	100%	100%	100%	100%	100%	50%
4	100%	100%	75%	75%	100%	100%
5	25%	50%	100%	75%	0	33%
6	0	0	0	0	0	0
7	100%	100%	50%	100%	100%	50%
8	100%	50%	100%	100%	100%	100%

Salmonella was significantly different over flock (*P*<0.0914)
N = 4 pools of five poults for MN Flocks 1 to 6
N = 2 pools of five poults for ND Flocks 7 and 8 (ND flocks had individual bird results but they were transformed into pooled results)
Flock 6 was removed from statistical data set

Table 3.10 Percent of pools positive for *Campylobacter spp.*

Flock	1 week		2 weeks		3 weeks	
	Heavy	Light	Heavy	Light	Heavy	Light
1	50%	0	25%	0	0	25%
2	0	25%	25%	25%	25%	0
3	100%	75%	75%	25%	25%	0
4	100%	100%	0	25%	0	25%
5	0	0	75%	100%	0	0
6	0	0	0	0	0	0
7	0	0	0	50%	100%	100%
8	0	0	0	0	0	0

*Campylobacter spp.*was not significantly different among flocks, weeks or weight groups
N = 4 pools of five poults for MN Flocks 1 to 6
N = 2 pools of five poults for ND Flocks 7 and 8 (ND flocks had individual bird results but they were transformed into pooled results)
Flocks 6 and 8 were removed from statistical data set

Table 3.11 Statistical results for plate counts for bacteria

Bacteria	Flock	Week	Weight Group	Flock x Week Interaction
Aerobic	*P*<0.0037	*P*<0.0001	NS[1]	*P*<0.0001
Anaerobic	*P*<0.0001	*P*<0.0004	*P*<0.0887	*P*<0.0001
Lactobacilli	*P*<0.0224	*P*<0.0001	NS	*P*<0.0001
Heterofermentative Lactobacilli	*P*<0.0003	*P*<0.0001	NS	*P*<0.0001

[1] NS = not significant (*P* > 0.05)
Multiple samples had no growth for each bacterium and were treated as missing values
See tables 3.14 to 3.17 for number of samples with no growth per group

Table 3.12 Statistical results for aerobic and anaerobic bacteria

	Aerobic		Anaerobic	
Flock	Week	Weight Group	Week	Weight Group
1	*P*<0.0005	NS[1]	NS	NS
2	*P*<0.0048	NS	NS	NS
3	*P*<0.0001	NS	*P*<0.0129	NS
4	*P*<0.0001	NS	NS	NS
5	*P*<0.0006	NS	*P*<0.0024	NS
6	*P*<0.0185	NS	*P*<0.0162	NS
7	*P*<0.0429	NS	*P*<0.0001	NS
8	*P*<0.0002	NS	*P*<0.0001	NS

[1] NS = not significant (*P* > 0.05)
See tables 3.14 and 3.15 for number of samples with no growth per group

Table 3.13 Statistical results for lactobacilli and heterofermentative lactobacilli

	Lactobacilli		Heterofermentative Lactobacilli	
Flock	Week	Weight Group	Week	Weight Group
1	*P*<0.0618	NS[1]	*P*<0.0041	NS
2	*P*<0.0049	NS	*P*<0.0008	NS
3	NS	NS	*P*<0.0307	NS
4	*P*<0.0001	*P*<0.0664	*P*<0.0180	NS
5	*P*<0.0008	NS	*P*<0.0002	NS
6	*P*<0.0001	NS	*P*<0.0002	NS
7	*P*<0.0001	NS	*P*<0.0759	NS
8	*P*<0.0001	NS	*P*<0.0001	NS

[1] NS = not significant (*P* > 0.05)
See tables 3.16 and 3.17 for number of samples with no growth per group

Table 3.14 Average counts for aerobic bacteria

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		(Log_{10} cfu/g)	Standard Error	(Log_{10} cfu/g)	Standard Error
1	1	7.67	0.13	7.76	0.29
	2	8.09	0.13	7.79	0.22
	3	7.64	0.25	8.07	0.18
	4	7.71	0.22	7.81	0.27
	5	7.78	0.48	8.29	0.11
	6	7.44	0.72	8.65	0.43
	7	8.00	0.28	7.27	0.31
	8	8.12	0.34	7.93	0.11
2	1	7.81[2]	0.02	8.05[3]	.
	2	7.50[1]	0.14	7.67[1]	0.18
	3	6.18	0.24	6.22	0.29
	4	5.97	0.23	6.54	0.29
	5	7.54[1]	0.12	6.71	0.06
	6	7.69	0.17	7.11	0.25
	7	6.82[4]	0.27	6.94	0.24
	8	6.99	0.24	6.89	0.23
3	1	7.03	0.20	6.66	0.14
	2	7.03[1]	0.19	7.41	0.17
	3	6.92	0.22	7.00	0.24
	4	7.08	0.32	7.22	0.1
	5	6.39[1]	0.33	6.2	0.53
	6	6.62[1]	0.33	6.72	0.15
	7	6.94	0.21	7.44	0.28
	8	7.66	0.21	7.82[2]	0.38

See Table 3.12 for significant differences
N = 4 pools of five poults for MN Flocks 1 to 6
N = 10 individual poults for ND Flocks 7 and 8
[1] No growth for 1 sample
[2] No growth for 2 samples
[3] No growth for 3 samples
[4] No growth for 4 samples

Table 3.15 Average counts for anaerobic bacteria

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		(Log_{10} cfu/g)	Standard Error	(Log_{10} cfu/g)	Standard Error
1	1	7.47	0.30	7.64	0.35
	2	7.89	0.12	7.14	0.30
	3	7.97	0.30	8.17	0.12
	4	7.40	0.19	7.80	0.39
	5	7.81	0.47	8.30	0.06
	6	7.60	0.61	8.82	0.36
	7	6.34	0.32	6.30	0.29
	8	8.28	0.31	8.62	0.16
2	1	7.63[1]	0.28	8.13[3]	.
	2	7.43[1]	0.09	7.34[2]	0.01
	3	7.75	0.39	7.51	0.42
	4	7.26	0.14	7.74	0.13
	5	7.59[1]	0.17	6.73[2]	0.22
	6	7.92	0.14	7.09	0.37
	7	6.94[2]	0.21	7.46	0.18
	8	6.96[2]	0.34	7.26	0.19
3	1	7.33	0.25	7.60[1]	0.09
	2	7.54	0.09	7.66	0.08
	3	7.12	0.15	7.25	0.06
	4	7.74	0.21	7.52	0.12
	5	6.48[1]	0.26	6.66[1]	0.48
	6	6.77[2]	0.48	6.67	0.27
	7	7.30	0.18	7.33	0.18
	8	7.68	0.20	7.97[3]	0.37

See Table 3.12 for significant differences
N = 4 pools of five poults for MN Flocks 1 to 6
N = 10 individual poults for ND Flocks 7 and 8
[1] No growth for 1 sample
[2] No growth for 2 samples
[3] No growth for 3 samples

Table 3.16 Average counts for lactobacilli

Age (weeks)	Flock	Heavy Weight Group (Log_{10} cfu/g)	Heavy Weight Group Standard Error	Light Weight Group (Log_{10} cfu/g)	Light Weight Group Standard Error
1	1	7.65	0.34	7.66	0.26
	2	8.46	0.14	7.69	0.22
	3	7.68	0.44	8.26	0.28
	4	8.31	0.12	8.26	0.21
	5	7.87	0.47	8.37	0.06
	6	8.52	0.22	8.98	0.12
	7	7.99	0.31	7.88	0.27
	8	8.72	0.34	8.68	0.20
2	1	7.71	0.15	7.75[1]	0.32
	2	8.10	0.27	7.75[1]	0.25
	3	7.66	0.38	7.42	0.45
	4	7.08	0.25	7.55	0.14
	5	7.36	0.33	6.79	0.20
	6	7.96	0.16	7.26[1]	0.26
	7	6.87[2]	0.16	7.12[2]	0.20
	8	7.29	0.21	7.22[1]	0.22
3	1	7.23	0.26	7.04	0.26
	2	6.95	0.23	7.45	0.14
	3	7.65	0.20	7.59	0.18
	4	7.12	0.15	7.62	0.23
	5	6.44[1]	0.29	6.46[1]	0.62
	6	6.57	0.29	7.27	0.33
	7	8.07	0.19	8.03	0.18
	8	7.32	0.21	7.82[3]	0.36

See Table 3.13 for significant differences
N = 4 pools of five poults for MN Flocks 1 to 6
N = 10 individual poults for ND Flocks 7 and 8
[1] No growth for 1 sample
[2] No growth for 2 samples
[3] No growth for 3 samples

Table 3.17 Average counts for heterofermentative lactobacilli

Age (weeks)	Flock	Heavy Weight Group (Log_{10} cfu/g)	Heavy Weight Group Standard Error	Light Weight Group (Log_{10} cfu/g)	Light Weight Group Standard Error
1	1	7.78	0.21	7.82	0.16
	2	8.08	0.22	7.73	0.13
	3	8.04	0.22	8.39	0.11
	4	8.12	0.17	7.92	0.20
	5	7.89	0.44	8.34	0.05
	6	8.56	0.27	9.08	0.15
	7	8.08	0.29	7.78	0.31
	8	8.84	0.32	8.93	0.15
2	1	7.95[1]	0.32	7.67[1]	0.12
	2	7.72[1]	0.22	8.05[1]	0.35
	3	7.73	0.37	7.56	0.33
	4	7.24	0.28	7.61	0.19
	5	6.85[2]	0.48	6.78[1]	0.26
	6	7.91	0.17	7.11	0.28
	7	7.23[2]	0.15	7.50	0.23
	8	7.26	0.25	7.24	0.23
3	1	7.02	0.25	7.20[1]	0.08
	2	6.77	0.20	7.21	0.21
	3	7.60	0.20	7.61	0.09
	4	7.37	0.17	7.64	0.20
	5	6.23	0.40	6.67[1]	0.31
	6	6.40[2]	0.11	7.37	0.45
	7	7.72	0.23	7.82	0.18
	8	7.68	0.19	7.72[2]	0.33

See Table 3.13 for significant differences
N = 4 pools of five poults for MN Flocks 1 to 6
N = 10 individual poults for ND Flocks 7 and 8
[1] No growth for 1 sample
[2] No growth for 2 samples

Table 3.18 Direction of change for bacteria counts from one to three weeks of age

Flock	Aerobic	Anaerobic	Lactobacilli	Heterofermentative Lactobacilli
1	Decreased	NS[1]	Decreased	Decreased
2	Decreased	NS	Decreased	Decreased
3	Decreased	Decreased	NS	Decreased
4	Decreased	NS	Decreased	Decreased
5	Decreased	Decreased	Decreased	Decreased
6	Decreased	Decreased	Decreased	Decreased
7	Decreased	Increased	Lowest at 2 wks	Decreased
8	Lowest at 2 wks	Lowest at 2 wks	Decreased	Decreased

[1] NS = not significant ($P > 0.05$)

Table 3.19 Bacteria plate counts from samples at placement

	Aerobic		Anaerobic		Lactobacilli		Heterofermentative Lactobacilli	
Flock	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	6.71	0.32	6.71	0.32	6.44	0.43	6.54	0.41
2	5.79	0.96	5.79	0.96	5.31	0.69	5.21	0.58
3	4.86	0.33	4.86	0.33	4.75	0.38	4.78	0.37
4	6.15	0.99	6.15	0.99	6.04	0.84	6.05	0.88
5	5.48	0.68	5.48	0.68	5.28	0.56	5.58	0.52
6[1]	.	.	.	.	.	.	.	.
7	5.41[2]	0.51	5.41[3]	0.51	5.79[3]	0.56	6.05[3]	0.51
8[4]	7.93[5]	0.08	7.93[6]	0.08	7.89	0.07	7.92	0.07

N = 4 for MN Flocks 1 to 6 and N = 20 for ND Flocks 7 and 8

[1] All Flock 6 plate counts were too numerous to count for samples at placement

[2] 13 samples were too numerous to count

[3] 12 samples were too numerous to count

[4] Flock 8 was sampled at three days of age not at placement

[5] 2 samples were too numerous to count

[6] 1 sample was too numerous to count

Table 3.20 Flocks listed had significantly higher counts than those that are not listed

Age (weeks)	Aerobic	Anaerobic	Lactobacilli	Heterofermentative Lactobacilli
0	8 (6 TNTC[1])	1 8 (6 TNTC)	8 (6 TNTC)	8 (6 TNTC)
1	NS	2 5 6 8	4 5 6 8	6 8
2	1 2 3 4	NS	1 2 3 5	1 2 3 4 6 7
3	2 8	1 2 4 8	3 7	3 4 7 8

[1] TNTC = too numerous to count, significant differences were unable to be determined for Flock 6 at placement

Table 3.21 Statistical results for presence of coccidia from histopathology

Tissue	Flock	Age	Weight Group	Interactions
Duodenum[1]	NS[5]	*P*<0.0078	NS	none
Jejunum[2]	*P*<0.0001	*P*<0.0001	NS	none
Ileum[3]	nim[6]	*P*<0.0001	NS	none
Ceca[4]	*P*<0.1138	*P*<0.0042	NS	none

[1] Flocks 1, 2, 5, 6 and 7 were removed from the statistical data
[2] Flocks 1, 2, 5 and 6 and week one were removed from the statistical data
[3] Flocks 1, 2, 5 and 6 were removed from the statistical data
[4] Flocks 1, 2, 5 and 6 and week two were removed from the statistical data
[5] NS = not significant ($P > 0.05$)
[6] nim = not in model

Table 3.22 Score levels for heterophilic and lymphocytic infiltrates

Statistical Data	Density	Distribution
0	Absent	Absent
0	Low	Focal
1	Moderate	Multifocal
1	High	Generalized

Table 3.23 Heterophilic density statistical results

Tissue	Flock	Age	Weight Group	Interactions
Duodenum[1]	NS[5]	NS	nim[6]	NS
Jejunum[2]	NS	NS	NS	NS
Ileum[3]	NS	NS	NS	NS
Ceca [4]	NS	NS	NS	NS

[1] Flocks 4, 5, 6, 7 and week 2 were removed from the statistical data
[2] Flocks 1, 2, 7 and 8 were removed from the statistical data
[3] Flocks 1, 3 and 7 were removed from the statistical data
[4] Flocks 2, 3, 4, 6 and 7 were removed from the statistical data and Flocks 1 and 2 only had samples collected at three weeks of age
[5] NS = not significant ($P > 0.05$)
[6] nim = not in model

Table 3.24 Heterophilic distribution statistical results

Tissue	Flock	Age	Weight Group	Flock* Age* Weight Group	Flock* Age	Other Interactions
Duodenum	*P*<0.0081	NS[2]	NS	NS	NS	NS
Jejunum	*P*<0.0001	NS	NS	*P*<0.0349	*P*<0.0012	NS
Ileum	*P*<0.0007	*P*<0.0679	NS	NS	NS	NS
Ceca[1]	NS	*P*<0.0054	NS	NS	NS	NS

[1] Flock 1 was removed from the statistical data and Flocks 1 and 2 only had samples collected at three weeks of age
[2] NS = not significant ($P > 0.05$)

Table 3.25 Lymphocytic density statistical results

Tissue	Flock	Age	Weight Group	Interactions
Duodenum	NS[3]	NS	NS	NS
Jejunum[1]	*P*<0.0356	*P*<0.0017	NS	NS
Ileum	NS	NS	*P*<0.1044	NS
Ceca [2]	NS	*P*<0.0013	*P*<0.0048	NS

[1] Flock 4 was removed from the statistical data
[2] Flocks 4 and 5 were removed from the statistical data and Flocks 1 and 2 only had samples collected at three weeks of age
[3] NS = not significant ($P > 0.05$)

Table 3.26 Lymphocytic distribution statistical results

Tissue	Flock	Age	Weight Group	Age* Weight Group	Other Interactions
Duodenum	*P*<0.0031	*P*<0.0001	NS[1]	NS	NS
Jejunum	*P*<0.0001	*P*<0.0001	NS	*P*<0.0427	NS
Ileum	*P*<0.0001	*P*<0.0001	*P*<0.0914	NS	NS
Ceca	Not scored				

[1] NS = not significant ($P > 0.05$)

Table 3.27 Bursa statistical results

Score	Type	Flock	Age	Weight Group	Age* Weight Group
Heterophilic	Distribution	*P*<0.0015	*P*<0.1035	NS[4]	NS
	Density[1]	*P*<0.0593	*P*<0.0577	NS	NS
Lymphocellular	Distribution[2]	*P*<0.0526	*P*<0.0002	*P*<0.0304	NS
depletion	Density[3]	*P*<0.0717	NS	NS	NS
Atrophy	Density	*P*<0.0005	NS	NS	*P*<0.0151

[1] Flock 7 was removed from the statistical data
[2] Flock 8 was removed from the statistical data
[3] Flocks 1 and 8 were removed from the statistical data
[4] NS = not significant ($P > 0.05$)

Table 3.28 Spleen statistical results

Score	Flock	Age	Weight Group	Interactions
Germinal Center Hyperplasia[1]	NS[5]	*P*<0.0021	NS	NS
Lymphocellular Depletion[2]	*P*<0.0951	NS	*P*<0.0951	NS
Red Pulp[3]	*P*<0.0106	*P*<0.0857	NS	NS
Ellipsoids[4]	*P*<0.0004	NS	NS	NS

[1] Flocks 3, 4, 7 and 8 were removed from the statistical data
[2] Flocks 1, 2, 3, 5, 6 and 8 were removed from the statistical data
[3] Flocks 1 and 2 were removed from the statistical data
[4] Flocks 1, 2, 4 and 6 were removed from the statistical data
[5] NS = not significant ($P > 0.05$)

Table 3.29 Average body weights for poults used for xylose analysis

		Heavy Weight Group			Light Weight Group		
Age (weeks)	Flock	Weight (g)	Standard Error	CV	Weight (g)	Standard Error	CV
2	1	265.8	2.6	3.0	207.6	3.7	5.7
	2	288.9	3.9	4.3	212.4	3.7	5.5
	3	192.7	1.8	3.0	161.0	3.0	5.8
	4	197.5	1.8	2.9	162.3	4.1	8.0
	5	295.9	5.4	5.8	234.6	5.2	7.7
	6	342.6	3.7	3.4	286.7	3.9	4.3
3	1	534.6	7.4	4.4	406.4	4.6	3.6
	2	583.2	3.7	2.0	421.5	6.7	5.0
	3	371.7	10.1	8.6	275.9	7.1	8.2
	4	351.7	4.8	4.3	276.8	10.4	11.9
	5	577.0	9.1	5.0	475.6	9.5	6.3
	6	651.0	8.3	4.0	550.5	6.0	3.5

N = 10 poults

Table 3.30 Average xylose absorption at 60 minutes

At Age (weeks)	Flock	Heavy Weight Group D-xylose (mg/dL)	SE	N[1]	Light Weight Group D-xylose (mg/dL)	SE	N[1]
2	1	23.76	3.07	8	33.84	2.02	7
	2	26.59	3.62	9	29.89	3.01	10
	3	31.08	2.11	10	30.44	3.14	10
	4	33.49	1.24	9	29.69	2.55	9
	5	30.03	5.32	9	19.05	2.44	4
	6	24.87	2.65	10	23.27	2.54	10
3	1	26.83	2.38	10	21.17	2.12	8
	2	23.38	2.06	9	31.01	4.65	10
	3	33.83	3.56	10	22.28	2.15	9
	4	38.94	3.05	10	34.15	2.57	10
	5	16.03	2.51	10	18.44	2.00	10
	6	18.93	0.95	8	29.04	4.28	10

[1] Ten samples collected per group, N is the number of successfully analyzed samples

Table 3.31 Statistical results for xylose absorption at 60 minutes

Flock	1	2	3	4	5	6
Weight Group	NS[1]	NS	NS	P<0.0527	NS	NS
Age	P<0.0641	NS	P<0.0392	P<0.0883	P<0.0666	NS
Age*Weight Group	P<0.0037	(NS)	P<0.0634	(NS)	P<0.0913	P<0.0606

[1] NS = not significant ($P > 0.05$)

Table 3.32 Average xylose absorption prior to gavage

Age (weeks)	Flock	Heavy Weight Group D-xylose (mg/dL)	SE	N[1]	Light Weight Group D-xylose (mg/dL)	SE	N[1]
2	1	9.098	2.506	9	7.887	0.765	10
	2	10.56	1.587	10	5.763	0.943	10
	3	5.711	0.510	7	6.199	0.420	8
	4	5.524	0.899	9	9.101	3.599	7
	5	18.12	5.599	6	9.576	1.431	9
	6	4.565	0.377	10	4.849	0.483	10
3	1	8.051	0.698	10	7.669	0.670	8
	2	7.686	1.013	9	10.42	3.518	9
	3	5.411	0.399	10	7.525	0.352	9
	4	5.148	0.367	10	4.226	0.489	10
	5	4.427	0.251	10	4.589	0.369	10
	6	5.123	0.490	10	6.836	0.542	10

[1] Ten samples collected per group, N is the number of successfully analyzed samples

Table 3.33 Differences between heavy and light weight groups for D-xylose concentrations found in plasma collected from poults 60 minutes after being gavaged with a 5 % D-xylose solution

	Age	
Flock	2 weeks	3 weeks
1	Light > Heavy	Heavy > Light
2	NS[1]	
3	NS	Heavy > Light
4	Heavy > Light	
5	NS	NS
6	NS	Light > Heavy

[1] NS = not significant ($P > 0.05$)

Table 3.34 Differences between two and three week old poults for D-xylose concentrations found in plasma collected from poults 60 minutes after being gavaged with a 5 % D-xylose solution

	Weight Group	
Flock	Heavy	Light
1	NS[1]	2 weeks > 3 weeks
2	NS	
3	NS	2 weeks > 3 weeks
4	3 weeks > 2 weeks	
5	2 weeks > 3 weeks	NS
6	2 weeks > 3 weeks	NS

[1] NS = not significant ($P > 0.05$)

Table 3.35 Differences between heavy and light weight groups for D-xylose concentrations found in plasma collected from poults before being gavaged with a 5 % D-xylose solution

	Age	
Flock	2 weeks	3 weeks
1	NS[1]	
2	Heavy > Light	NS
3	NS	Light > Heavy
4	NS	
5	NS	NS
6	NS	

[1] NS = not significant ($P > 0.05$)

Table 3.36 Differences between two and three week old poults for D-xylose concentrations found in plasma collected from poults before being gavaged with a 5 % D-xylose solution

	Weight Group	
Flock	Heavy	Light
1	NS[1]	
2	NS	NS
3	NS	3 weeks > 2 weeks
4	NS	
5	2 weeks > 3 weeks	2 weeks > 3 weeks
6	NS	

[1] NS = not significant ($P > 0.05$)

FIGURES

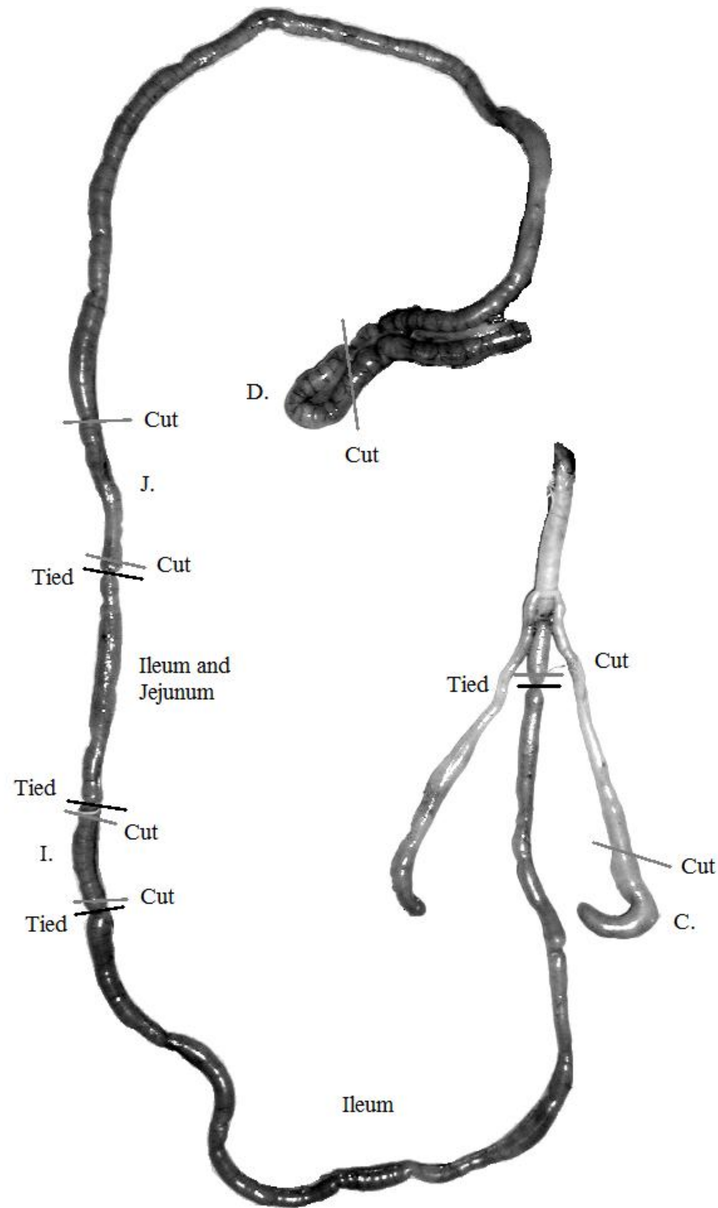
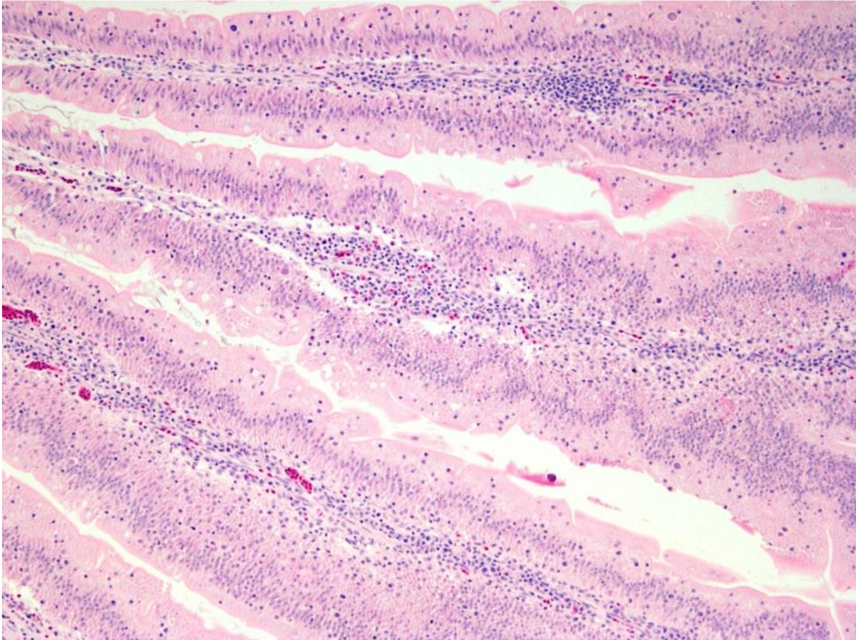


Figure 3.1 Picture of gut sections collected for contents and histopathology
Tied (grey line): where floss was used
Cut (black line): where specimen was cut
Histopathology sections are identified as D. duodenum, I. ileum, J. jejunum, C. ceca
Sections collected for bacteria analysis are the section labeled ileum and jejunum
Section used for virus and protozoa analyses and for inoculums is labeled ileum

a.



b.

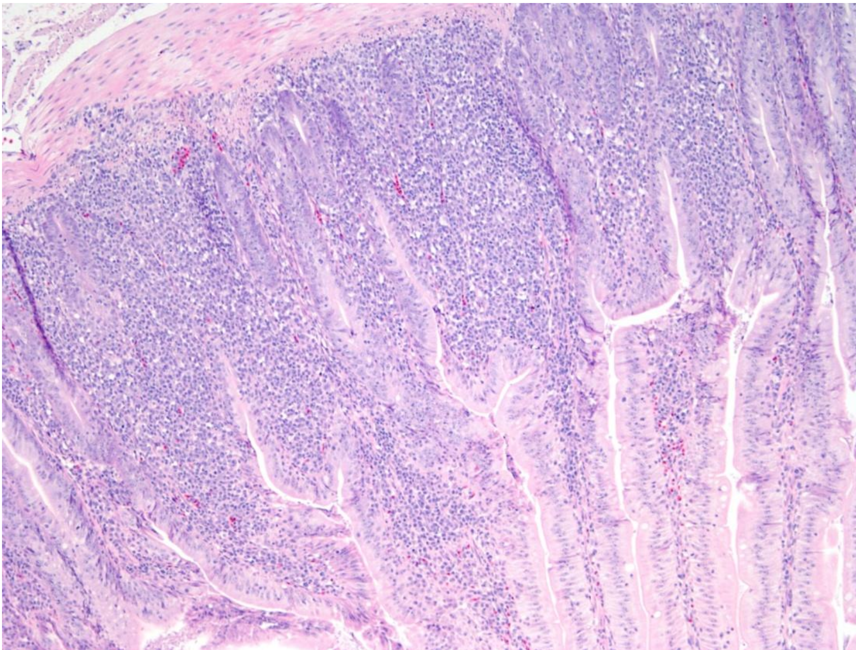


Figure 3.2 Duodenum from three week old light weight poults

a. Tissue from Flock 4 with a low density of lymphocytic infiltrates (100x)

b. Tissue from Flock 2 with a moderate density of lymphocytic infiltrates (100x)

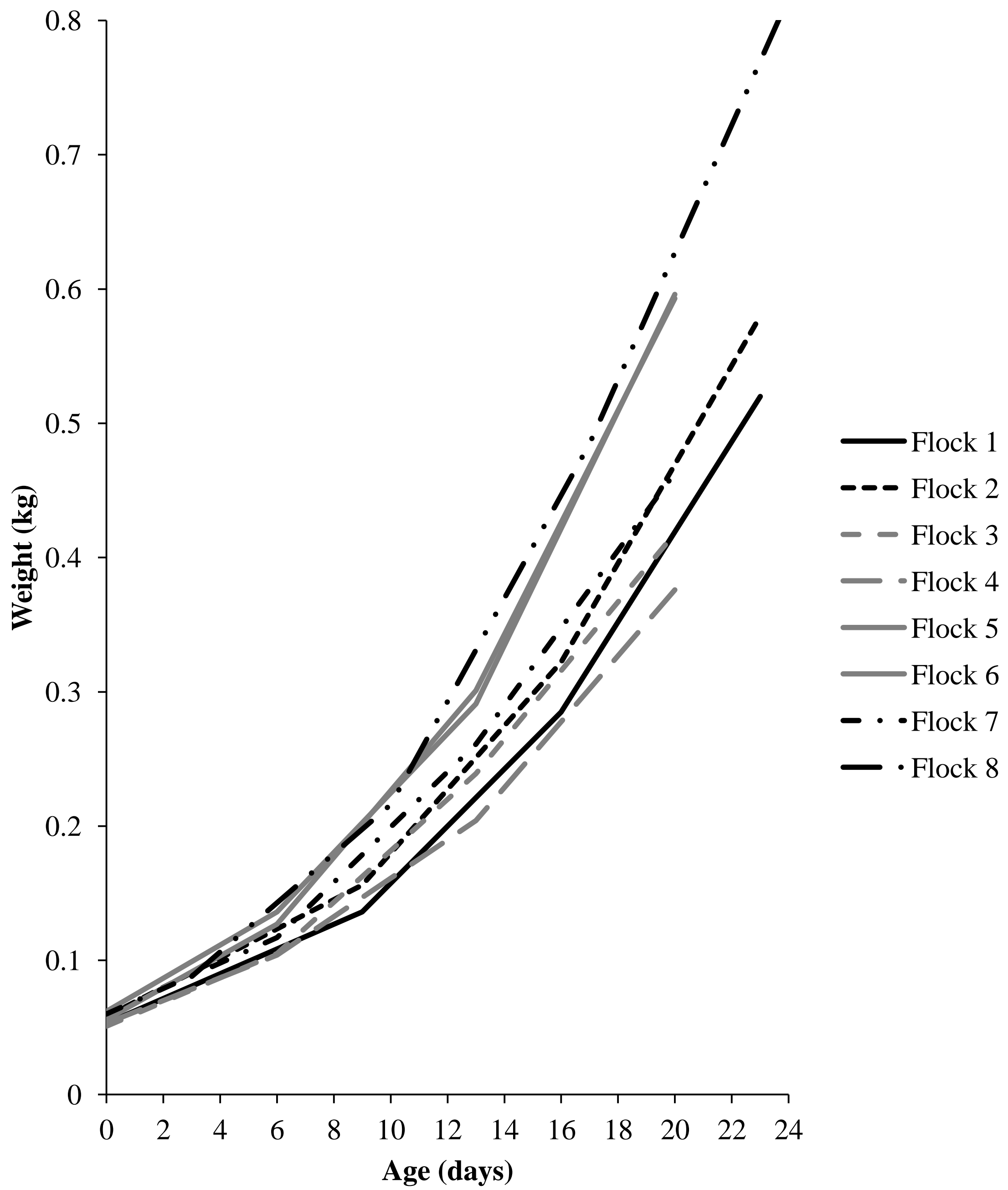


Figure 3.3 Average poult weights over age

MN commercial flocks (1 through 4), MN research flocks (5 and 6) and ND commercial flocks (7 and 8) Nicholas performance standards for male turkeys 88 x 700 (2010) at 7, 14, and 21 days of age are 150, 400 and 790 grams

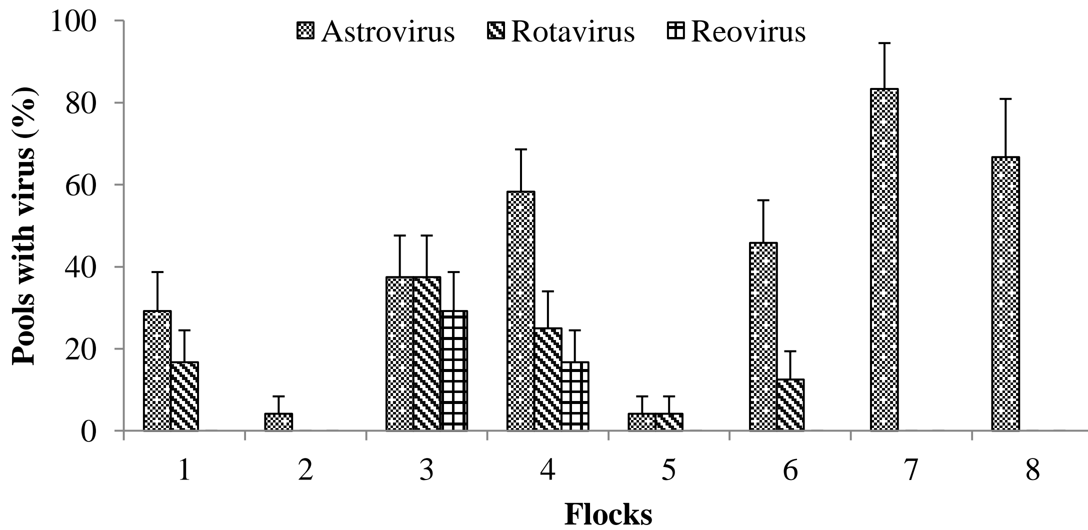


Figure 3.4 Virus presence among flocks
Results are means averaged over age and weight group with standard error bars
N = 24 pools of five poults for Flocks 1 through 6, N = 12 pools of five poults for Flocks 7 and 8
Astrovirus was significantly different among flocks (P<0.0059)
Rotavirus was significantly different among flocks (P<0.0914) (Flocks 2, 7 and 8 and data from week three were removed from statistical data set)
Reovirus was not significantly different among flocks (Flocks 1, 2, 5, 6, 7 and 8 and data from week three were removed from statistical data set)

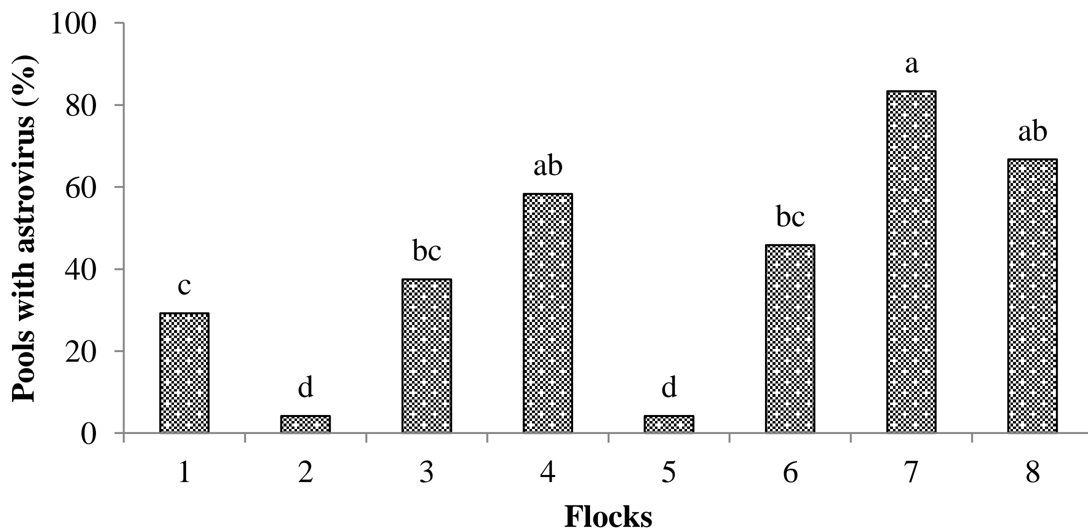


Figure 3.5 Astrovirus presence among flocks
Results are means averaged over age and weight group
N = 24 pools of five poults for Flocks 1 through 6, N = 12 pools of five poults for Flocks 7 and 8
Astrovirus was significantly different among flocks (P<0.0059)
a, b, c, d: bars with different letters are significantly different ($P < 0.05$)

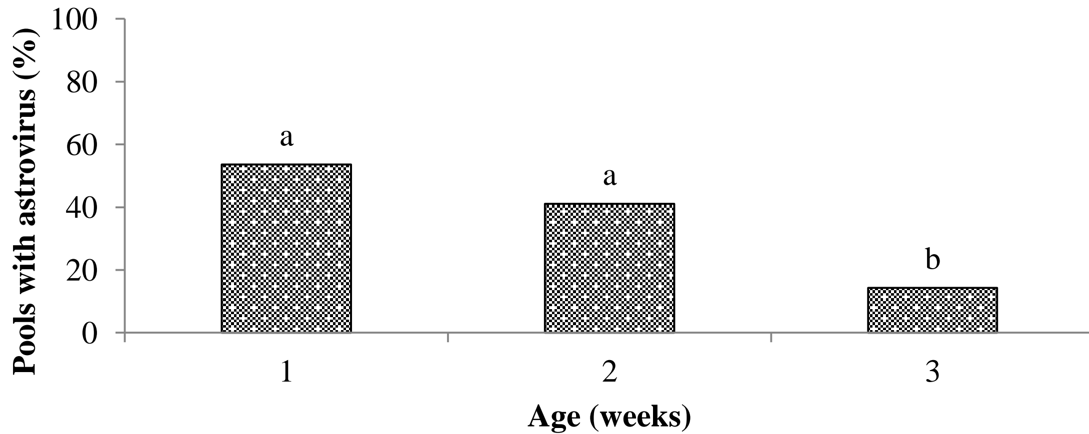


Figure 3.6 Astrovirus presence over age
Results are means averaged over flock and weight group
N = 56 pools of five poults
Astrovirus significantly decreased over age (*P*<0.0043)
a, b, c, d: bars with different letters are significantly different (*P* < 0.05)

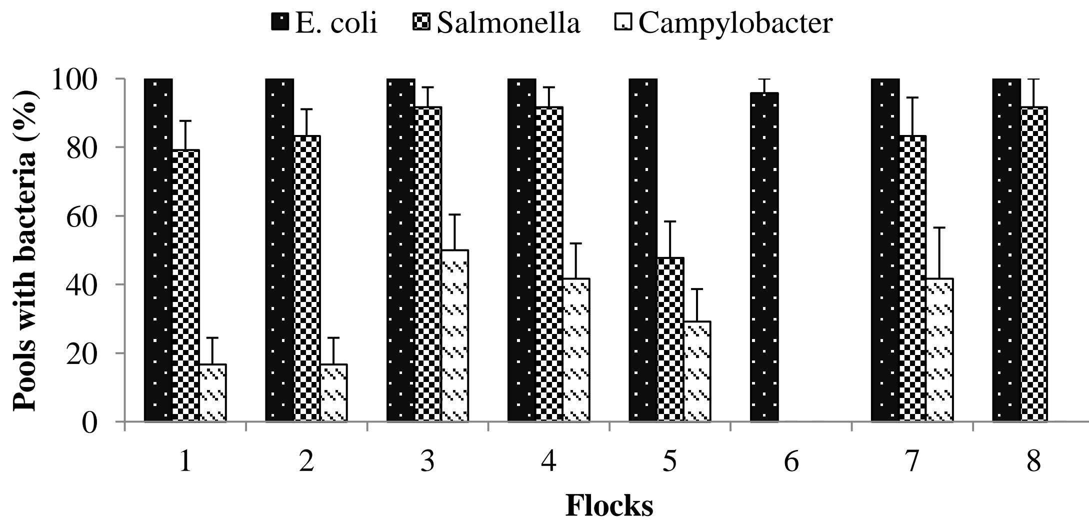


Figure 3.7 Bacteria presence among flocks
Results are means averaged over age and weight group with standard error bars
N = 24 pools of five poults for Flocks 1 through 6
N = 12 pools of five poults for Flocks 7 and 8
Salmonella was significantly different among flocks (*P*<0.0914) (Flock 6 was removed from statistical data set)
*Campylobacter spp.*was not significantly different among flocks (Flocks 6 and 8 were removed from statistical data set)
E. Coli was not significantly different among flocks

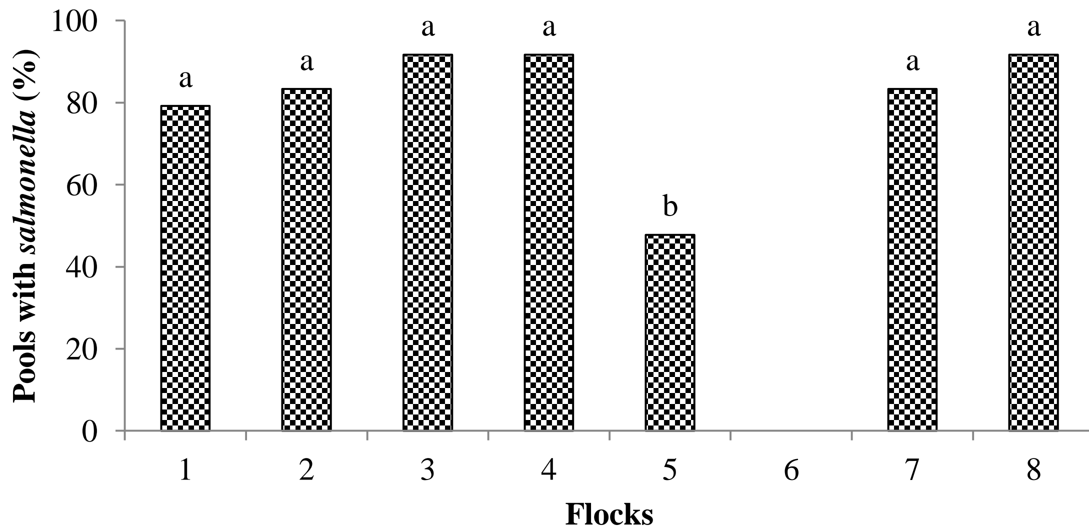


Figure 3.8 *Salmonella spp.* presence among flocks
Results are means averaged over age and weight group
N = 24 pools of five poults for Flocks 1 through 6
N = 12 pools of five poults for Flocks 7 and 8
Salmonella was significantly different among flocks (P<0.0914) (Flock 6 was removed from statistical data set)
a, b, c, d: bars with different letters are significantly different ($P < 0.05$)

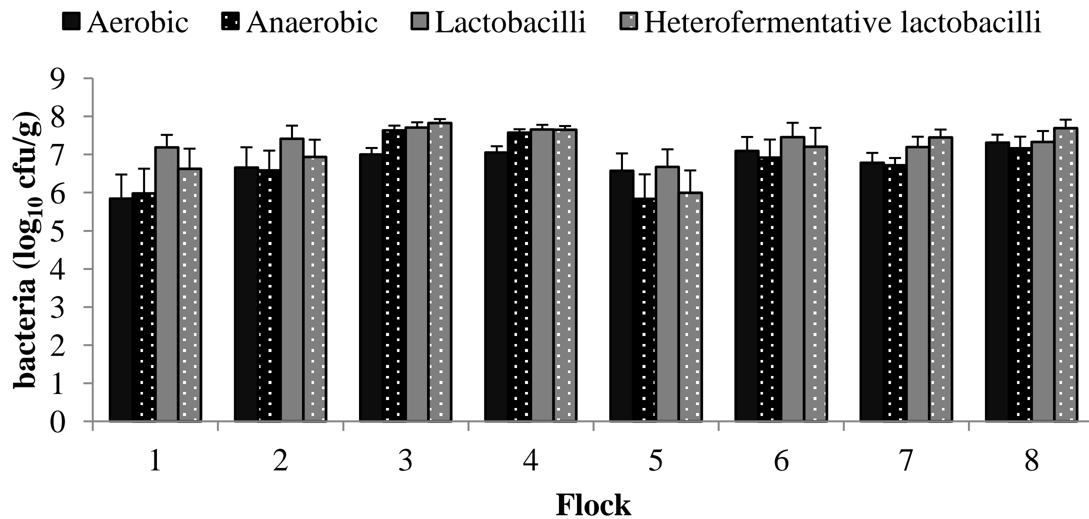


Figure 3.9 Total plate counts for bacteria
Results are means averaged over age and weight group with standard error bars
N = 24 pools of five poults for Flocks 1 through 6
N = 60 poults for Flocks 7 and 8
Significant interaction between flock and age was present for all bacteria (P<0.0001)

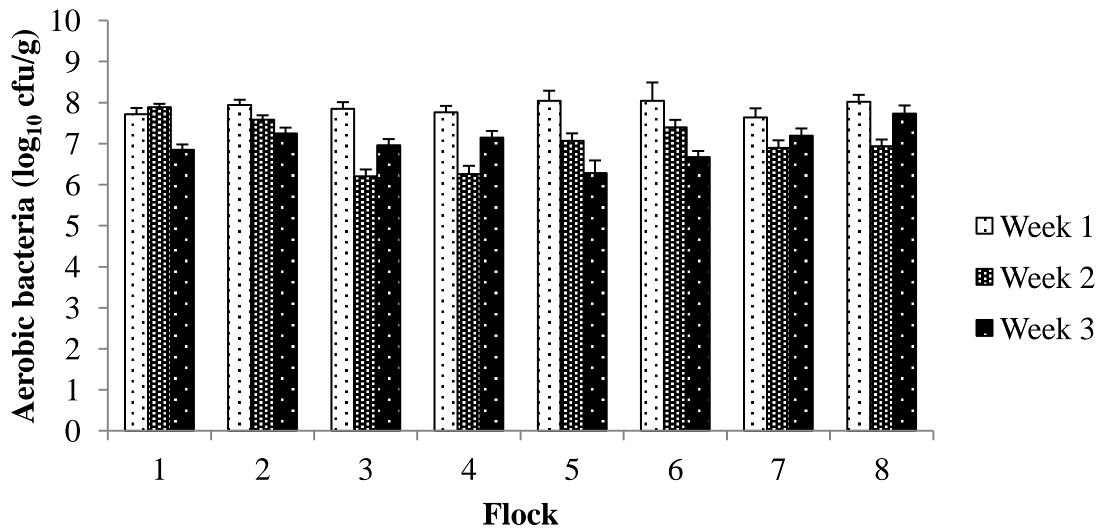


Figure 3.10 Aerobic bacteria by flock and age
Results are means averaged over weight group with standard error bars
N = 8 pools for Flocks 1 through 6
N = 20 poults for Flocks 7 and 8
Aerobic bacteria was significantly different over age for each flock ($P<0.05$)
See Table 3.14 for number of samples with no growth

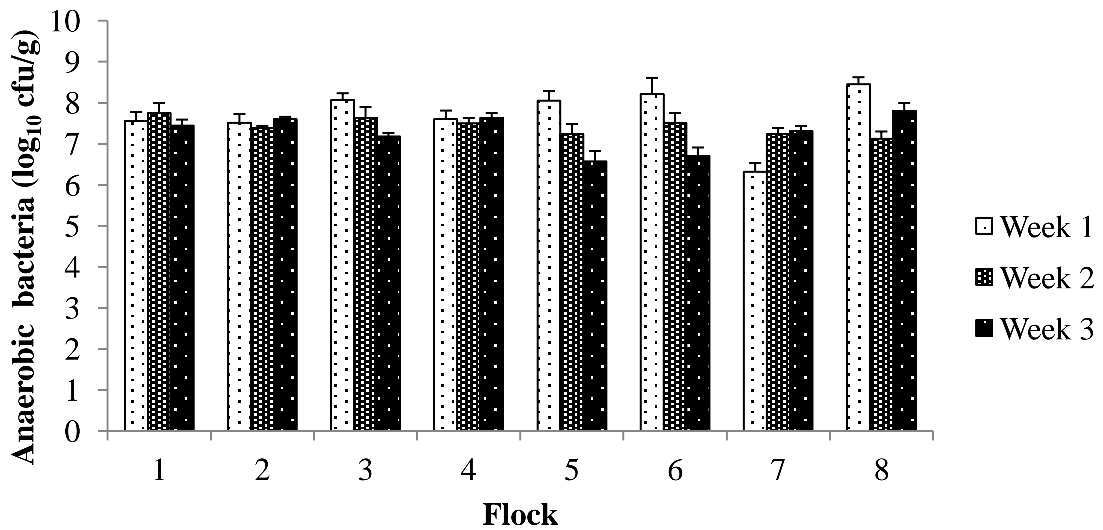


Figure 3.11 Anaerobic bacteria by flock and age
Results are means averaged over weight group with standard error bars
N = 8 pools for Flocks 1 through 6
N = 20 poults for Flocks 7 and 8
Aerobic bacteria was significantly different over age for Flocks 3, 5, 6, 7 and 8 ($P<0.05$)
See Table 3.15 for number of samples with no growth

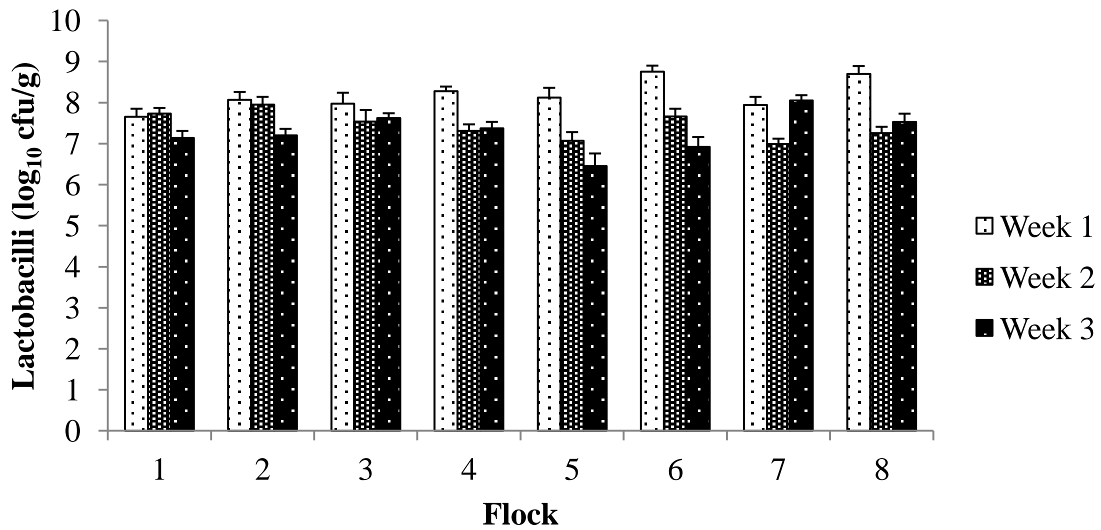


Figure 3.12 Lactobacilli by flock and age
Results are means averaged over weight group with standard error bars
N = 8 pools for Flocks 1 through 6, N = 20 poults for Flocks 7 and 8
Lactobacilli was significantly different over age for Flocks 2, 4, 5, 6, 7 and 8 (P<0.05)
Lactobacilli trended differently over age for Flock 1 (P<0.0618)
See Table 3.16 for number of samples with no growth

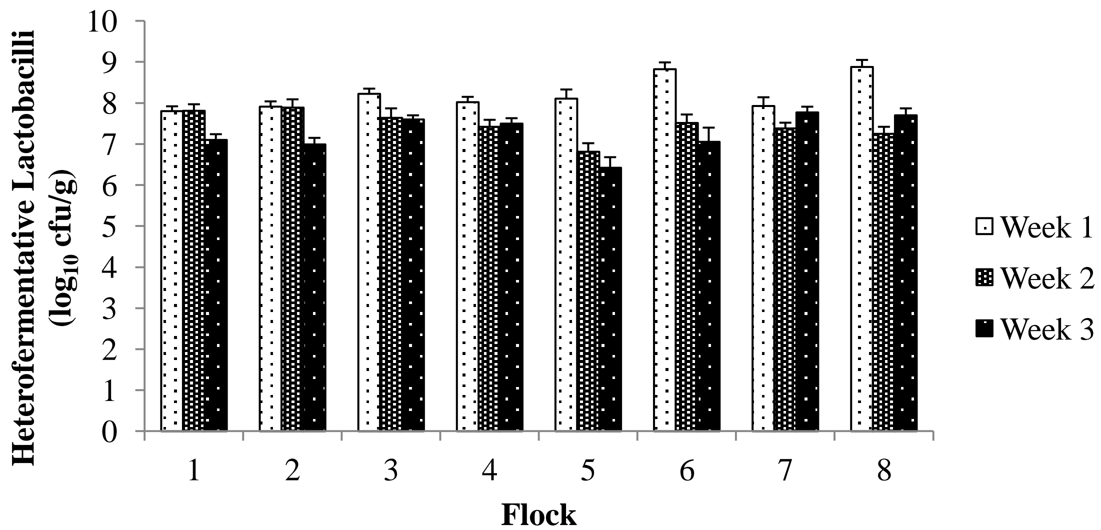


Figure 3.13 Heterofermentative lactobacilli by flock and age
Results are means averaged over weight group with standard error bars
N = 8 pools for Flocks 1 through 6, N = 20 poults for Flocks 7 and 8
Heterofermentative lactobacilli was significantly different over age for Flocks 1, 2, 3, 4, 5, 6 and 8 (P<0.05)
Heterofermentative lactobacilli trended differently over age for Flock 7 (P<0.0759)
See Table 3.17 for number of samples with no growth

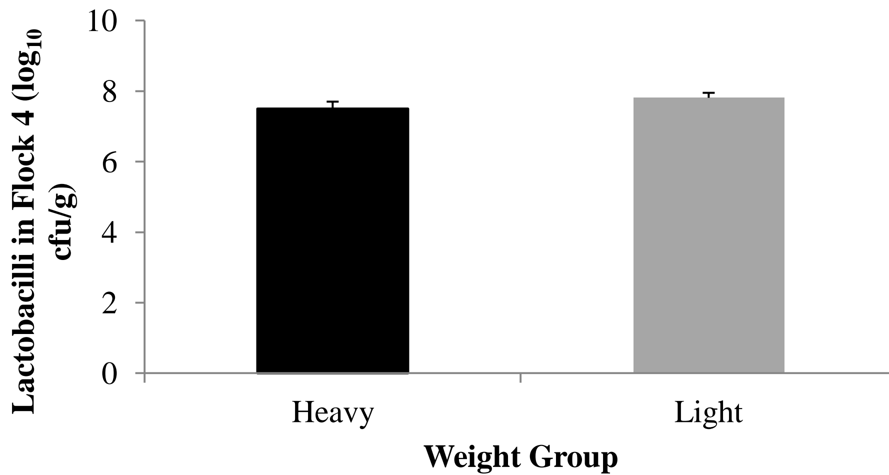


Figure 3.14 Lactobacilli in Flock 4 by weight group
Results are means averaged over age with standard error bars, N = 12 pools
Lactobacilli trended differently between weight groups for Flock 4 (P<0.0664)

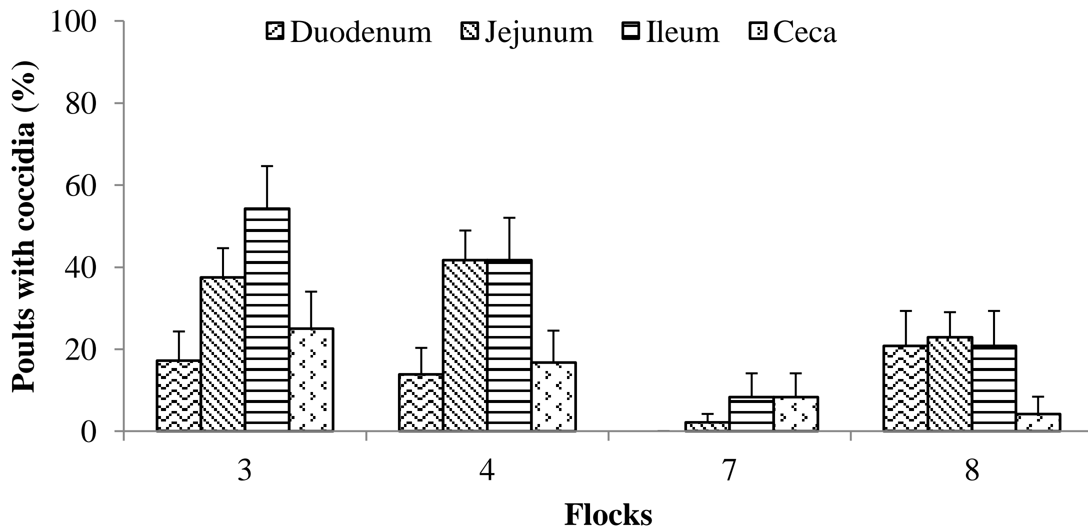


Figure 3.15 Coccidia present in gut tissue samples by flock
Results are means averaged over age and weight group with standard error bars
N = 24 for duodenum and ileum and ceca and N = 48 for jejunum
No significant differences were seen among flocks for the duodenum (Flocks 1, 2, 5, 6 and 7 were removed from the statistical data)
Significant differences were seen among flocks for the jejunum (P<0.0001) (Flocks 1, 2, 5 and 6 and week one were removed from the statistical data)
No significant differences were seen among flocks for the ileum (Flocks 1, 2, 5 and 6 were removed from the statistical data)
Trending differences were seen among flocks for the ceca (Flocks 1, 2, 5 and 6 and week two were removed from the statistical data)

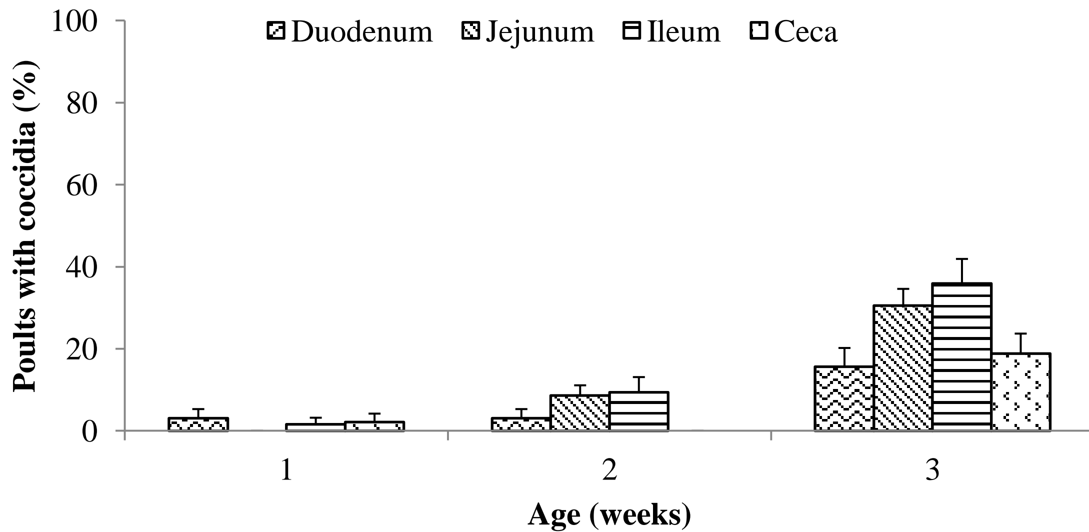


Figure 3.16 Coccidia present in gut tissue samples over age
Results are means averaged over flock and weight group with standard error bars
N = 64 for duodenum and ileum, N = 48 (at 1 and 2 weeks) and 64 (at 3 weeks) for ceca and N = 128 for Jejunum
Significant differences were seen over age for all tissues (*P*<0.05)
Duodenum (Flocks 1, 2, 5, 6 and 7 were removed from the statistical data)
Jejunum (Flocks 1, 2, 5 and 6 and week one were removed from the statistical data)
Ileum (Flocks 1, 2, 5 and 6 were removed from the statistical data)
Ceca (Flocks 1, 2, 5 and 6 and week two were removed from the statistical data)

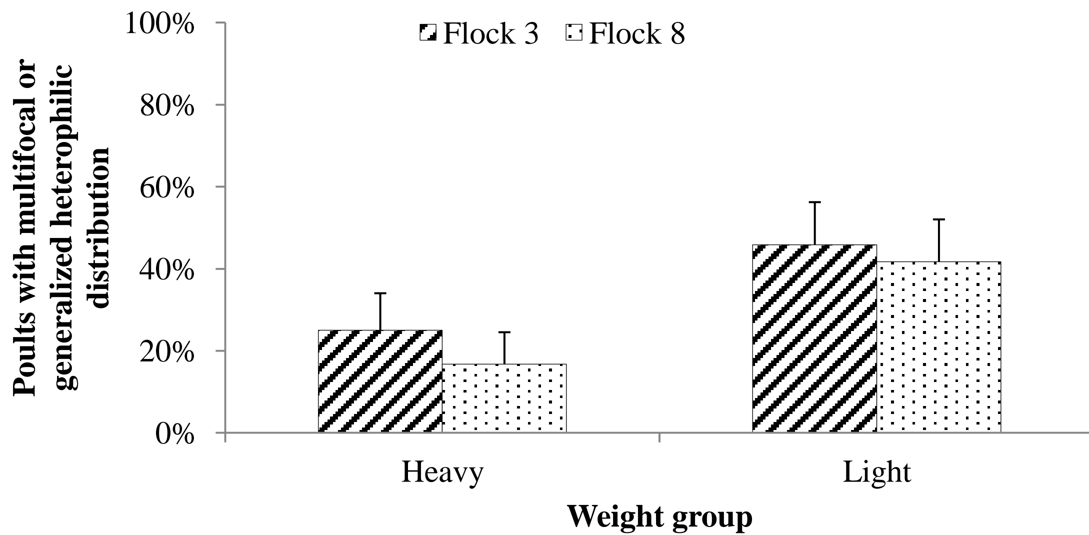


Figure 3.17 Distribution of heterophilic infiltrates in the jejunum in Flocks 3 and 8 by weight group
Results are means averaged over age with standard error bars, N = 24
Infiltrates were different between weight groups in Flocks 3 and 8 (*P*<0.1098, *P*<0.0474)

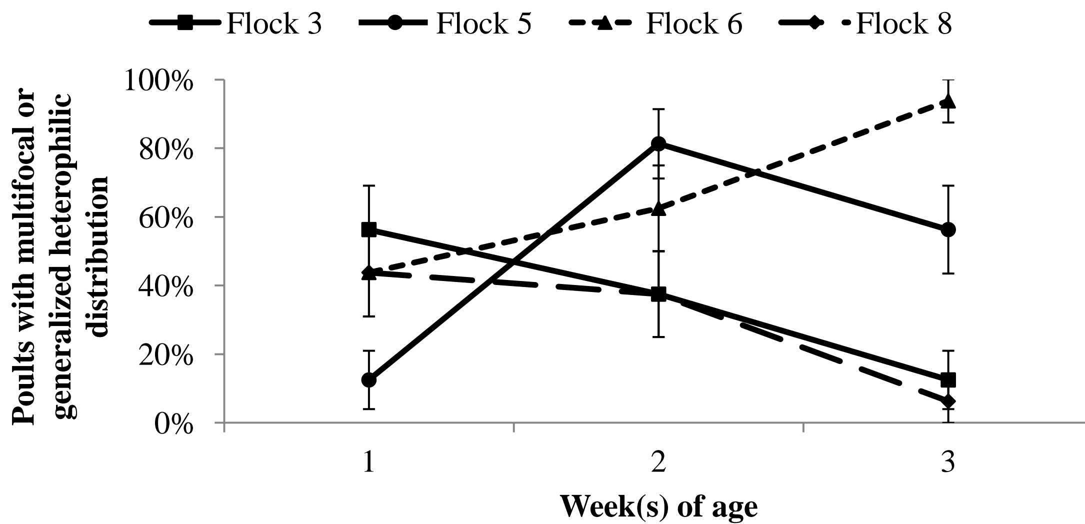


Figure 3.18 Distribution of heterophilic infiltrates in the jejunum in Flocks 3, 5, 6 and 8 over age
Results are means averaged over flock and weight group with standard error bars, N = 16
Infiltrates were different over age in Flocks 3, 5, 6 and 8 ($P<0.0470$, $P<0.0024$, $P<0.0349$, $P<0.0789$)

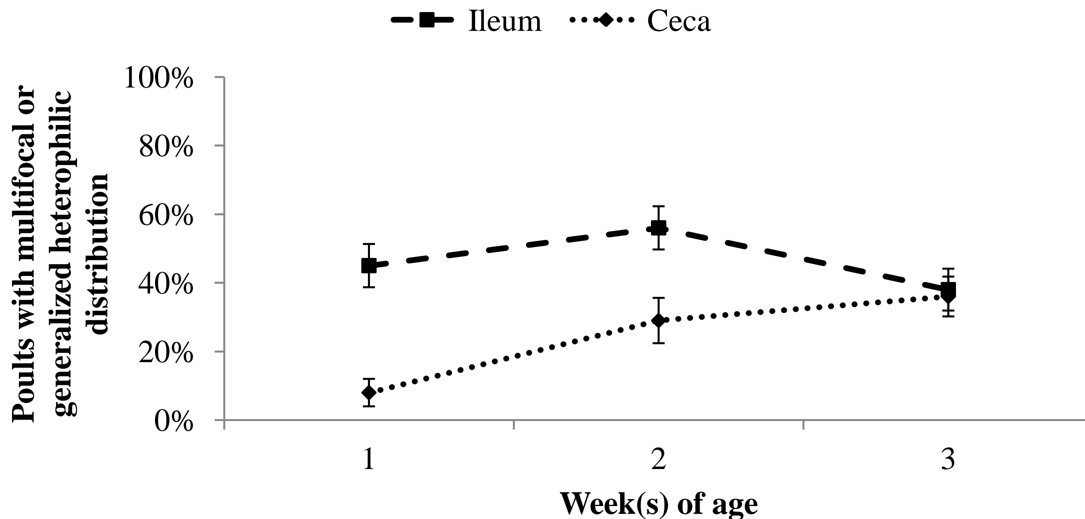


Figure 3.19 Distribution of heterophilic infiltrates in the ileum and ceca over age
Results are means averaged over flock and weight group with standard error bars
N = 64 for ileum
N = 48 (at 1 and 2 weeks) and 64 (at 3 weeks) for ceca
Infiltrates were different over age in the ileum ($P<0.0679$)
Infiltrates were different over age in the ceca ($P<0.0054$) (Flock 1 was removed from the statistical data and no samples were collected in Flocks 1 and 2 at one and two weeks of age)

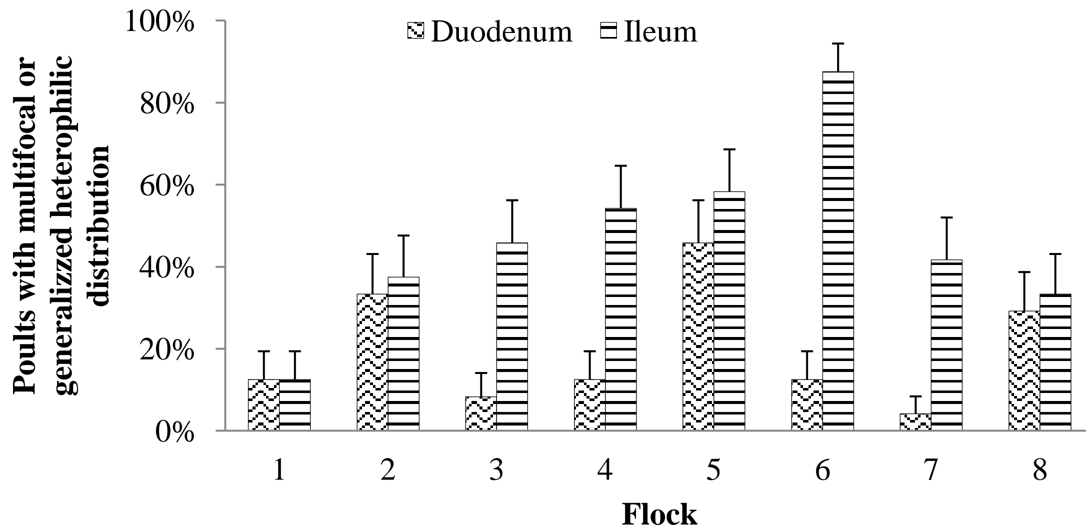


Figure 3.20 Distribution of heterophilic infiltrates in the duodenum and ileum by flock
Results are means averaged over age and weight group with standard error bars, N = 48
Infiltrates were different among flocks in the duodenum and ileum ($P<0.0081$, $P<0.0007$)

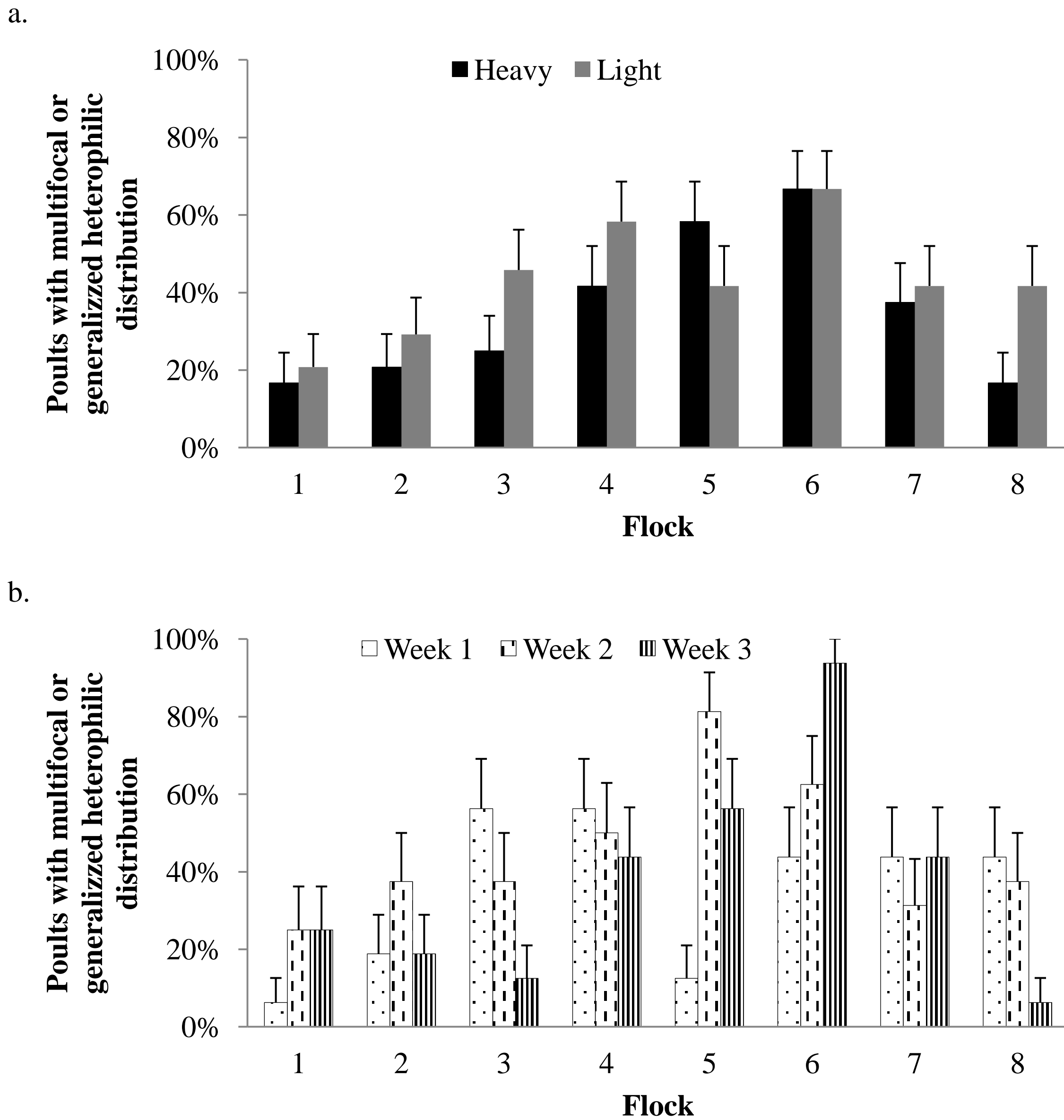


Figure 3.21 Distribution of heterophilic infiltrates in the jejunum by flocks
a. Results are means averaged over age with standard error bars, N = 24
Infiltrates were different between weight groups in Flocks 3 and 8 (P<0.1098, P<0.0474)
b. Results are means averaged over weight group with standard error bars, N = 16
Infiltrates were different over age in Flocks 3, 5, 6 and 8 (P<0.0470, P<0.0024, P<0.0349, P<0.0789)

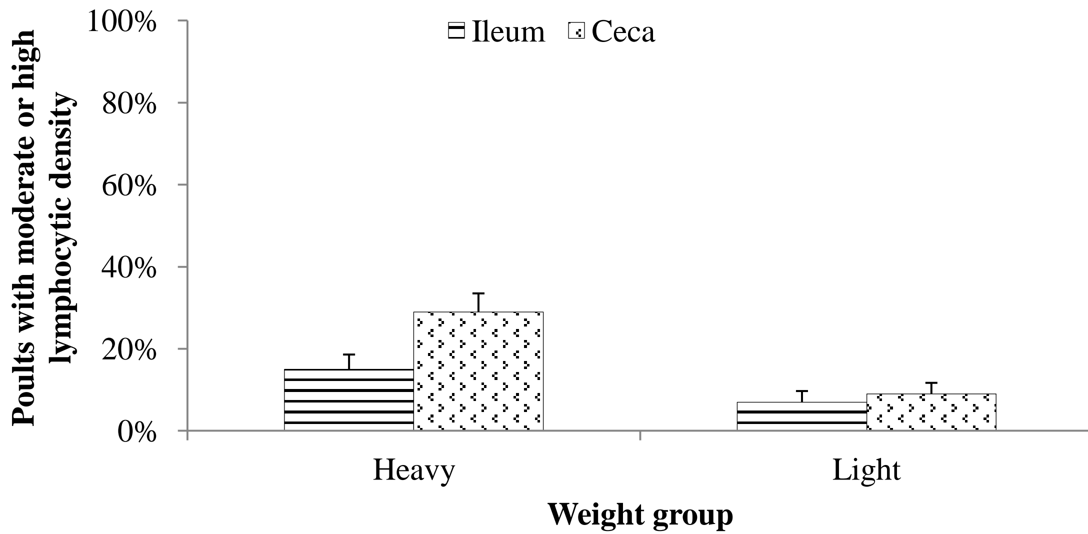


Figure 3.22 Density of lymphocytic infiltrates in the ileum and ceca by weight group
Results are means averaged over flock and age with standard error bars
N = 80 in ceca, N = 96 in ileum
Infiltrates were different between weight groups in the ileum (P<0.1044)
Infiltrates were different between weight groups in the ceca (P<0.0048) (Flocks 4 and 5 were removed from the statistical data set and no samples were collected from Flocks 1 and 2 at one and two weeks of age)

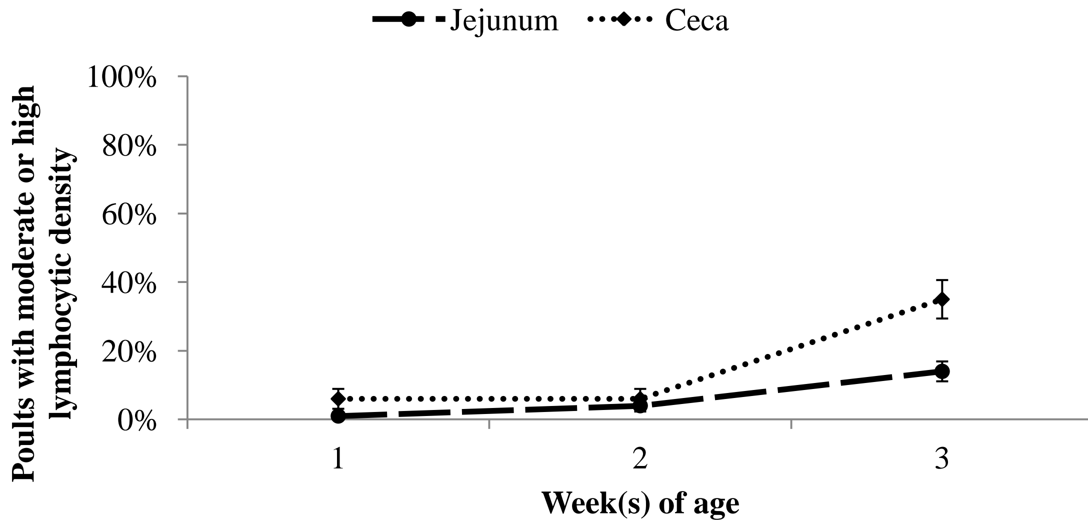


Figure 3.23 Density of lymphocytic infiltrates in the jejunum and ceca over age
Results are means averaged over flock and weight group with standard error bars
N = 128 in jejunum, N = 32 (at 1 and 2 weeks) and 48 (at 3 weeks) for ceca
Infiltrates were different over age in the jejunum (P<0.0017)
Infiltrates were different over age in the ceca (P<0.0013) (Flocks 4 and 5 were removed from the statistical data set and no samples were collected from Flocks 1 and 2 at one and two weeks of age)

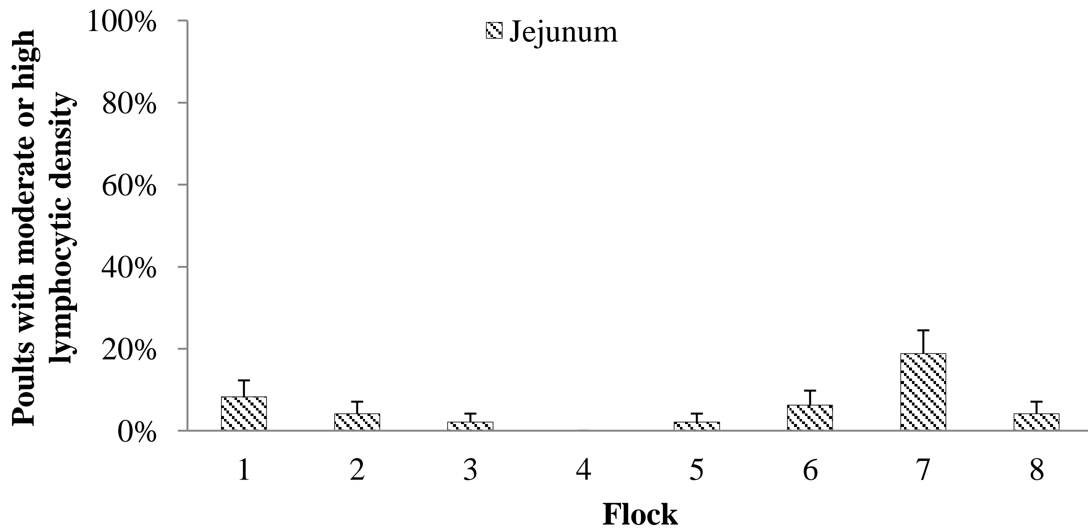


Figure 3.24 Density of lymphocytic infiltrates in the jejunum by flock
Results are means averaged over age and weight group with standard error bars, N = 48
Infiltrates were different over age in the jejunum (*P*<0.0356)

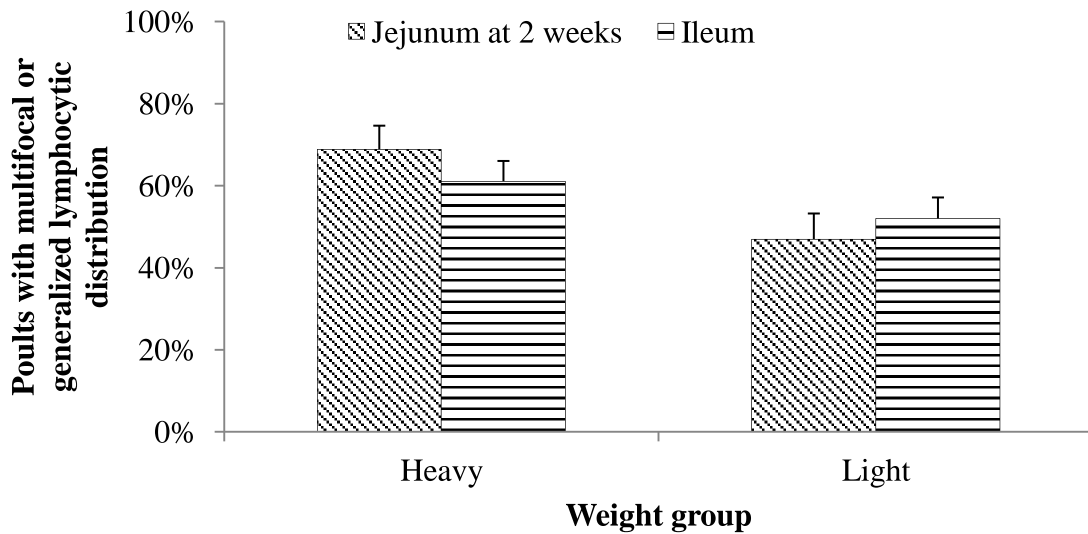


Figure 3.25 Distribution of lymphocytic infiltrates in the jejunum at 2 weeks and ileum by weight group
Results are means with standard error bars averaged over flock for jejunum, N = 64
Infiltrates were different between weight groups at two weeks of age in the jejunum (*P*<0.0131)
Results are means with standard error bars averaged over flock and age for ileum, N = 96
Infiltrates were different weight groups in the ileum (*P*<0.0914)

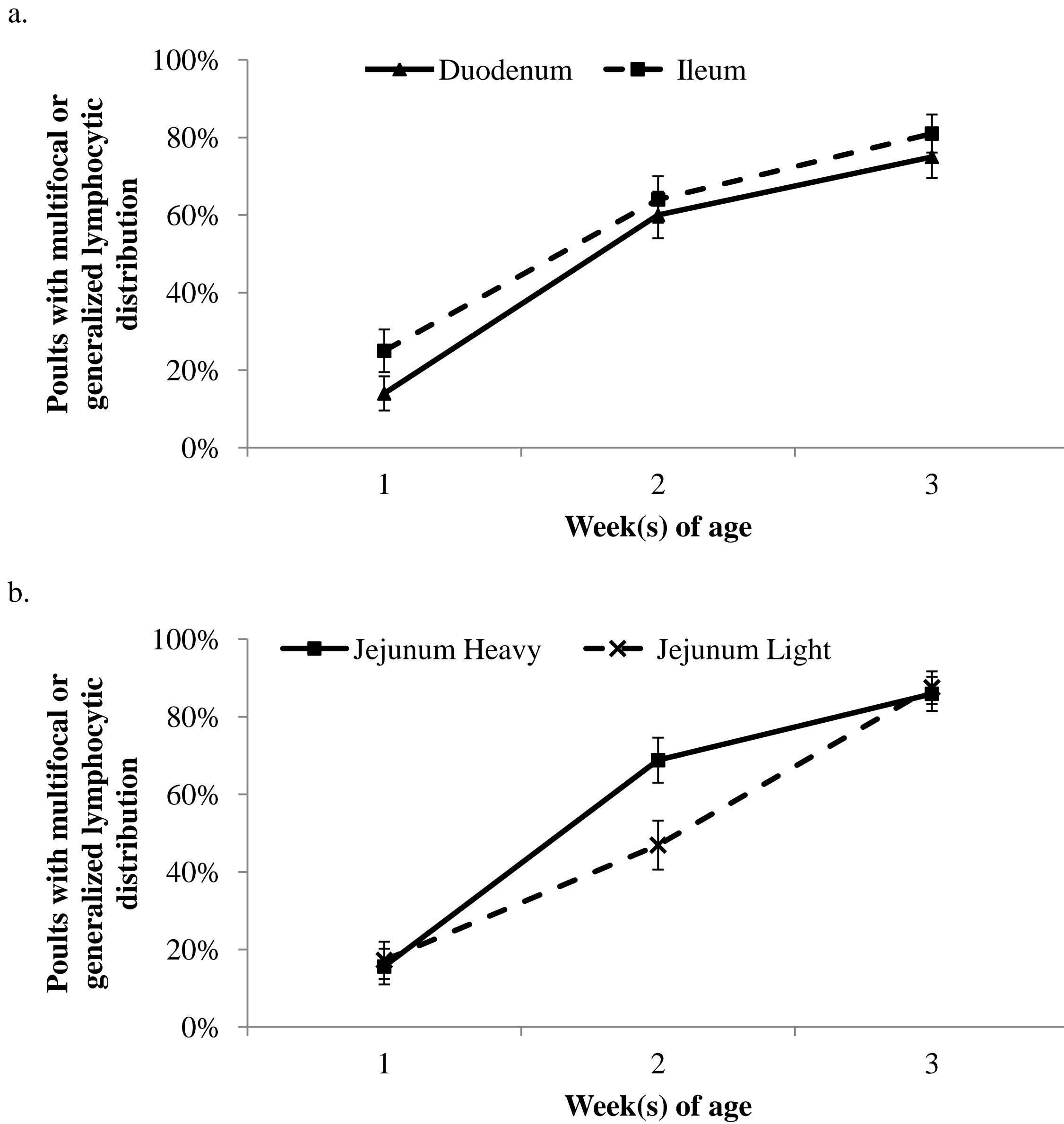


Figure 3.26 Distribution of lymphocytic infiltrates over age
a. Results are means with standard error bars averaged over flock and weight group, N = 64
Infiltrates were different over age for the duodenum and ileum ($P<0.0001$)
b. Results are means with standard error bars averaged over flock for the jejunum by weight group, N = 64
Infiltrates were different over age in the jejunum for heavy and light weight poults ($P<0.0001$)

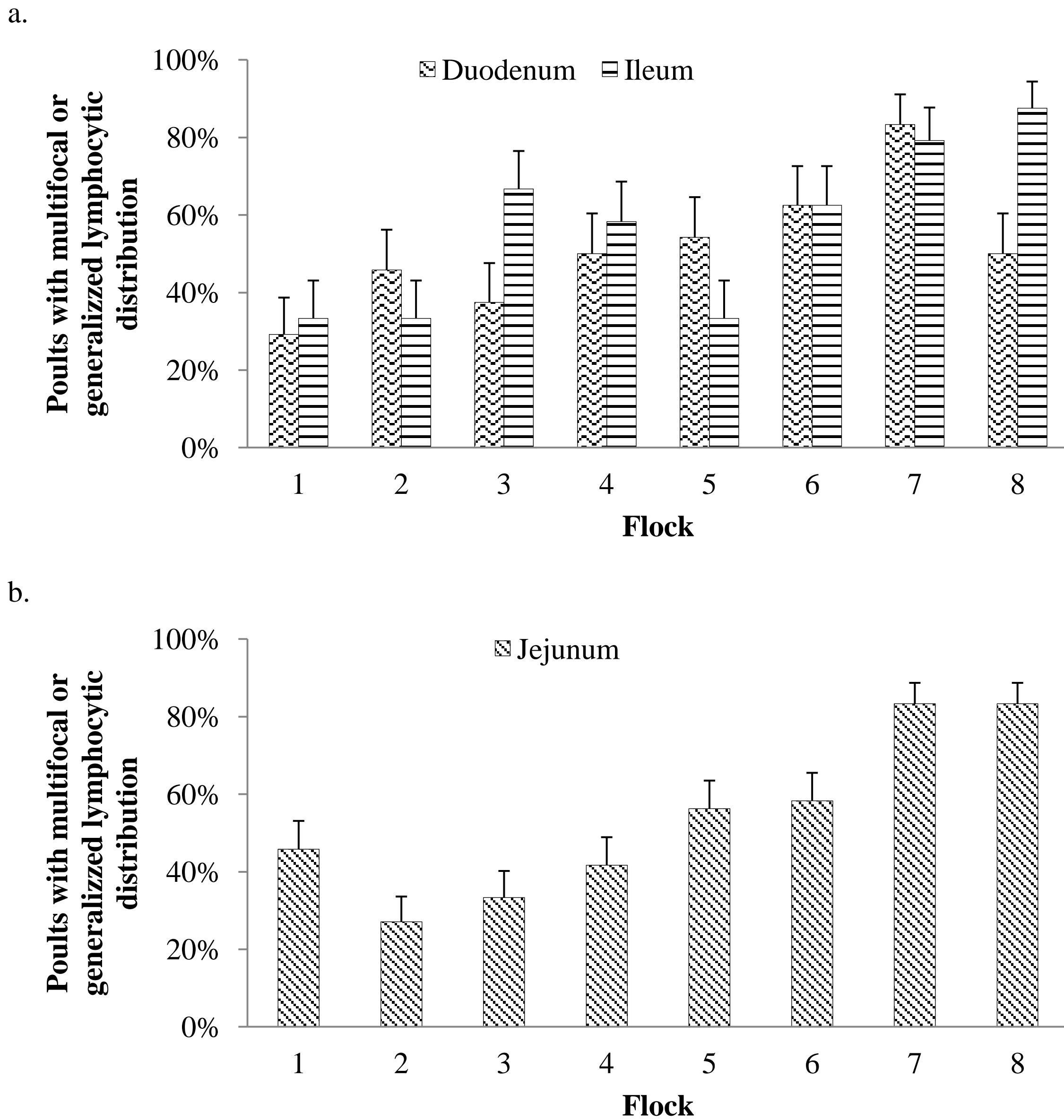


Figure 3.27 Distribution of lymphocytic infiltrates over flock

a. Results are means with standard error bars averaged over age and weight group, N = 24

Infiltrates were different among flocks for the duodenum and ileum (*P*<0.0031, *P*<0.0001)

b. Results are means with standard error bars averaged over age and weight group for the jejunum, N = 48

Infiltrates were different among flocks in the jejunum (*P*<0.0001)

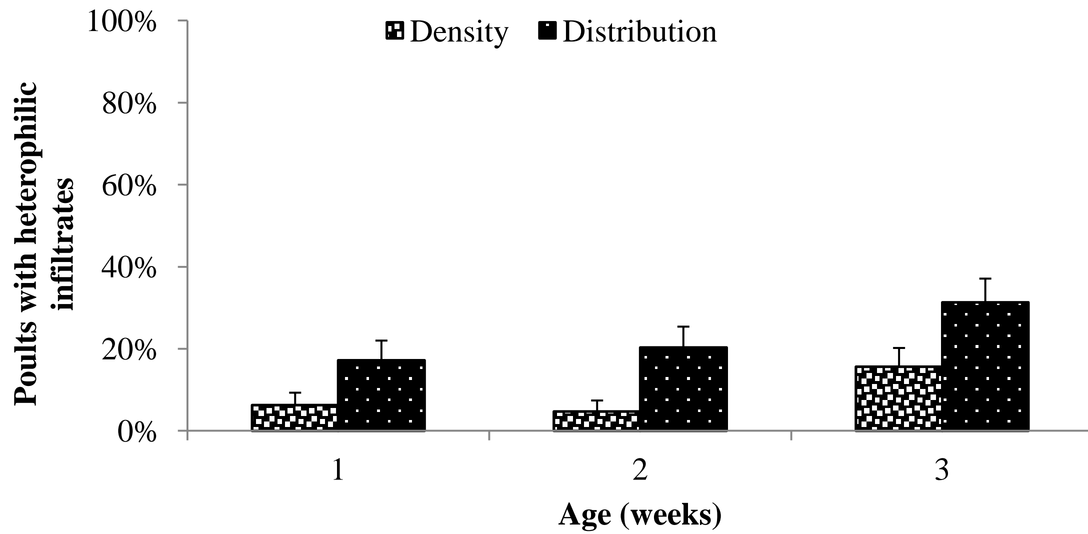


Figure 3.28 Density and distribution of heterophilic infiltrates in the bursa over age
Results are means with standard error bars averaged over flock and weight group, N = 64
Infiltrates were different over age for density and distribution (*P*<0.0577, *P*<0.1035) Flock 7 was removed from the statistical data set for density of heterophilic infiltritrates in the bursa

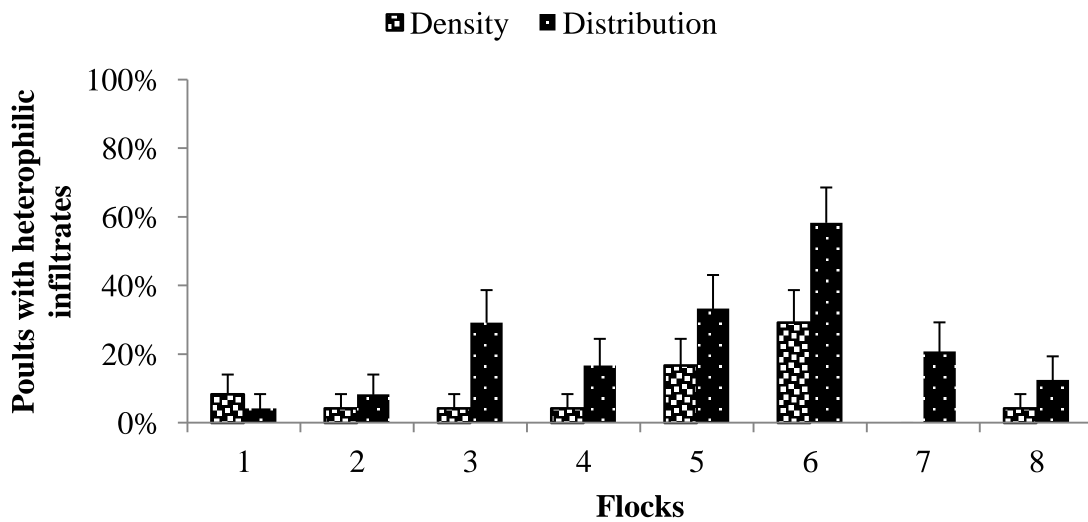


Figure 3.29 Density and distribution of heterophilic infiltrates in the bursa by flock
Results are means with standard error bars averaged over age and weight group, N = 24
Infiltrates were different among flocks for density and distribution (*P*<0.0593, *P*<0.0015) Flock 7 was removed from the statistical data set for density of heterophilic infiltritrates in the bursa

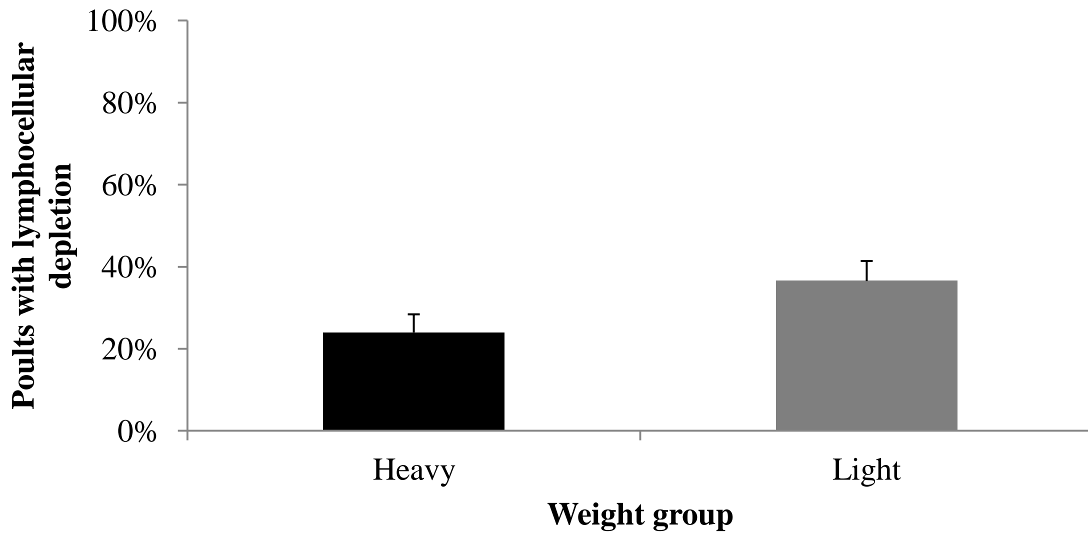


Figure 3.30 Distribution of lymphocellular depletion in the bursa by weight group
Results are means with standard error bars averaged over age and flock, N = 96
Infiltrates were different between weight groups for distribution (P<0.0304) (Flock 8 was removed from the statistical data set)

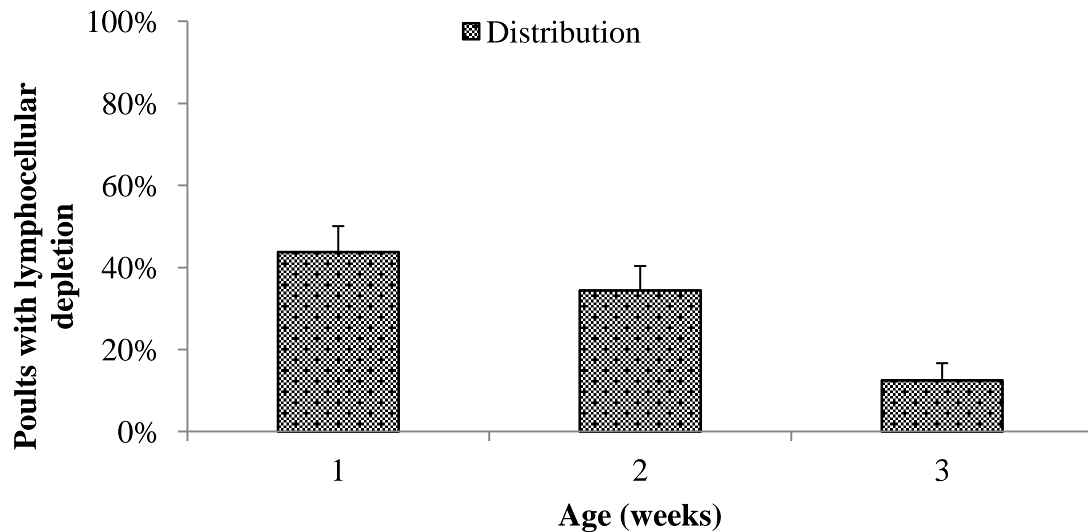


Figure 3.31 Distribution of lymphocellular depletion in the bursa over age
Results are means with standard error bars averaged over weight group and flock, N = 64
Infiltrates were different over age for distribution (P<0.0002) (Flock 8 was removed from the statistical data set)

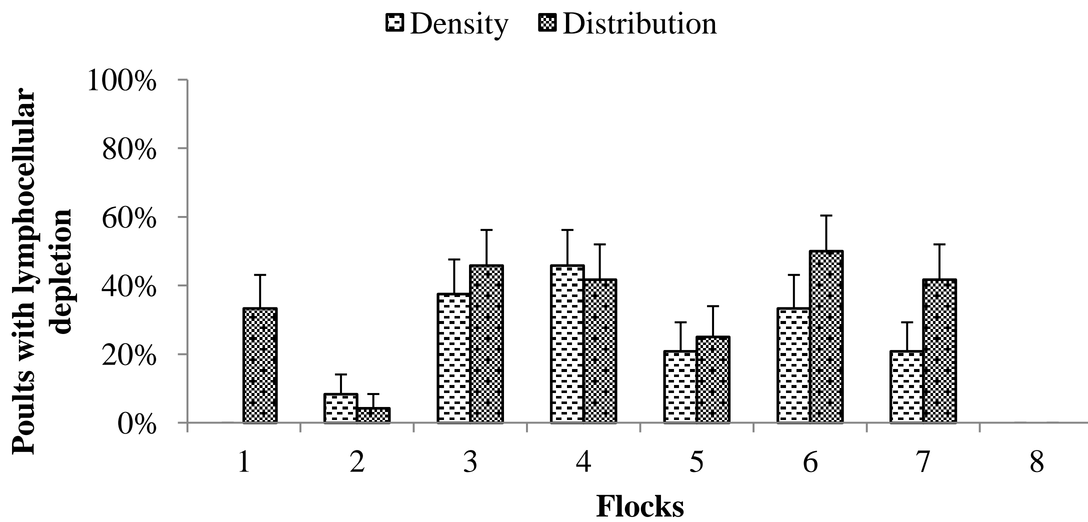


Figure 3.32 Density and distribution of lymphocellular depletion in the bursa by flock
Results are means with standard error bars averaged over weight group and age, N = 24
Infiltrates were different over age for density (P<0.0717) (Flocks 1 and 8 were removed from the statistical data set)
Infiltrates were different over age for distribution (P<0.0526) (Flock 8 was removed from the statistical data set)

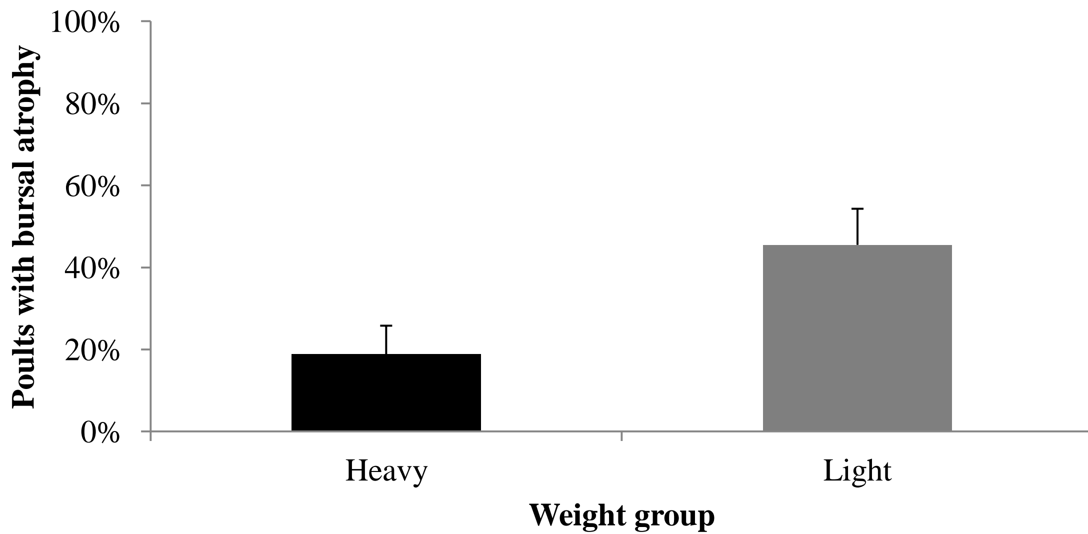


Figure 3.33 Atrophy of the bursa at three weeks of age by weight group
Results are means with standard error bars averaged over flock at three weeks of age, N = 32
Atrophy at three weeks was different between weight groups (P<0.0124)

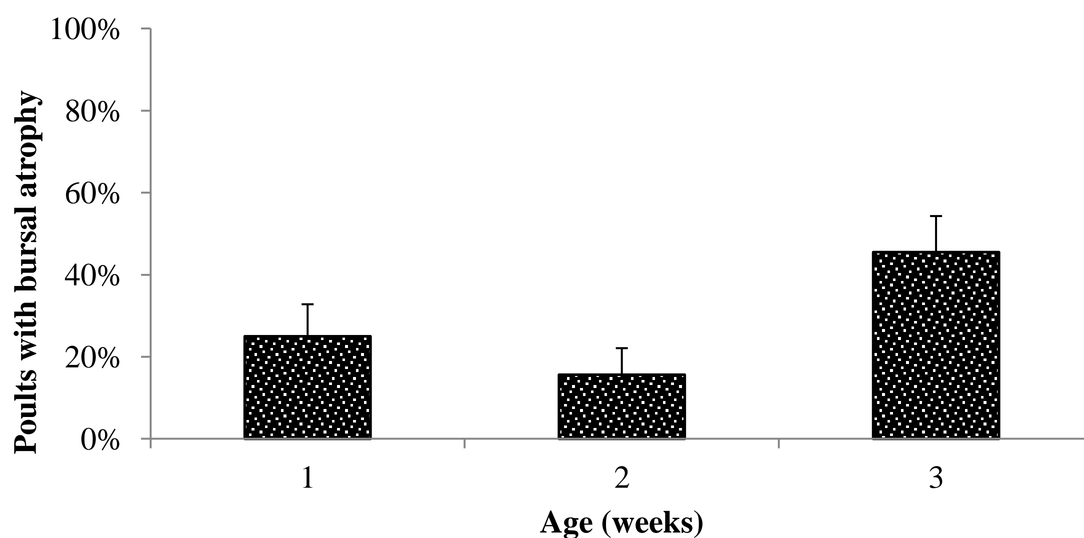


Figure 3.34 Atrophy of the bursa for light weight poults over age
Results are means with standard error bars averaged over flock and weight group, N = 32
Atrophy was different over age for light weight poults (*P*<0.0308)

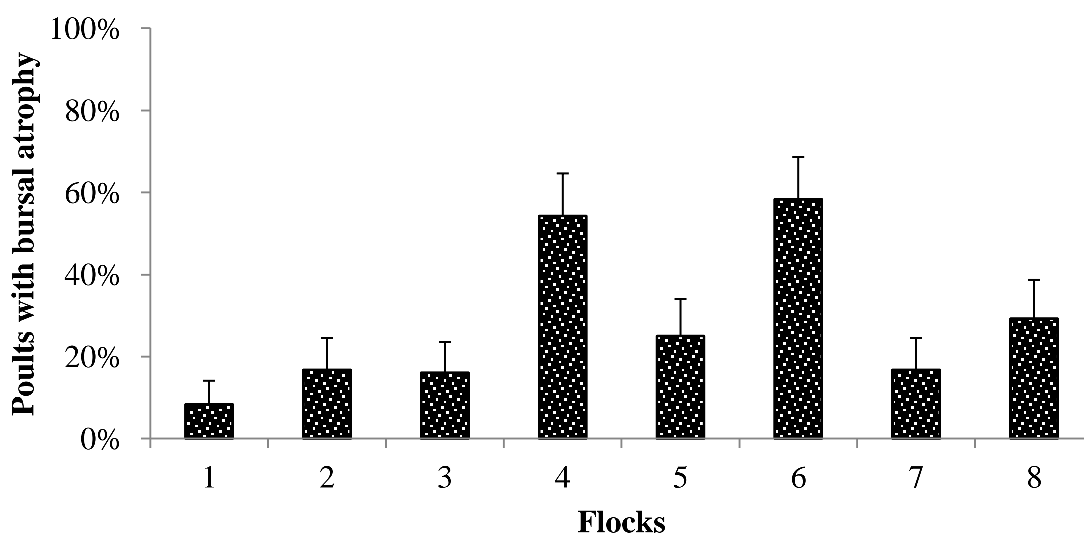


Figure 3.35 Atrophy of the bursa by flock
Results are means with standard error bars averaged over age and weight group, N = 24
Atrophy was different among flocks (*P*<0.0005)

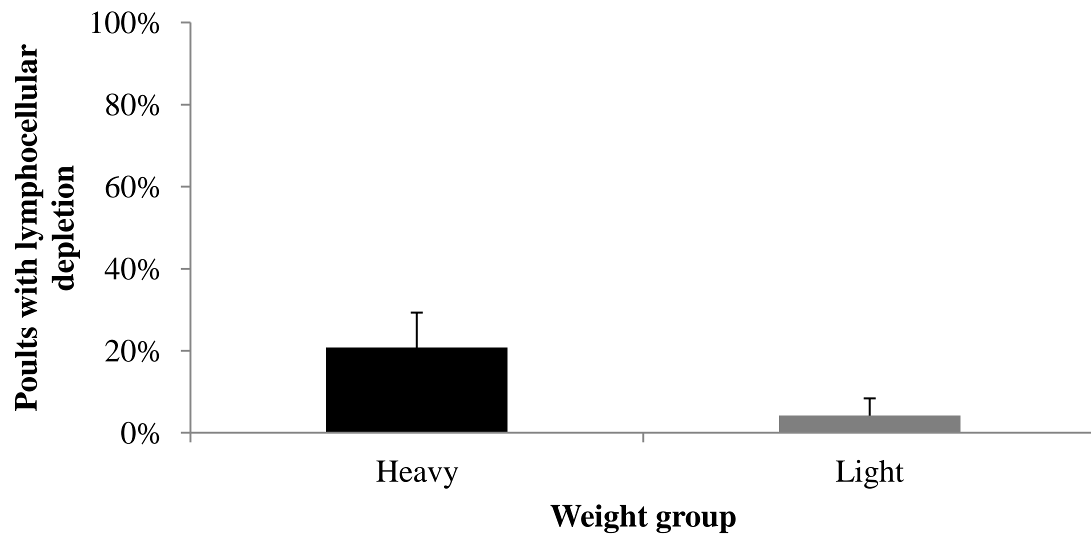
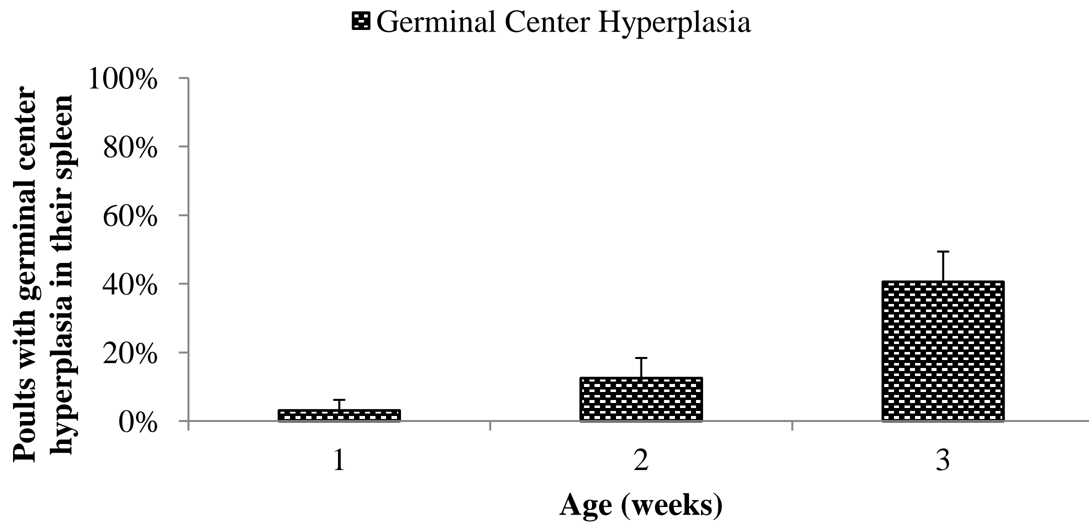


Figure 3.36 Lymphocellular depletion in the spleen for Flocks 4 and 7
Results are means with standard error bars averaged over age and flock, N = 24
Lymphocellular depletion was different between weight groups for Flocks 4 and 7 (P<0.0951) (No lymphocellular depletion was found in the remaining flocks)

a.



b.

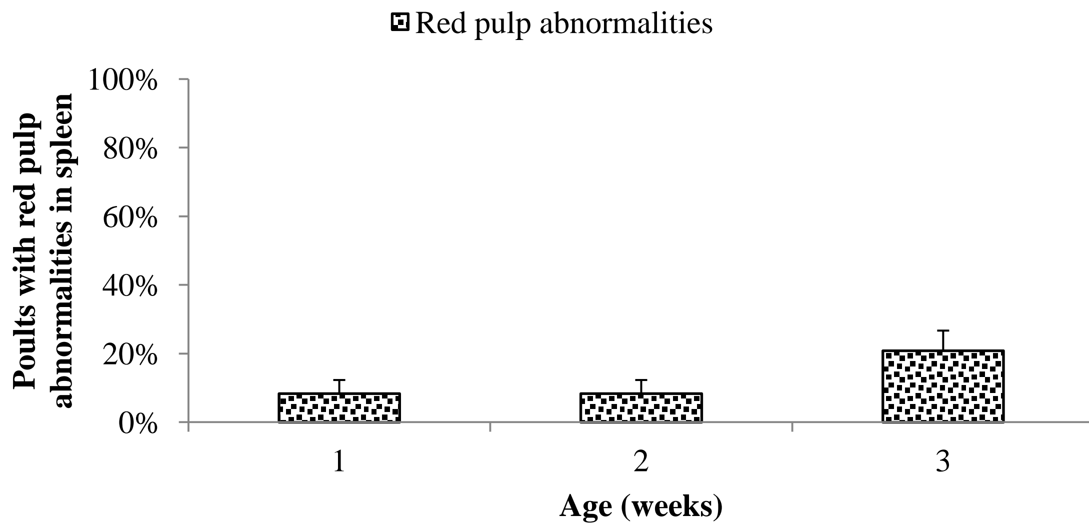


Figure 3.37 Germinal center hyperplasia and red pulp abnormalities in the spleen over age

Results are means with standard error bars averaged over weight group and flock, N = 64

a. Germinal center hyperplasia was different over age in the spleen ($P<0.0021$) (Flocks 3, 4, 7 and 8 were removed from the statistical data set)

b. Red pulp abnormalities were different over age in the spleen ($P<0.0857$) (Flocks 1 and 2 were removed from the statistical data set)

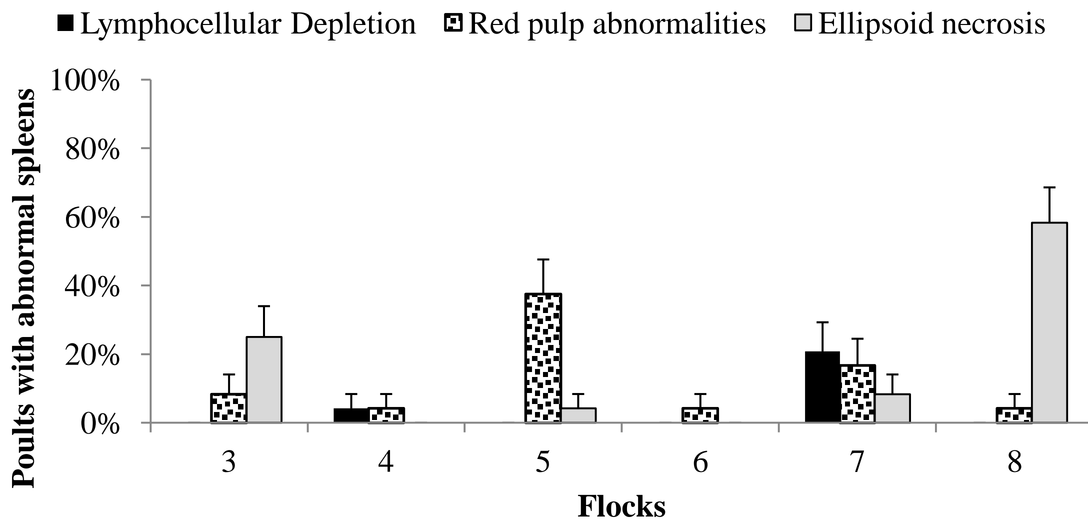


Figure 3.38 Lymphocellular depletion, red pulp abnormalities and ellipsoid necrosis in the spleen by flock
Results are means with standard error bars averaged over weight group and age, N = 24
Lymphocellular depletion was different between Flocks 4 and 7 (P<0.0951) (No lymphocellular depletion was found in the remaining flocks
Red pulp abnormalities were different among flocks (P<0.0106) (Flocks 1 and 2 were removed from the statistical data)
Ellipsoid necrosis was different among flocks (P<0.0004) (Flocks 1, 2, 4 and 6 were removed from the statistical data)

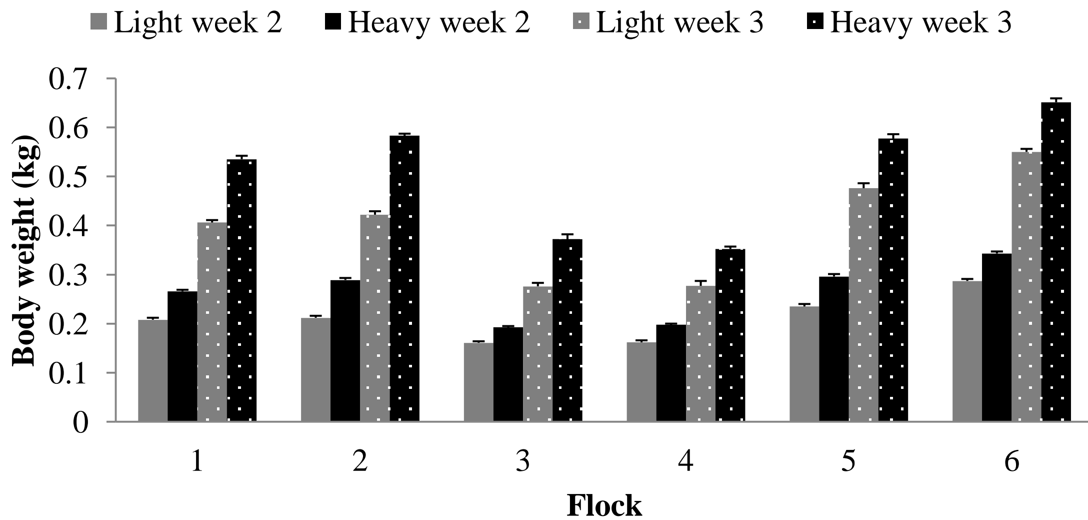


Figure 3.39 Body weights of poults used for D-xylose absorption
Results are means with standard error bars, N = 10

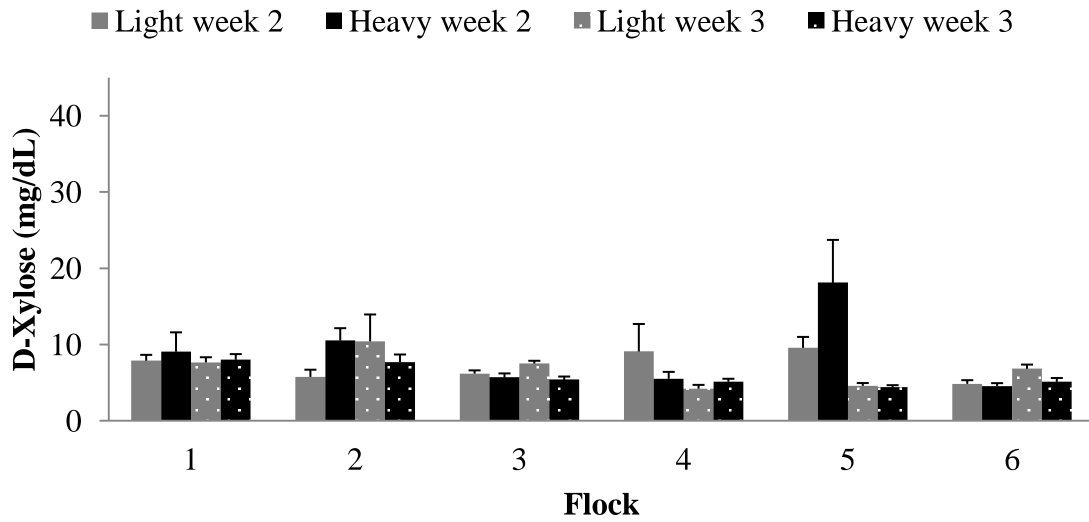


Figure 3.40 Xylose in plasma from poults prior to gavaging
Results are means with standard error bars, N = 6 to 10

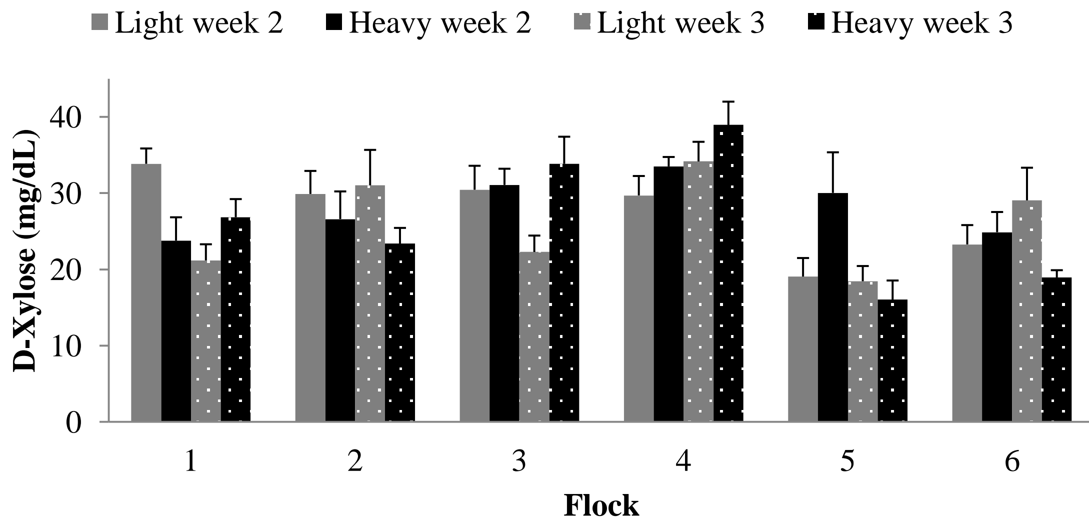


Figure 3.41 Xylose in plasma from poults 60 minutes after gavaging by flock
Results are means with standard error bars, N = 4 to 10
A significant interaction was found between weight group, age and flock (*P*<0.0001)

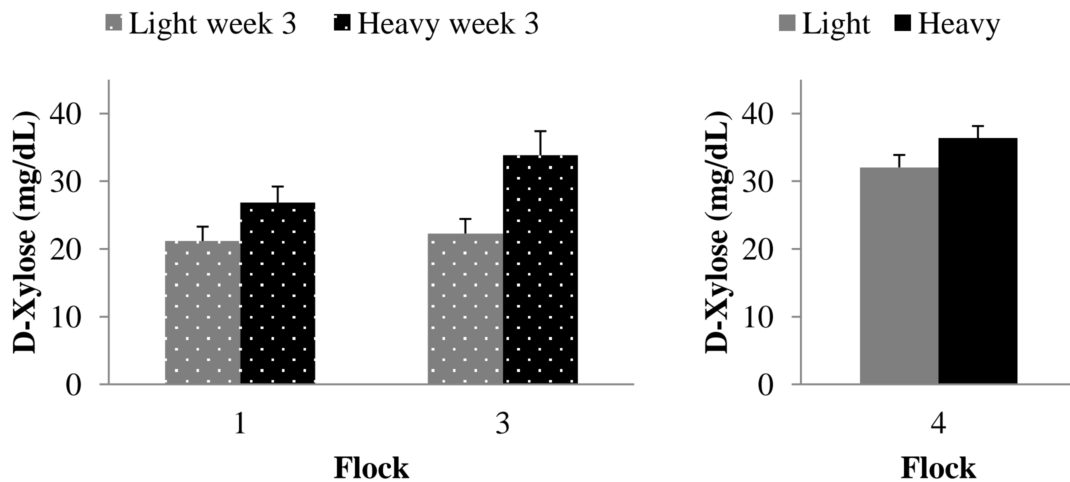


Figure 3.42 Xylose in plasma from poults 60 minutes after gavaging in flocks 1, 3 and 4 by weight group
Results are means with standard error bars
Heavy weight poults had significantly higher concentrations of D-xylose in Flocks 1 and 3 at three weeks of age (*P*<0.1031, *P*<0.0150), N = 8 to 10
Heavy weight poults had significantly higher concentrations of D-xylose in Flock 4 averaged over age (*P*<0.0527), N = 19

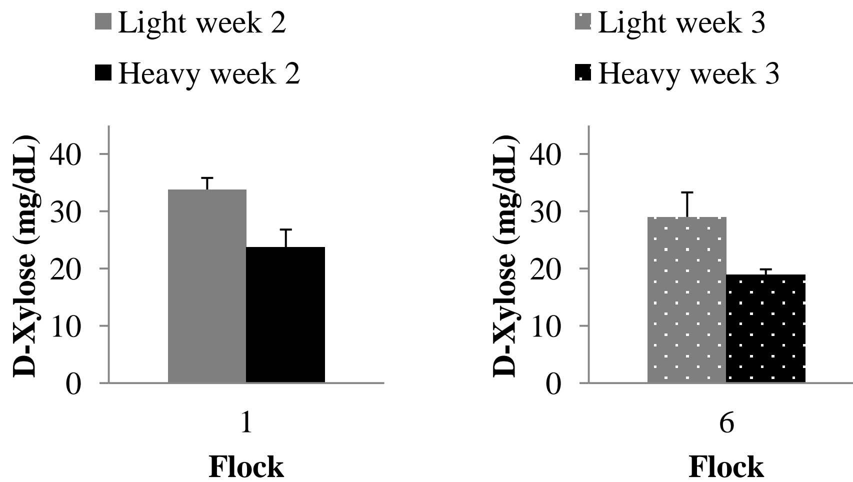


Figure 3.43 Xylose in plasma from poults 60 minutes after gavaging in flocks 1and 6 by weight group
Results are means with standard error bars
Light weight poults had significantly higher concentrations of D-xylose in Flock 1 at two weeks of age (*P*<0.0198), N = 7 and 8
Light weight poults had significantly higher concentrations of D-xylose in Flock 6 at three weeks of age (*P*<0.0552), N = 10 and 8

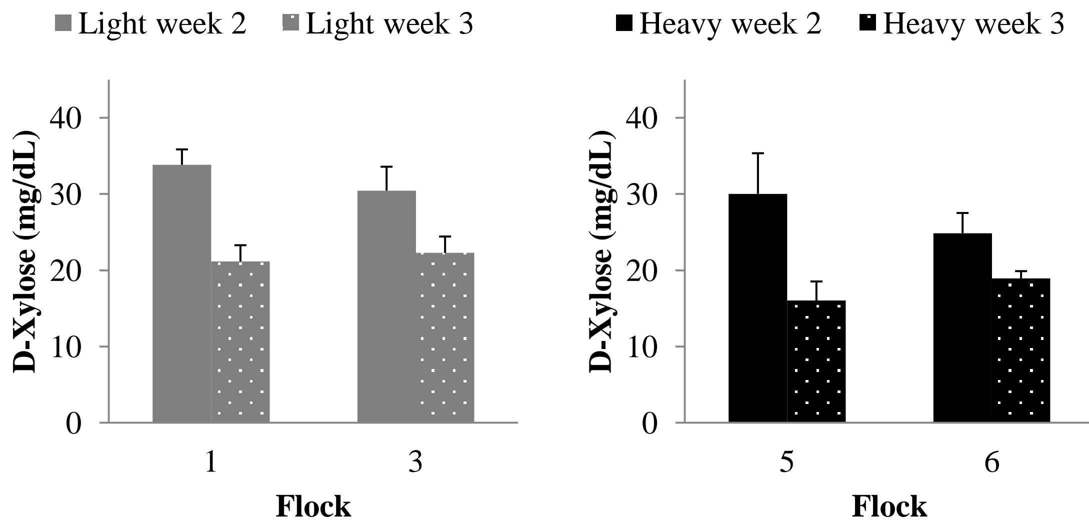


Figure 3.44 Xylose in plasma from poults 60 minutes after gavaging in Flocks 1, 3, 5 and 6 by age
Results are means with standard error bars
Two week old light weight poults had higher concentrations of D-xylose than three week old light weight poults in Flocks 1 and 3 (P<0.0009, P<0.0515), N = 7 to 10
Two week old heavy weight poults had higher concentrations of D-xylose than three week old heavy weight poults in Flocks 5 and 6 (P<0.0249, P<0.0737), N = 8 to 10

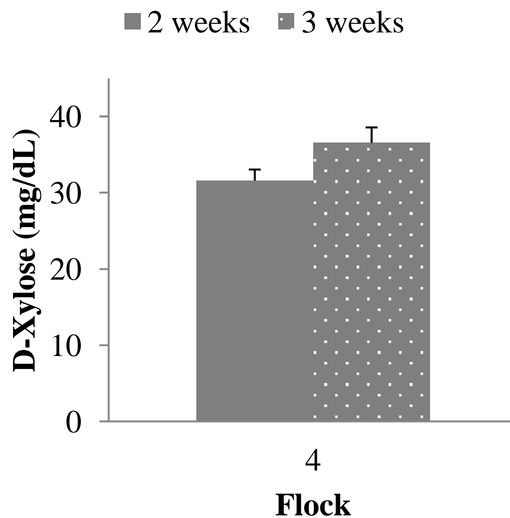


Figure 3.45 Xylose in plasma from poults 60 minutes after gavaging in Flock 4 by age
Results are means with standard error bars
Three week old poults had higher concentrations of D-xylose than two week old poults in Flock 4 (P<0.0883), N = 18 and 20

Chapter 4: Inoculation Trial

INTRODUCTION

The objective of the inoculation trial was to determine if differences in performance existed between poults inoculated with gut contents as compared to the poults the contents had been collected from in the field trial. Different inoculum groups included poults inoculated with the six different MN flocks, each including a heavy and a light weight group. These were compared with uninoculated control poults. There was not enough material available from the ND poults; consequently they were not included in this trial. The inoculums were from ileal samples collected from two and three week old poults in the previous field trial. These samples were pooled and sterile phosphate buffered saline (PBS) was added to equalize the ratio of gut contents and PBS from the amount added during the contents removal from the intestinal tissue in the stomacher. The final inoculums consisted of 0.82 grams of material per milliliter. Inoculated poults received two milliliters of inoculums and controls received two milliliters of sterile PBS. The total amount of gut contents given to each treatment poult was 1.64 grams.

MATERIALS AND METHODS

Study Design

Nicholas male poults with beaks trimmed were raised from zero to seven days of age in brooder battery cages and fed a commercial poult starter. At seven days of age 312 poults with body weights near average (130.3 g, SE 6.0) were kept for the inoculation study. Poults were divided into 52 pens with six poults in each group. Each group was housed in a separate cage of the battery brooder unit. These poults were inoculated at seven days of age with gut contents taken from the previous field trials. There were 12 different treatment groups and one control group. The treatment groups were inoculated with contents from either heavy or light weight groups from the six MN flocks. Each treatment group consisted of 24 birds separated into four pens of six poults.

Four brooder battery units with 16 individual cages each were used. The units had eight cages on either side at four different levels. The two cages on the same level shared the same watering pan so the poults in them had to be from the same inoculation group. For example the Flock 1 inoculation treatments took up the eight cages on one side of a battery brooder unit. These cages were paired into four different levels that were then randomly assigned to either the heavy or light weight groups. Battery unit one had Flock 1 light and heavy treatment groups on one side and Flock 2 on the other. Battery unit three had Flock 3 on one side and Flock 4 inoculation groups on the other. Flocks 5 and 6 inoculation groups were each on different sides of battery unit three. Four pens of controls were allocated to different pens and levels of a final fourth battery unit.

At zero and seven days of age, prior to inoculation, ten randomly selected poults were euthanized via carbon dioxide inhalation and gut contents collected as outlined in the field trial. At 14 days of age, seven days post inoculation (PI), three birds per pen were randomly selected and euthanized and gut contents and tissue samples collected as outlined in the field trial methods. At 15 days of age, eight days PI, plasma samples were collected from the remaining three poults per pen for the determination of D-xylose absorption following the same procedure as outlined in Chapter 3. Body weights of all poults were recorded at zero, six, seven and 13 days of age. Feed intake was tracked from seven to 13 days of age for each pen (group of six poults). All live animal procedures were approved by the University of Minnesota Institutional Animal Care Committee (IACUC).

Sample Collection

Samples were collected as outlined in Chapter 3 for the field trial in the methods and materials section unless described otherwise in this chapter. The same procedure for the collection of gut contents and tissue samples was used as for the MN flocks from Chapter 3 except for the number of poults sampled. Ten poults were sampled at zero days of age and placed in two separate pools. Ten poults were sampled at seven days of age and placed in two separate pools prior to inoculation. At 14 days of age three poults per pen were sampled and pooled with 156 total poults being sampled. There were four pools

each which corresponded to a pen of three poults per treatment or control group. Plasma collection was done at 15 days of age (eight days post inoculation) by the second method listed in Chapter 3. The second method of blood collection used a 23 gauge needle and 3cc syringe to draw blood out of the vein and deposit it into the heparinized Eppendorf tubes.

Laboratory Procedures

For analysis of bacteria the pools collected at 14 days of age and seven days of age were used. The bacteria present in the two pools from seven day old poults were determined individually. For samples collected at 14 days of age the four pens (three poults each) for each treatment or control group were pooled together and bacterial analysis was completed at NDSU as outlined in Chapter 3. Virus analysis of samples collected at zero, seven and 14 days of age was completed for each pen as described in Chapter 3. Tissue samples were collected at 14 days of age and were prepared and scored as outlined in Chapter 3 with four samples per treatment group for all tissues except the jejunum which had eight samples per treatment group. Presence of coccidia was determined during the histopathology scoring and not by fecal flotation in this trial.

Statistical Analysis

SAS version 9.2 was used to run all statistical analysis. Procedures Mixed was used for total plate counts of bacteria. Glimmix was used for the presence of virus and bacteria and the absorption of D-xylose The Logistic procedure was used to determine differences in tissue scores. The same models were used for this inoculation trial as for the field trial in Chapter 3 except as mentioned here. The statistical data compared the samples from the fourteen day old poults to each other. This included both the inoculated poults and uninoculated controls. Similar to the field trial, if an entire group (flock) did not have any scores it was removed from the data set to make the statistical model work. The variables in the statistical model were inoculum flock groups and weight groups.

Proc Logistic was used to analyze tissue scores. Unlike the statistical process for the field trial scores in Chapter 3, the data was not converted to binomial format as the

statistical model would not work for the limited amount of data in this trial. The experimental unit was eight jejunum per treatment group and four tissue samples for the remaining tissues. Once again the “&dat” and “&var” would be replaced with the data set and variable being analyzed using the SAS macro program. All of the different tissue scores used this program except lymphocytic infiltrates in the ceca. Models for distribution and density of lymphocytic infiltrates only had one factor in the final model. The model for distribution consisted of flock, and for density only, weight group. After the model, contrast statements were used to determine the odds ratio for difference between two different inoculums groups (heavy vs light or Flock 1 vs Flock 2).

```
Proc Logistic data=&dat; Class WTGP FLOCK &var;
Model &var = WTGP FLOCK;

Contrast 'wtgp h l' wtgp 1 -1 / estimate=exp;
Contrast 'wtgp h n' wtgp 1 0 / estimate=exp;
Contrast 'wtgp l n' wtgp 0 1 / estimate=exp;
Contrast 'flock 1 2' flock 1 -1 0 0 0 0 / estimate=exp;
Contrast 'flock 1 3' flock 1 0 -1 0 0 0 / estimate=exp;
Contrast 'flock 1 4' flock 1 0 0 -1 0 0 / estimate=exp;
Contrast 'flock 1 5' flock 1 0 0 0 -1 0 / estimate=exp;
Contrast 'flock 1 6' flock 1 0 0 0 0 -1 / estimate=exp;
Contrast 'flock 1 C' flock 1 0 0 0 0 0/ estimate=exp;
Contrast 'flock 2 3' flock 0 1 -1 0 0 0 / estimate=exp;
Contrast 'flock 2 4' flock 0 1 0 -1 0 0 / estimate=exp;
Contrast 'flock 2 5' flock 0 1 0 0 -1 0 / estimate=exp;
Contrast 'flock 2 6' flock 0 1 0 0 0 -1 / estimate=exp;
Contrast 'flock 2 C' flock 0 1 0 0 0 0 / estimate=exp;
Contrast 'flock 3 4' flock 0 0 1 -1 0 0 / estimate=exp;
Contrast 'flock 3 5' flock 0 0 1 0 -1 0 / estimate=exp;
```

```
Contrast 'flock 3 6' flock 0 0 1 0 0 -1 / estimate=exp;
Contrast 'flock 3 C' flock 0 0 1 0 0 0 / estimate=exp;
Contrast 'flock 4 5' flock 0 0 0 1 -1 0 / estimate=exp;
Contrast 'flock 4 6' flock 0 0 0 1 0 -1 / estimate=exp;
Contrast 'flock 4 C' flock 0 0 0 1 0 0 / estimate=exp;
Contrast 'flock 5 6' flock 0 0 0 0 1 -1/ estimate=exp;
Contrast 'flock 5 C' flock 0 0 0 0 1 0 / estimate=exp;
Contrast 'flock 6 C' flock 0 0 0 0 0 1/ estimate=exp;
```

Proc Glimmix was used for analysis of virus presence. The experimental unit for the virus analysis was a pool of three birds from each pen. There were four pens per treatment group. Astrovirus was found in all samples from day 14 and reovirus was not found in any samples, so no statistics were determined for either. The following model is for rotavirus. The interaction between flock and weight group was not significant.

```
Proc Glimmix data=virus; Class WTGP FLOCK;
Model Rotavirus = WTGP FLOCK / dist = bin solution;
```

For presence of specific bacteria no statistics were determined since E. coli was once again found in all but one sample (Flock 3 light weight group) and no S*almonella spp., Clostridium spp.* or *Campylobacter spp.* were found in any of the samples. The experimental unit for bacteria presence and total plate counts was one group of pooled intestines for each treatment or control group at 14 days of age. The pools contained gut contents from 12 different poults (three per pen). For total plate counts of bacteria the mixed procedure in SAS was used. An interaction between flock and weight group was unable to be tested because of the small data set.

```
Proc Mixed data=baclog105; class FLOCK WTGP;
Model APT = FLOCK WTGP; lsmeans FLOCK WTGP / pdiff;
```

For plasma xylose concentration, average daily gain, feed intake, feed conversion and body weight differences the SAS procedure Glimmix was used. For feed intake and conversion the statistical unit was the pen with six birds per pen and four pens per treatment group. For average daily gain and body weights the experimental unit was individual bird with 24 birds per treatment group. For xylose absorption the experimental unit was the individual bird with 12 birds per treatment group. The following is the model that was used for these variables.

```
Proc Glimmix data=&dat; Class FLOCK WTGP;
Model &var = FLOCK WTGP; LSmeans FLOCK WTGP / pdiff;
```

RESULTS

Body Weights

Poult body weights at placement averaged 61.4 grams (SE ± 0.2) and ranged from 44 to 76 grams. At seven days of age poults chosen for the study averaged 130.3 grams (SE ± 0.6) (Figure 4.1). At 13 days of age (six days post inoculation) poults had an average weight of 187.7 g (SE ± 1.34) see Table 4.1. Significant differences were seen between flock sources of inoculums on body weights of poults at 13 days of age ($P < 0.0001$) see Figure 4.2 and Table 4.2. Controls weighed an average of 17 and 33 grams more than poults gavaged with inoculums from research (flocks 5 and 6) and commercial flocks (1-4), respectively. Poults gavaged with inoculums from the research flocks (5 and 6) weighed on average 16 g more than poults gavaged with contents from the MN commercial Flocks 1-4. No differences were seen for body weight at 13 days of age between poults gavaged with inoculums from heavy and light weight poults.

From seven to 13 days of age, average daily gain (ADG) per poult showed the same statistical differences as body weights at 13 days of age (Table 4.3). Significant differences were seen between flock sources of inoculums for ADG of poults from seven to 13 days of age ($P < 0.0013$). Average daily gain was 14.3 g (SE ± 0.5) for control poults (Figure 4.3). Controls gained an average of 3.6 and 6 g more than poults gavaged with inoculums from research flocks (5 and 6) and MN commercial flocks (1-4). Poults gavaged with inoculums from the research Flocks 5 and 6 gained on average 2.4 g more than poults gavaged with contents from the MN commercial Flocks 1-4. No differences were seen between poults gavaged with inoculums from heavy and light weight poults for average daily gain from seven to 13 days of age.

Feed Intake and Conversion

Feed intake per poult averaged over all treatment and control groups was 22.17 grams per day (SE ± 0.39). No differences were observed for feed intake between weight group or flock inoculums (Figure 4.4 and Table 4.4). Feed conversion (g feed/g gain) was not different between poults receiving inoculums from light or heavy poults. Feed

conversion was different among poults receiving inoculums from different flocks ($P < 0.0010$) see Figure 4.5 and Table 4.5. Control poults had the best feed conversion of 1.79 and were significantly different than poults gavaged with contents from Flocks 1-5 but not from Flock 6. The poults gavaged with contents from the research flocks (5 and 6) had significantly better feed conversion than poults gavaged with contents from MN commercial Flocks 1, 2 and 4 but not Flock 3.

Virus

Astrovirus was not found in poults at zero or seven days of age prior to inoculation. At 14 days of age all pools were positive for astrovirus including the control birds (Figure 4.6). No differences were seen between weight groups or flocks for inoculated or control poults at 14 days of age for presence of astrovirus since they were all positive. Reovirus was not found in any pools including samples collected at zero, seven and 14 days of age.

Rotavirus was not found in poults at zero or seven days of age prior to inoculation. At 14 days of age rotavirus was present in at least one pen in each of the groups inoculated with contents from MN commercial flocks (1-4). One pen of poults inoculated with contents from light weight poults from research Flock 5 was also positive for rotavirus (Figure 4.7). The other research flock (Flock 6) and the control poults had no presence of rotavirus detected at 14 days of age. No statistical differences were seen between heavy and light weight inoculums groups or among flock inoculums groups.

Bacteria

E. coli, *Campylobacter* and *Salmonella* were not found in any samples from 7 days of age pre inoculation. Samples were not tested at zero days of age for presence of bacteria. *E. coli* was found in all pens at 14 days of age except for the poults inoculated with contents from light weight poults from Flock 3 (Figure 4.9). *Campylobacter* and *Salmonella* were found originally in the gut contents on the field trial samples but none were detected in the inoculated poults (Figures 4.10 and 4.11).

Total bacteria plate counts for lactobacilli, heterofermentative lactobacilli, anaerobic and aerobic bacteria are shown in Figure 4.12. Total bacteria plate counts for lactobacilli were not significantly different among inoculum flock groups and the control group at day 14. Seven day old poults averaged 6.45 log_{10} cfu/g for lactobacilli plate counts which were less than the average of 8.06 log_{10} cfu/g at 14 days of age. Total bacteria plate counts for lactobacilli were significantly different among inoculum weight groups with the heavy group having a lower count than the light weight poults but neither being different than the controls ($P < 0.0624$) See Figure 4.13.

Total bacteria plate counts for anaerobic bacteria were significantly different among inoculum flock groups and controls at 14 days of age ($P < 0.0261$) see Figure 4.14. Seven day old poults averaged 6.29 log_{10} cfu/g for anaerobic bacteria plate counts which was less than the average of 7.22 log_{10} cfu/g at 14 days of age. Group 5 had the highest counts and was statistically similar to groups 1, 2, 6 and the controls. Group 5 had significantly higher counts than group 3 and 4. Control poults and groups 1, 2, 5 and 6 had significantly higher anaerobic counts than groups 3 and 4.

For aerobic bacteria and heterofermentative lactobacilli counts no significant differences were seen between inoculation groups. Seven day old poults averaged 6.29 log_{10} cfu/g for aerobic bacteria plate counts which was less than the average of 7.04 log_{10} cfu/g at 14 days of age. Seven day old poults averaged 6.29 log_{10} cfu/g for heterofermentative lactobacilli plate counts which was less than the average of 7.90 log_{10} cfu/g at 14 days of age.

Histopathology

Heterophilic Infiltrates in the Gut Tissue. In the small intestine differences were seen between the controls and inoculated groups for heterophilic density in the jejunum ($P < 0.0670$) (Figure 4.15). Control poults had on average a score of 1.0 for heterophilic density which was higher than poults inoculated with light or heavy weight contents which had an average score of 0.6 ($P < 0.0208$, $P < 0.0219$). Poults inoculated with contents from heavy weight poults were not different than poults inoculated with contents from light weight poults. Heterophilic distribution was different between the controls and

inoculated groups in the ileum ($P < 0.0909$) see Figure 4.16. Control poults had an average score of 2.8 which was higher than the light weight poults with a score of 2.2 ($P < 0.0422$). Control poults had an average score of 2.8 which was higher than the heavy weight poults with a score of 1.9 ($P < 0.0805$). Poults in the heavy group were not significantly different from the light group.

Heterophilic infiltrates varied among inoculum flocks and controls for both density and distribution in the ileum. In the ileum heterophilic density scores were significantly lower in inoculum group 2 than in groups 1, 3, 4, 6 and controls ($P < 0.0308$) (Figure 4.17a). For heterophilic distribution in the ileum, inoculum group 2 had significantly lower scores than all of the other inoculums groups and the control group ($P < 0.0124$). Inoculum group 5 also had significantly lower scores than group 6 and controls. Heterophilic infiltrates varied among inoculum flocks and controls for both density and distribution in the ceca (Figure 4.17b). For the ceca heterophilic density and distribution were similarly different between inoculum groups ($P < 0.0896$, $P < 0.0649$). Inoculum groups 1 and 5 had significantly lower heterophilic scores in the ceca than groups 2, 6 and controls.

Lymphocytic Infiltrates in the Gut Tissue. The lymphocytic infiltrates differences in the duodenum were different between weight groups and controls for both density and distribution ($P < 0.0470$, $P < 0.0083$). Lymphocytic infiltrates were significantly higher for density and distribution in the light weight group than controls or heavy weight inoculum group. The heavy weight poults also had significantly higher density and distribution of lymphocytes than the control group (Figure 4.18).

Differences among the inoculums groups for lymphocytic distribution were seen in the ceca ($P < 0.0123$). In the ceca, flock 1 had significantly lower scores than groups 3, 4, 5 and 6. The statistical data did not include group 2 or the controls as all scores for lymphocytic distribution were zero. Differences among inoculums groups for lymphocytic distribution were seen in the duodenum ($P < 0.0587$). In the duodenum no scores were found in group 1 and it was removed from the statistical model. Inoculum groups 2 and 3 had lower scores than group 6 and the controls (Figure 4.19).

Immune Tissue. Lymphocellular necrosis of the bursa was present in all flocks and was significantly different among weight groups and controls for distribution ($P < 0.0040$). Poults in the light weight groups had significantly higher scores for distribution of lymphocytic infiltrates than heavy weight poults ($P < 0.0009$). Heavy and light weight poults were not different from control poults most likely because there were only 4 tissues scored for the control group (Figure 4.20).

Inoculum group differences were seen for both distribution and density of lymphocytic necrosis ($P < 0.0009$, $P < 0.0025$) see Figure 4.21. For distribution groups 1, 3 and 4 had higher scores than groups 2 and 5. In addition, groups 1 and 4 had higher scores than group 6 and the controls. For density groups, 1, 3 and 4 had higher scores than groups 2 and 5. In addition, group 4 also had higher scores than groups 1, 3, 6 and the controls. The spleen tissue was also collected and scored but no significant differences were found between inoculums flock, weight groups and controls.

Xylose

The plasma samples taken prior to gavaging the mean of all pre-gavage (T0) samples was 4.05 mg D-xylose/dL (SE ± 0.16) and the individual values ranged from 0.31 to 12.41 mg D-xylose/dL. The treatment group averages ranged from 3.12 to 5.01 mg D-xylose/dL per treatment group (Table 4.6, Figure 4.22a). At time zero no differences were seen between flocks for amount of D-xylose present. There was a significant difference between the heavy, light and control inoculum group's levels of D-xylose at T0 ($P < 0.0088$). Poults in the control and heavy groups both had significantly more xylose present in their plasma than the light weight group (Figure 4.23). No differences were seen between inoculums flock groups and controls for time zero samples.

Plasma samples taken 60 minutes after gavaging the mean concentration was 25.14 mg D-xylose/dL (SE ± 0.95) and the values ranged from 3.72 to 51.34 mg D-xylose/dL for each individual sample. The treatment group averages ranged from 18.97 to 37.35 mg D-xylose/dL per treatment group (Table 4.7, Figure 4.22b). No differences between the control, heavy or light weight inoculum groups were seen. Significant

differences among inoculum groups for flock differences were seen ($P < 0.0001$). Group 5 had higher levels of D-xylose at 60 minutes post gavage than groups 1, 2, 3, 6 and controls. Group 5 and 4 absorbed a similar amount. The controls absorbed similar amounts to all groups except group 5 (Figure 4.25).

DISCUSSION

The purpose of this study was to determine if poult performance and health measures differ when the poults are challenged with inoculums from commercial poults experiencing LTS. In comparison, we also inoculated poults with contents from research raised poults and had a group of control birds that were just inoculated with PBS. The results primarily indicated that flock source of inoculums had more of an effect on bird performance and health measures than whether this content was from heavy or light weight groups. There were some differences seen between the weight groups and control group and some were expected.

Low body weights were seen in the inoculated poults compared to the non-inoculated controls. As observed in the field trial, poults inoculated with contents from the four MN commercial had in general poorer performance, followed by the poults inoculated with contents from the two research flocks. However, the differences were not as great as occurred in the field trial especially for Flocks 3 and 4 as compared to Flocks 5 and 6. The combination of rotavirus and astrovirus in the MN commercial flocks may have been responsible for the decreased bird performance observed in the inoculated Flock groups 1-4 as compared to the research flocks. Additive affects of pathogen exposure has been seen in previous work done on PES which demonstrated that enteric viruses may first infect the poult and cause damage which then increases the chance for bacteria to infect the damaged tissue (Jindal et al., 2009c). Alternatively, the pathogenicity of the viruses may have been different. It has been shown that there are differences in pathogenicity of bacteria and virus. In the case of rotavirus, some types of rotavirus that infect chickens and some that infect turkeys, if transmitted to a different species, may not cause as severe or any disease symptoms (McNulty et al., 1980). Poults in the field trial, especially from Flocks 3 and 4, were also exposed to Salmonella and Campylobacter as well as reovirus which probably contributed to the more severe growth depression in the field.

Feed conversion was poorer for the inoculated poults from Flocks 1-4 as compared to uninoculated controls with the research flock results intermediate. Since

feed conversion is not typically measured in the brooder barn, there is no comparison to be made to the field situation. As feed intake was similar among inoculated and control groups, the poorer growth is the result of decreased utilization of the feed. Klasing (2007) has demonstrated the nutritional cost of stimulating the immune system as would occur when poults were inoculated with gut contents.

Lactobacilli were higher in the light weight group than the heavy weight group. The controls were not significantly different than either of the weight groups. In a previous study birds given probiotics had higher levels of lactobacilli in the ceca (Awad et al., 2009). Nine days after a challenge with *E. coli* the lowest amount of *E. coli* was found in the probiotic group. Since we did not measure amount of *E. coli* present we are unable to determine if the higher levels of lactobacilli in the light weight poults corresponded to lowered *E. coli* amounts. Another study shows that probiotic supplementation with lactobacilli did not improve body weights compared to the controls. This is similar to our study as we did not see a difference between body weights for poults that had received the inoculums from light weight groups compared to the controls. The probiotic treatment group did show increased villus height to crypt ratio in both the duodenum and ileum compared to controls (Awad et al., 2009). Though the addition of a synbiotic in this trial did improve body weights and significantly increased villus height to crypt ratio in the ileum, it did not improve the duodenum. It would seem that the affect of lactobacilli on a turkey poult is influenced by many factors and is not directly related to increased body weights. In contrast, previous research in turkey hens showed that the addition of a probiotic can increase body weights and average daily gain but not feed conversion (Torres-Rodriguez et al., 2007). Once again our study does not match these findings as we did not have a difference between weight groups and controls for body weight or feed conversion.

For histopathology scoring the gut tissues samples showed an unexpected result of more heterophilic infiltrates being found in the control poults. Heterophilic infiltrates are a symptom of the acute immune response and are associated with an immediate disease response. Poults with higher numbers are thought to be currently involved with an

immediate disease response. It is also possible that the controls may have been undergoing a challenge possibly due to astrovirus or *E.coli*. It is also possible that the immune system in the control poults had been activated within the last 12 hours and therefore we were seeing the first wave of heterophilic infiltrates to combat the challenge (Harmon, 1998). In comparison the heavy and light weight groups may have been undergoing their challenge for a while since it was seven days since they had been inoculated and the heterophilic response had decreased.

For lymphocytic infiltrates previous research has shown that these cells are associated with a mature immune system and are also present when poults are undergoing a chronic immune response (Malewitz and Calhoun, 1958). Lymphocytic infiltrates for density and distribution were seen the most in poults that had received inoculums from the light weight groups as compared to inoculum from heavy weight groups. Both heavy and light weight inoculated groups also had more lymphocytes present than the control group. It is possible that the poults receiving the inoculum from the light weight groups had a heightened immune response because of the increased challenge compared to the heavy weight and control poults. The control poults may have not had as many lymphocytes present because they had not been undergoing a challenge for long enough to activate the production of mature lymphocytes. The immune response started to develop and progressed through the acute immune response and had moved into the chronic disease response. This explanation at first seems unlikely since the inoculation challenge was only seven days long but after further review it seems that the beginning of the chronic disease response does occur seven days after inoculation (Malewitz and Calhoun, 1958). This and the fact that the scores for lymphocytic infiltrates were low could indicate the light weight poults had arrived at the chronic disease response prior to the other groups. The lymphocytes are then showing the very beginning of the chronic disease response in the light weight poults. Control poults had the least number of lymphocytes present which was not expected when looking at it from a gut maturation process (Yegani and Korver, 2008). The expected results would be that the controls

would have a more mature immune system and therefore more lymphocytes present than the light weight poults and in fact it was not the case.

In the bursa, lymphocellular necrosis was highest in poults receiving the inoculum from the light weight groups compared to the heavy weight group. This shows that the light weight poults were undergoing some sort of an attack on their lymphocytes by a pathogen. The output of lymphocytes in the gut tissue and the necrosis of lymphocytes seen in the bursa may be related to the same disease organism or it could be completely different. Lymphocellular necrosis of the bursa results in a decreased ability to respond to immune challenges and most likely will decrease the rate at which the remaining immune tissue (gut associated) will develop. The lymphocellular infiltrates seen in the gut may be from progenitors that developed in the bursa prior to the onset of the necrosis. The necrosis of the bursa in the light weight poults should also have an effect on the production of more mature lymphocytes. Because this was a short term study (one week duration), the effects of a challenge in the bursa would have continued on if the poults remained alive and would have impacted the continued immune response in the gut.

Though not significantly different from the heavy or light poults, the control poults did show the least amount of necrosis of the bursa. The low incidence of necrosis in the bursa in the controls could indicate that their immune development was not being compromised by infection. The next question would be how the lack of lymphocellular necrosis in the control poults bursa relates to the decreased levels of lymphocytic infiltrates in the control poults gut tissue. One would expect that the decreased necrosis would more likely coincide with increased levels of lymphocytes if the lymphocytes are indeed showing the gut maturation process. On the other hand the lymphocytes that were seen in the gut tissue may be more related to the absence of a chronic disease challenge.

Plasma xylose concentration varied with flock although values from five of the six inoculum groups did not differ from the control. The expected results would be that the controls would have absorbed the most (had the highest xylose concentration) followed by the group inoculated with contents from research poults and finally the lowest would be the poults inoculated with contents from the commercial flocks. Possible

reasons why the expected results were not seen according to a previous study the absorption capacity is only transiently affected after inoculation. Hayhow and Saif (1993) repeated a study with slightly different aged turkey poults. In that study they directly inoculated poults with rotavirus and enterovirus. In the first study poults in the control group absorbed more D-xylose at two, four and six days post inoculation (five, seven and nine days of age) but not at eight days post inoculation (11 days of age) than the treatment group that received both viruses. The groups that only received one of the viruses had significantly decreased absorption as compared to the controls only at six days post inoculation (nine days of age). In the second trial slightly older poults were used that ranged from three to 14 days post inoculation (six to 17 days of age) and no differences were seen in xylose absorption between any of the virus and control groups. It is possible that the results we obtained were showing that poults were recovering from the direct challenge on gut tissue that would have affected the absorptive capacity if xylose samples had been collected at four or five days instead of eight days post inoculation (Group 4 and 5). The remaining Groups 1-4, 6 and the controls may have still been undergoing a challenge that was directly affecting the absorption capabilities of the poults.

A detected weight group and control difference for samples prior to inoculation showed control and heavy weight groups with significantly higher levels of xylose (or other reactive substance) than the light weight groups. Previous research has only used the pre-inoculation sample for determining a base value but never to specifically determine if there are differences present. In a study with normal turkeys small amounts of xylose were found that average 2.3 mg/dl (Goodwin et al., 1984). In the first trial of another study the pre gavage blood samples were reported and ranged from 1.592 to 5.877 mg xylose /dL (Reynolds and Saif, 1986). Our values are in that same range from 3.15 to 5.01 mg xylose /dL. This study did find differences between its astrovirus inoculated group and controls from xylose levels 90 minutes post gavage but did not report any statistics for the differences between the pre gavage samples. A small amount of xylose can be present in poults from their normal diet. Xylose makes up a part of the

water insoluble non-starch polysaccharides component of some feeds. This possible interference is decreased with the 12 hour fast but seems that there is still a residual amount left in the blood stream. The question then is whether the differences seen in a fasted bird's blood are related to the absorption of nutrients or if there is a completely different explanation.

TABLES

Table 4.1 Body weight, gain, feed intake and feed conversion for 12 treatment groups and control group

Mean (SE)	Heavy weight groups from inoculum flocks					
	1	2	3	4	5	6
Body weights [1] 7 days PI (g)	173.7 (4.0)	181.3 (3.5)	185.5 (3.5)	187.2 (4.7)	190.6 (6.1)	196.9 (3.6)
ADG[2] (g/ bird per day)	7.74 (0.51)	8.47 (0.56)	8.87 (0.65)	9.18 (1.26)	9.65 (1.34)	10.94 (0.37)
Feed Intake[3] (g/bird per day)	20.0 (0.7)	22.4 (1.0)	22.2 (1.5)	21.7 (1.3)	22.8 (1.4)	23.2 (0.6)
Feed Conversion[4] (feed/gain)	2.55 (0.08)	2.74 (0.10)	2.17 (0.08)	2.88 (0.21)	2.15 (0.07)	2.09 (0.14)

	Light weight groups from inoculum flocks						Controls
	1	2	3	4	5	6	
Body weights [1] 7 days PI (g)	179.3 (3.6)	173.2 (3.1)	182.0 (5.8)	179.5 (3.8)	203.9 (4.0)	193.6 (3.9)	213.5 (5.2)
ADG[2] (g/bird per day)	8.40 (0.33)	7.54 (0.56)	8.02 (1.56)	8.22 (0.57)	11.56 (0.29)	10.54 (0.59)	14.31 (0.51)
Feed Intake[3] (g/bird per day)	21.3 (0.4)	20.5 (0.8)	18.5 (2.4)	23.5 (1.9)	24.9 (1.0)	21.9 (0.9)	25.3 (0.6)
Feed Conversion[4] (feed/gain)	2.61 (0.13)	2.67 (0.10)	2.58 (0.36)	2.45 (0.21)	2.24 (0.09)	2.12 (0.04)	1.79 (0.06)

At 7 days of age, before inoculation body weights of poults averaged 130.3 g (SE 6.0)
Performance objectives for 1 to 2 week old poults from Nicholas male turkeys (2010)
[1] Body weight 150 to 400 grams[2] Average daily gain 21 to 29 grams
[3] Feed intake 21 to 41 grams
[4] Feed Conversion 1.05 to 1.11

Table 4.2 Statistical results for body weight, gain, feed intake and feed conversion

	Flock	WtGp	Interactions		
Body weights	P<0.0001	NS[1]	NS	NS	NS
ADG	P<0.0013	NS	NS	NS	NS
Feed Intake	NS	NS	NS	NS	NS
Feed Conversion	P<0.0001	NS	NS	NS	NS

[1]ns = not significant ($P > 0.05$)

Table 4.3 Average daily gain per poult per day from 7 to 13 days of age

Group		Average daily gain (g)			
		Pen 1	Pen 2	Pen 3	Pen 4
Heavy	1	6.42	8.65	8.44	7.44
	2	6.83	9.24	8.73	9.06
	3	7.34	9.18	10.44	8.50
	4	6.55	11.92	10.63	7.60
	5	10.83	10.33	5.72	11.71
	6	10.54	10.68	10.51	12.04
Light	1	8.18	7.57	9.01	8.84
	2	8.65	8.35	6.60	6.58
	3	11.04	6.28	10.22	4.54
	4	8.73	6.56	9.17	8.41
	5	12.30	11.23	10.97	11.75
	6	10.06	11.87	11.04	9.18
Controls		14.85	15.43	13.81	13.16

N = 6 poults per pen

Table 4.4 Average feed intake per poult per day from 7 to 13 days of age

Group		Feed intake (g)			
		Pen 1	Pen 2	Pen 3	Pen 4
Heavy	1	18.3	21.4	19.4	20.8
	2	20.0	23.3	21.7	24.7
	3	26.7	20.6	21.7	20.0
	4	19.4	25.0	22.8	19.7
	5	25.8	22.2	19.2	23.9
	6	23.3	22.2	22.5	24.7
Light	1	21.9	20.3	21.9	21.1
	2	22.5	21.1	19.4	18.9
	3	22.8	12.8	22.2	16.4
	4	25.3	19.7	21.1	27.8
	5	26.7	26.1	22.2	24.4
	6	22.8	23.6	19.4	21.7
Controls		26.7	25.3	23.9	25.3

N = 6 poults per pen

Table 4.5 Feed conversion per poult per day from 7 to 13 days of age

Group		Pen 1	Pen 2	Pen 3	Pen 4
Heavy	1	2.86	2.47	2.31	2.80
	2	2.93	2.53	2.48	2.73
	3	3.63	2.24	2.08	2.35
	4	2.97	2.10	2.14	2.60
	5	2.39	2.15	2.40	2.04
	6	2.21	2.08	2.14	2.05
Light	1	2.68	2.68	2.44	2.39
	2	2.60	2.53	2.95	2.87
	3	2.06	2.04	2.17	2.41
	4	2.89	3.01	2.30	3.30
	5	2.17	2.33	2.03	2.08
	6	2.26	1.99	1.76	2.36
Controls		1.80	1.64	1.81	1.92

N = 6 poults per pen

Table 4.6 Baseline values for xylose concentration from 15 day old poults prior to gavage

	Heavy		Light	
Group	Xylose (mg/dL)		Xylose (mg/dL)	
1	3.915	(0.395)	3.743	(0.324)
2	5.010	(1.352)	3.445	(0.705)
3	4.628	(0.906)	4.485	(0.476)
4	4.603	(0.508)	3.125	(0.446)
5	4.656	(0.528)	3.487	(0.275)
6	3.893	(0.538)	3.346	(0.297)
Controls		4.831	(0.697)	

Values are means (standard errors) N = 8 to 12

Table 4.7 Xylose concentration in plasma from 15 day old poults 60 minutes after gavaging with 5 % D-xylose solutions

	Heavy		Light	
Group	Xylose (mg/dL)		Xylose (mg/dL)	
1	19.93	(4.82)	19.56	(3.04)
2	18.97	(2.52)	20.59	(4.42)
3	25.24	(2.02)	22.73	(3.62)
4	28.23	(3.32)	32.13	(1.84)
5	33.50	(3.39)	37.35	(2.69)
6	23.48	(3.89)	20.80	(2.01)
Controls		23.55	(2.76)	

Values are means (standard errors) n = 8 to 12

FIGURES

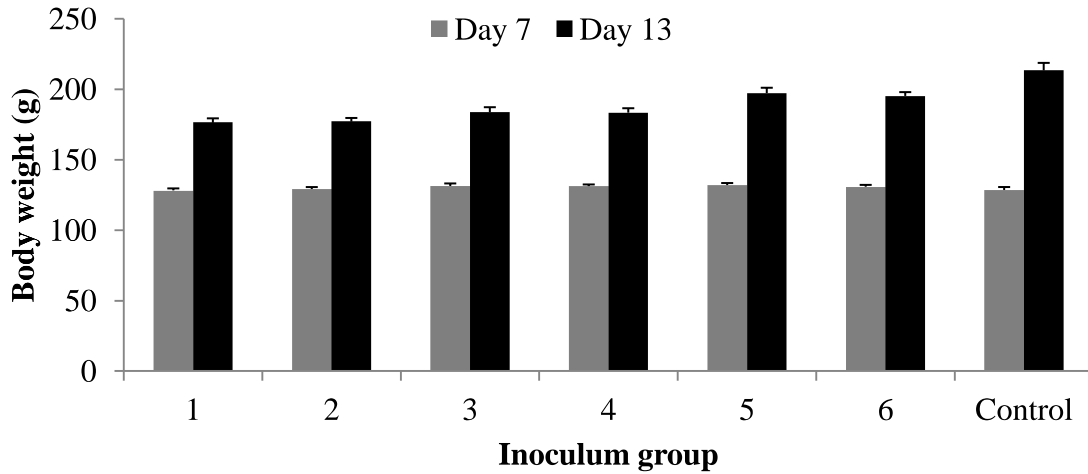


Figure 4.1 Body weights from seven and 13 day old poults
Results are means with standard error bars
N = 48 poults per treatment group and N = 24 poults in control group
Day 7 weights from pre-inoculation and 13 day weights from 6 days post inoculation
Inoculum groups 1 to 6 are groups that were inoculated with gut contents from field study (Flocks 1 to 6) and controls were inoculated with sterile PBS
Performance objectives for 1 and 2 week old poults from Nicholas male turkeys (2010) body weight 150 to 400 grams

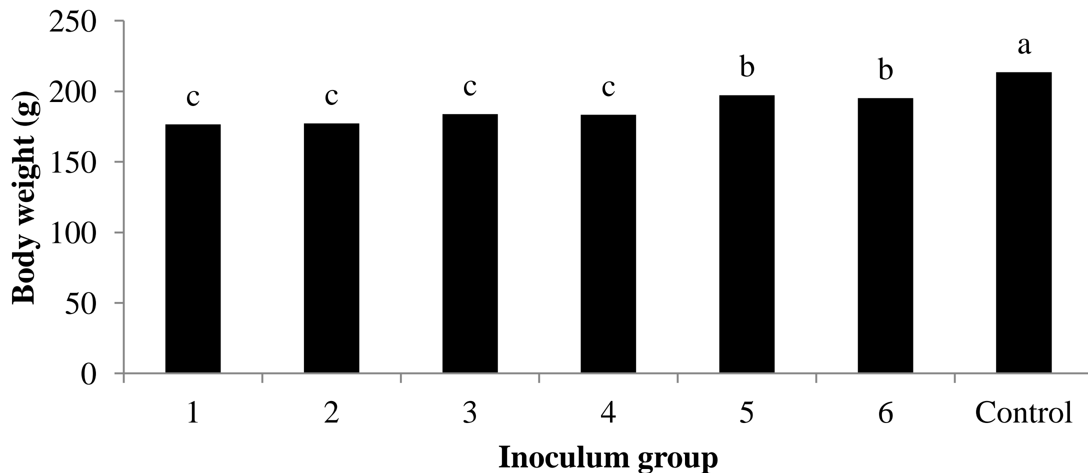


Figure 4.2 Body weights at 13 days of age (6 days post inoculation)
Results are means with standard error bars
N = 48 poults per treatment group and N = 24 poults in control group
Performance objectives for 2 week old poults from Nicholas male turkeys (2010) body weight 400 grams
Body weights were significantly different among inoculums flock groups ($P < 0.0001$)
a,b,c: bars with different letters are significantly different ($P < 0.05$)

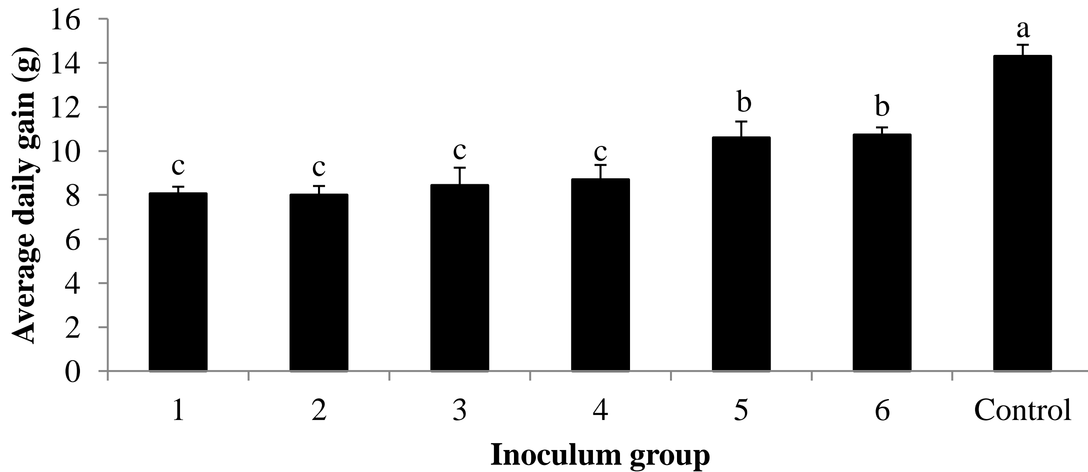


Figure 4.3 Average daily gain per poult from seven to 13 days of age
Results are means with standard error bars
N = 48 poults per treatment group and N = 24 poults in control group
Inoculum groups 1 to 6 are groups that were inoculated with gut contents from field study (Flocks 1 to 6) and controls were inoculated with sterile PBS
Performance objectives for 2 week old poults from Nicholas male turkeys (2010) average daily gain 29 grams
Average daily gain was significantly different among inoculums flock groups ($P < 0.0013$)
a,b,c: bars with different letters are significantly different ($P < 0.05$)

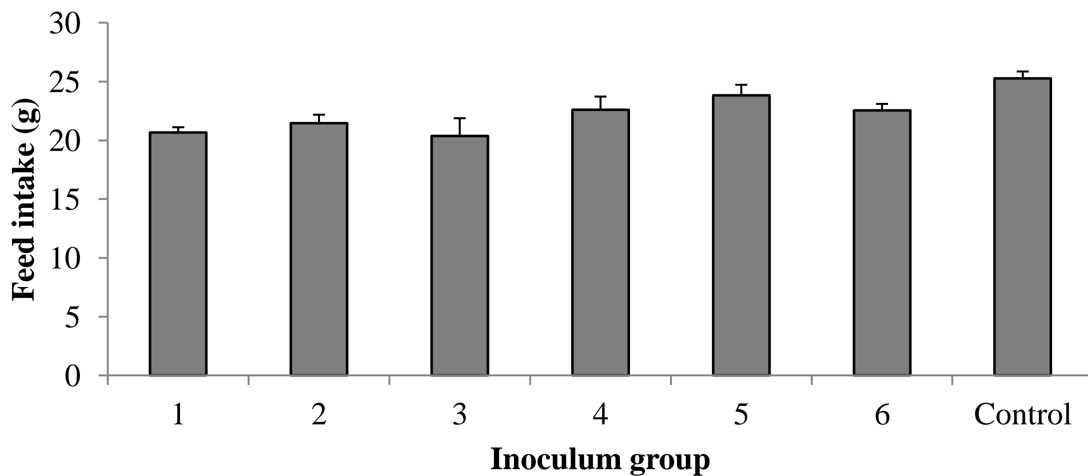


Figure 4.4 Feed intake per poult per day from seven to 13 days of age
Results are means with standard error bars
N = 8 pens per treatment group and N = 4 pens for control group
Performance objectives for 2 week old poults from Nicholas male turkeys (2010) feed intake 41 grams
Feed intake was not significantly different among inoculums flock groups

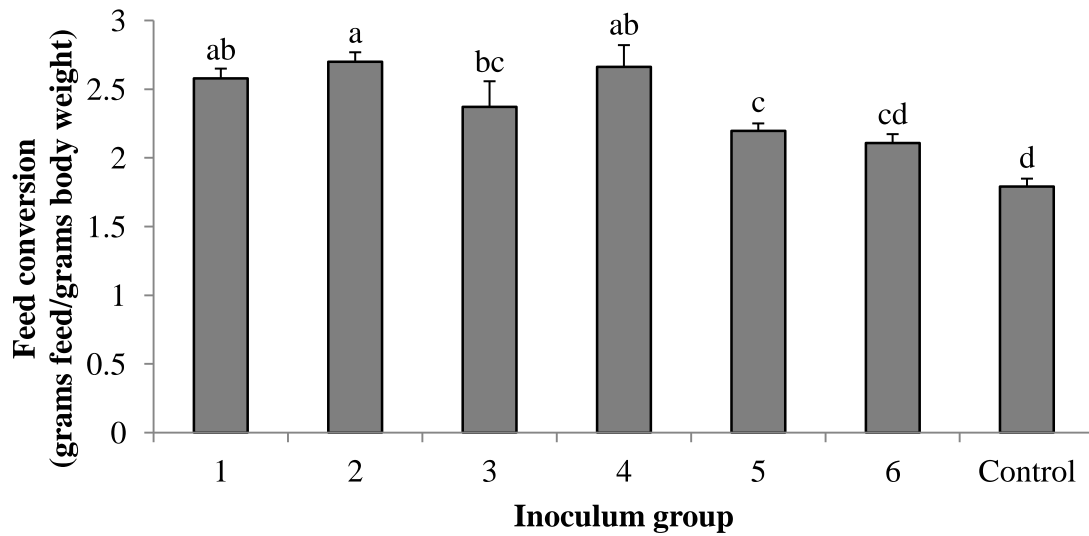
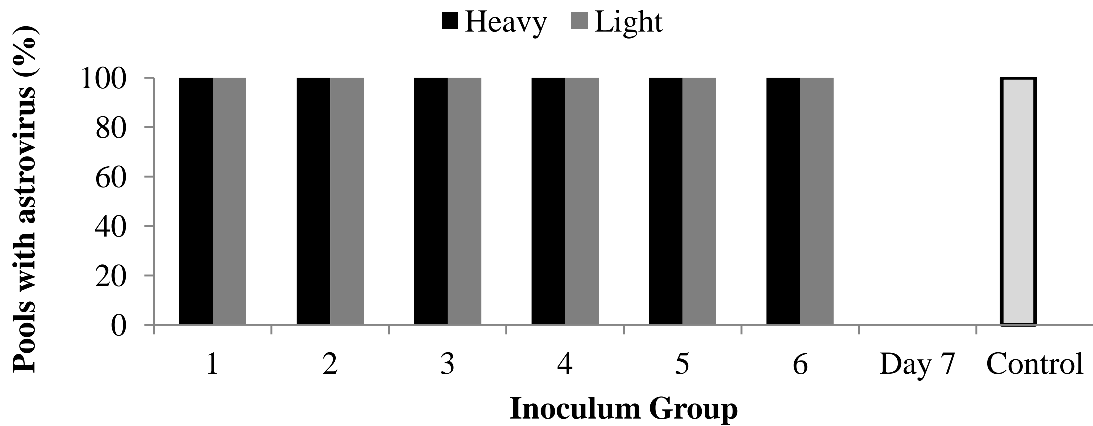
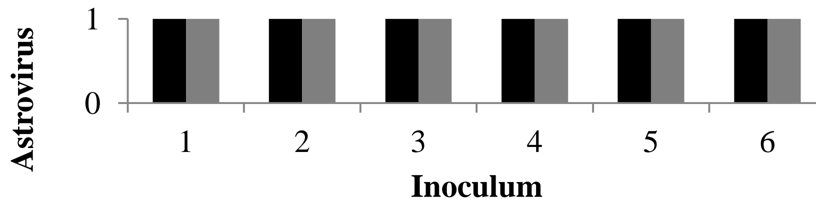


Figure 4.5 Feed conversion from seven to 13 days of age
Results are means with standard error bars
N = 8 pens per treatment group and N = 4 pens for control group
Performance objectives for 2 week old poults from Nicholas male turkeys (2010) Feed Conversion 1.11
Feed conversion was significantly different among inoculums flock groups ($P < 0.0001$)
a,b,c: bars with different letters are significantly different ($P < 0.05$)

a.



b.



c.

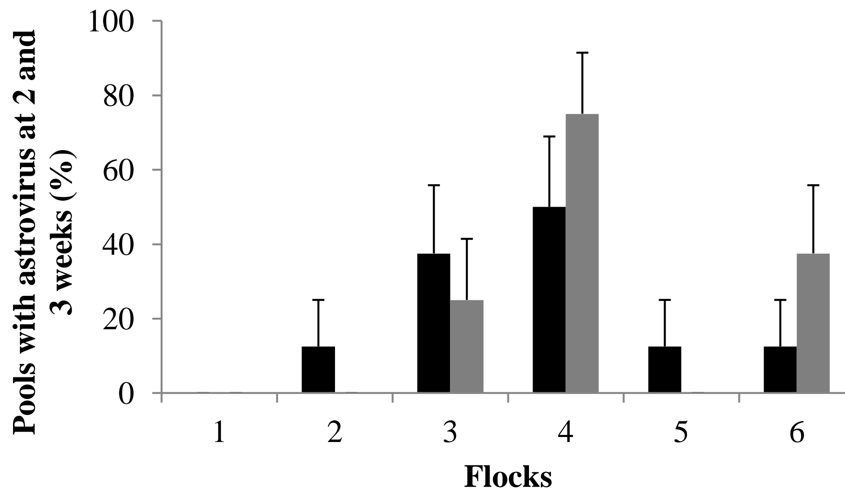


Figure 4.6 Astrovirus presence

Results are means with standard error bars

a. Astrovirus in 14 day old poults from inoculation trial N = 4 pens

No astrovirus was found at 0 or 7 days of age but controls at 14 days of age were all positive

b. Astrovirus present or absent in pooled inoculums, N = 1

c. Percent of poults from field trial that were positive for astrovirus at two and three weeks of age N = 8, these were pooled to make the inoculums

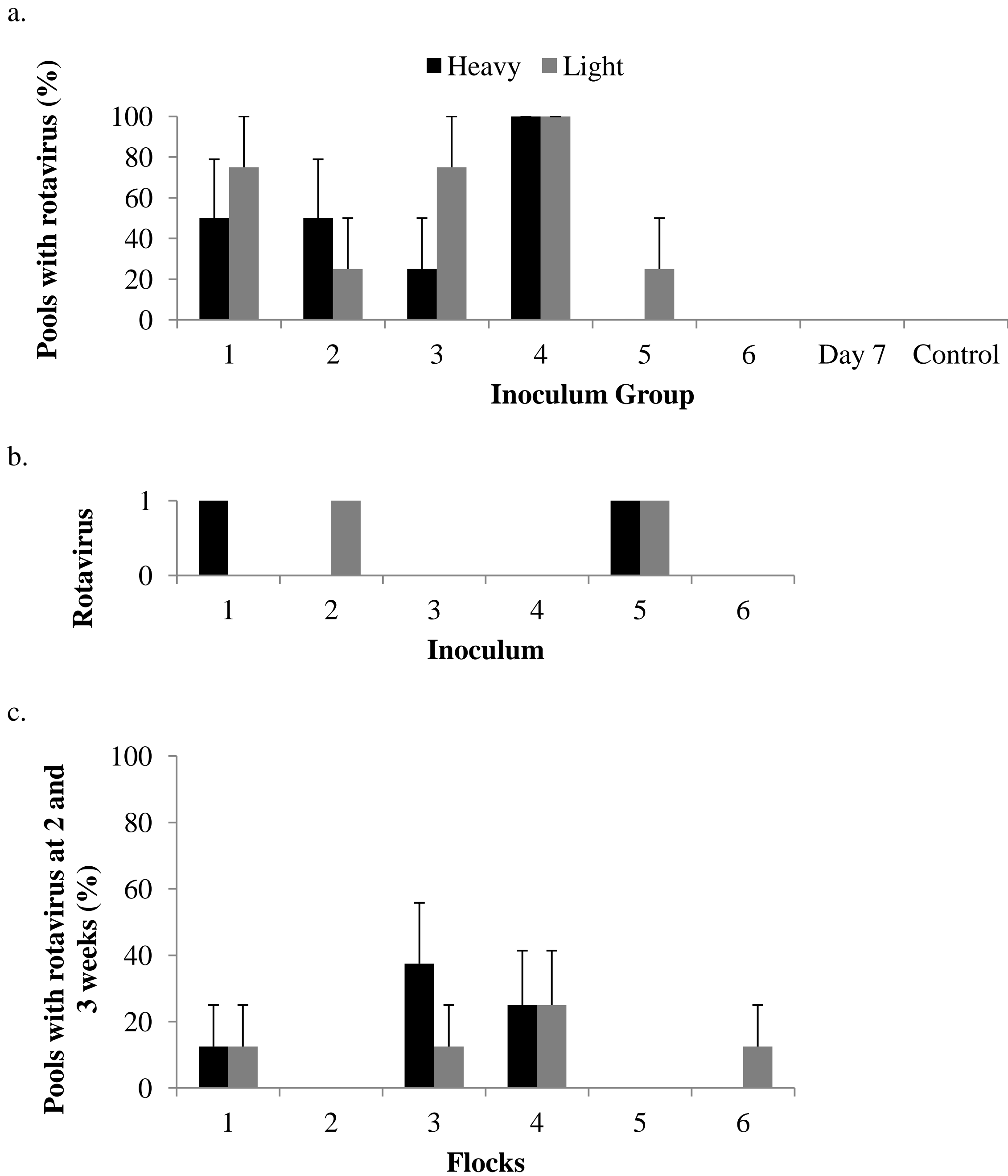


Figure 4.7 Rotavirus presence
Results are means with standard error bars
a. Rotavirus in 14 day old poults from inoculation trial N = 4 pens
No rotavirus was found at 0 or 7 days of age or in controls at 14 days of age
b. Rotavirus present or absent in pooled inoculums, N = 1
c. Percent of poults from field trial that were positive for rotavirus at two and three weeks of age
N = 8, these were pooled to make the inoculums

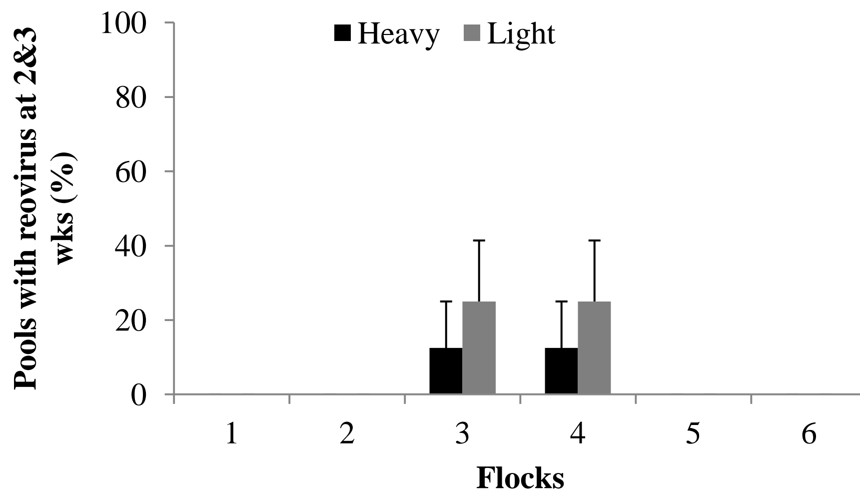
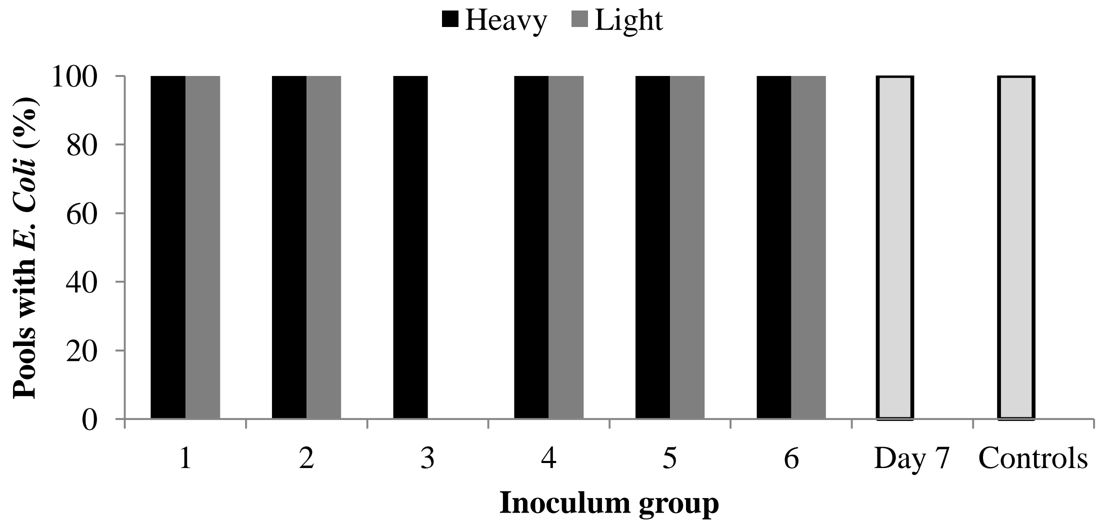


Figure 4.8 Reovirus presence
Results are means with standard error bars
Percent of poults from field trial that were positive for reovirus at two and three weeks of age
N = 8, these were pooled to make the inoculums
No reovirus was found in inoculums after these were pooled and no reovirus was found in any inoculated poults

a.



b.

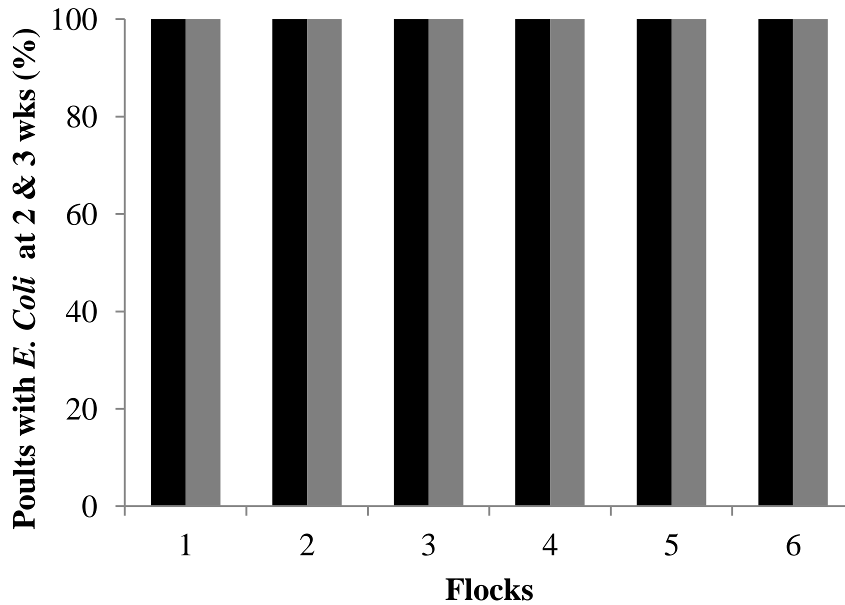


Figure 4.9 *E. coli* presence
Results are means
a. *E. coli* in 14 day old poults from inoculation trial N = 1 pool of 4 pens
E. coli was found at 7 days of age and in controls at 14 days of age
b. Percent of poults from field trial that were positive for *E. coli* at two and three weeks of age
N = 8, these were pooled to make the inoculums

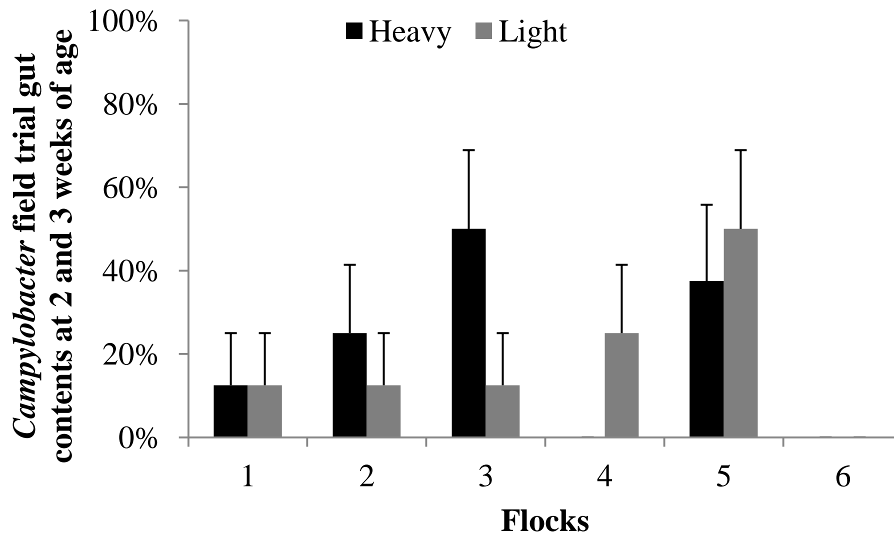


Figure 4.10 *Campylobacter* presence
Results are means with standard error bars, N = 8
Percent of poults from field trial that were positive for *Campylobacter* at two and three weeks of age, these were pooled to make the inoculums
No *Campylobacter* was found in poults from inoculation trial

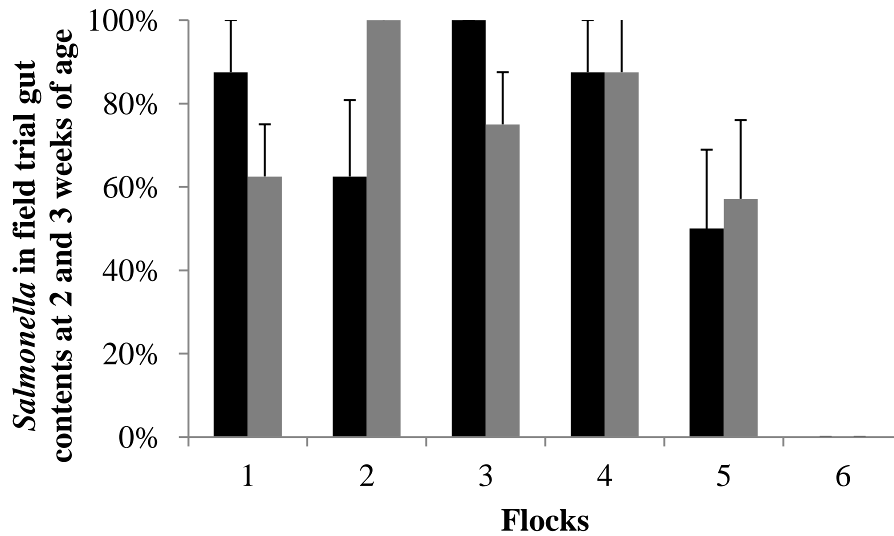
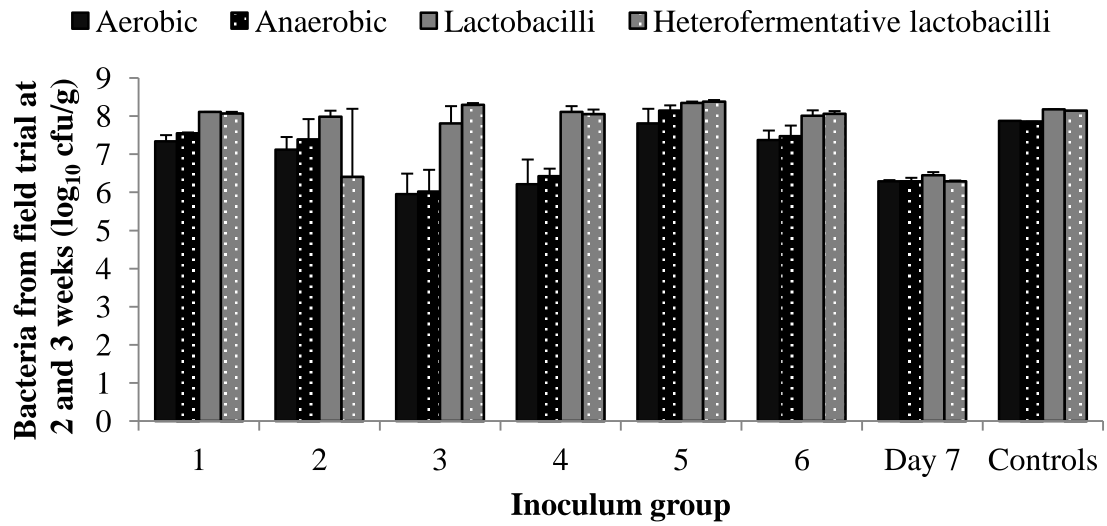


Figure 4.11 *Salmonella* presence
Results are means with standard error bars, N = 8
Percent of poults from field trial that were positive for *Salmonella* at two and three weeks of age, these were pooled to make the inoculums
No *Salmonella* was found in poults from inoculation trial

a.



b.

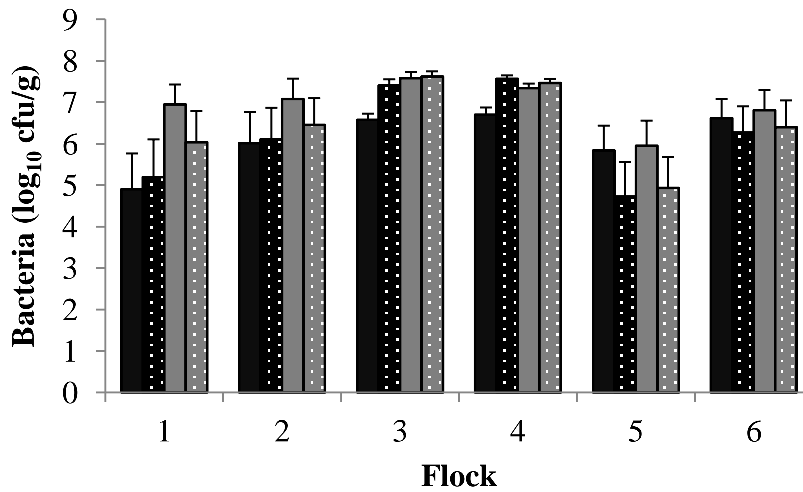


Figure 4.12 Bacteria total plate counts
Results are means with standard error bars
a. Bacteria total plate counts for 7 and 14 day old poults from inoculation trial
N = 2 pools of 4 pens per treatment group and 1 pool of four pens for controls
b. Bacteria total plate counts averaged over two and three weeks of age
N = 24 pools of five poults these were pooled to make the inoculums

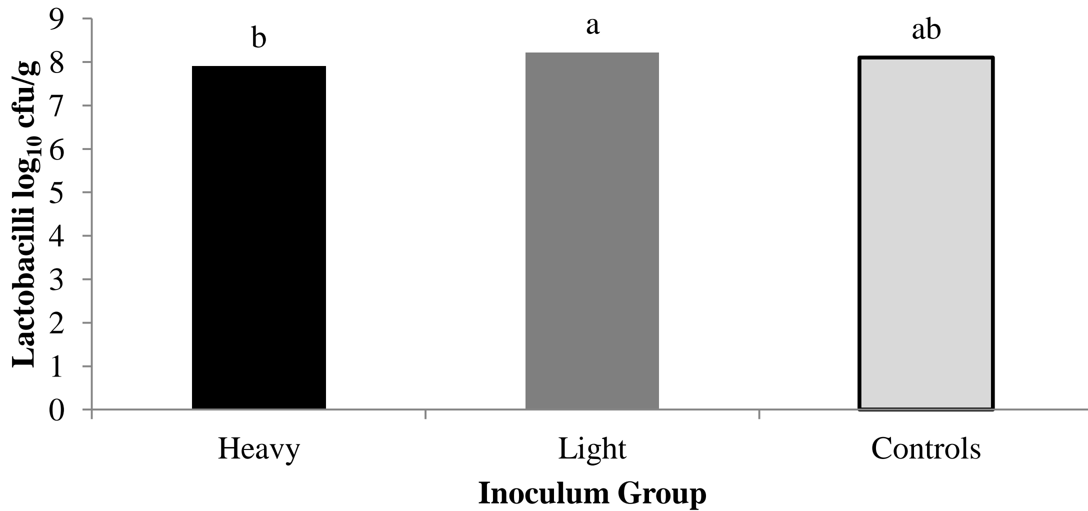


Figure 4.13 Lactobacilli total plate counts by weight group
Results are means, N = 6 for weight groups and N = 1 for controls
Differences were seen between weight groups ($P < 0.0624$)
a,b: Means with different letters are significantly different ($P < 0.05$)

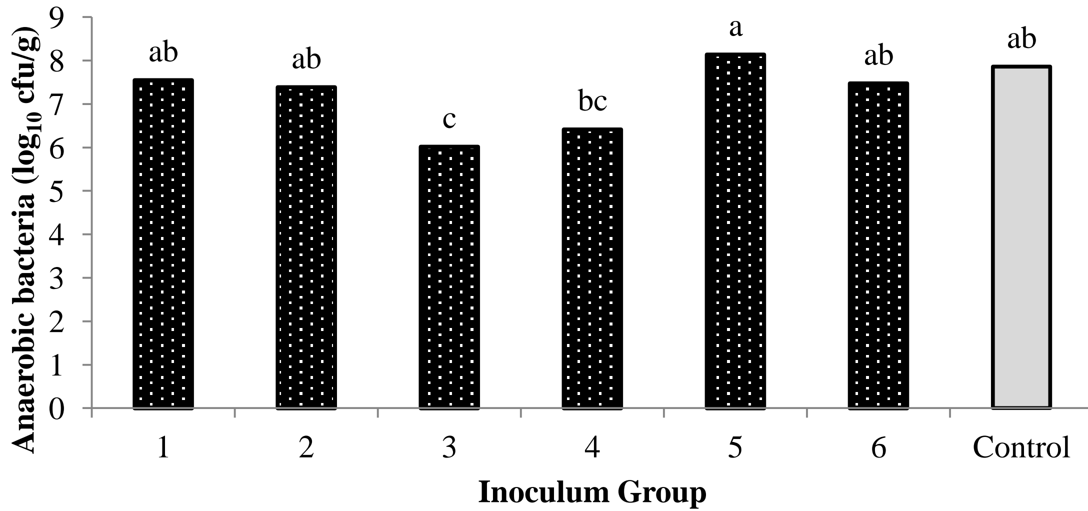


Figure 4.14 Anaerobic bacteria by flock
Results are means, N = 2 per treatment group and N = 1 for controls
Differences were seen among flocks ($P < 0.0261$)
a,b,c: Means with different letters are significantly different ($P < 0.05$)

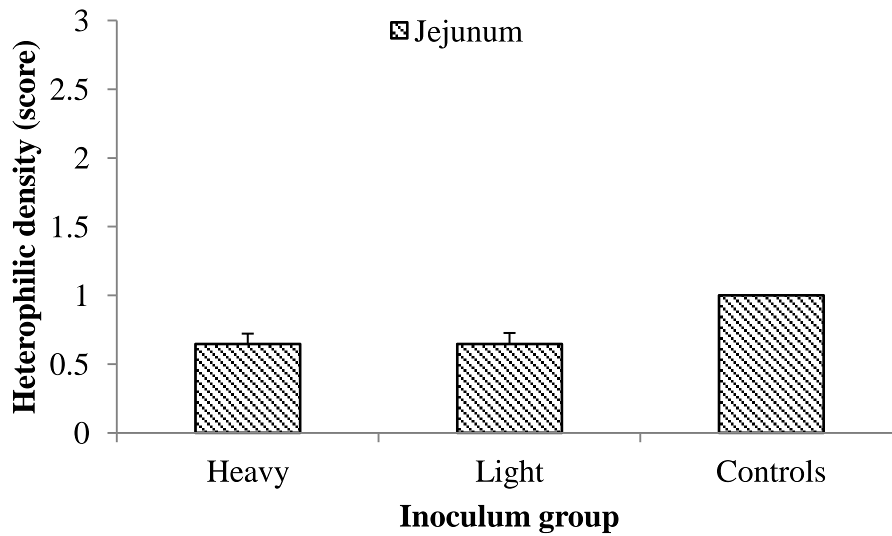


Figure 4.15 Heterophilic density in the jejunum by weight groups
Results are means with standard error bars, N = 48 per treatment group and N = 8 for controls
Controls had higher density than heavy and light weight groups ($P < 0.0670$)

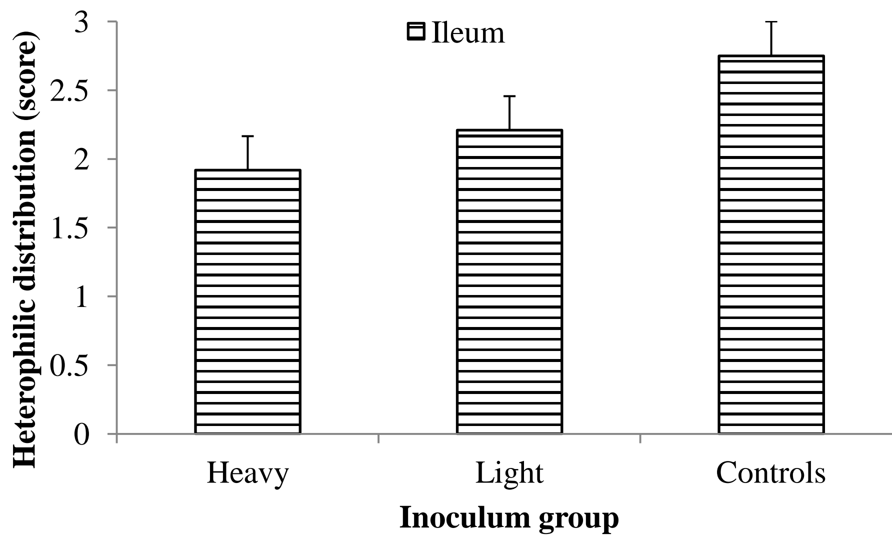


Figure 4.16 Heterophilic distribution in the ileum by weight groups
Results are means with standard error bars, N = 24 per treatment group and N = 4 for controls
Controls had higher density than heavy and light weight groups ($P < 0.0839$)

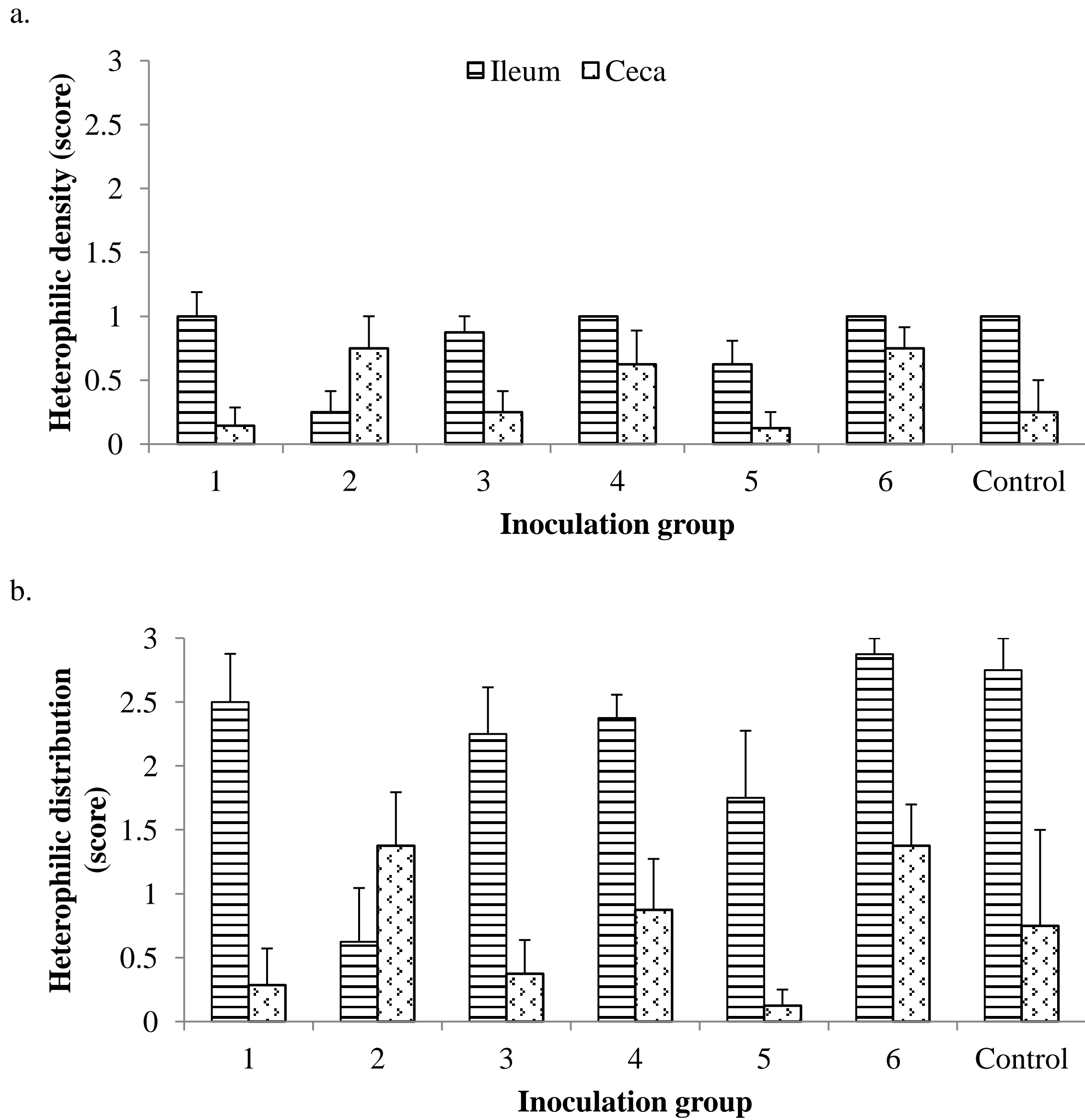


Figure 4.17 Heterophilic density and distribution in the ileum and ceca by flock
Results are means with standard error bars, N = 8 per treatment group and N = 4 for controls
a. Differences were seen among flocks for density in the ileum and ceca ($P < 0.0308$, $P < 0.0896$)
b. Differences were seen among flocks for distribution in the ileum and ceca ($P < 0.0124$, $P < 0.0649$)

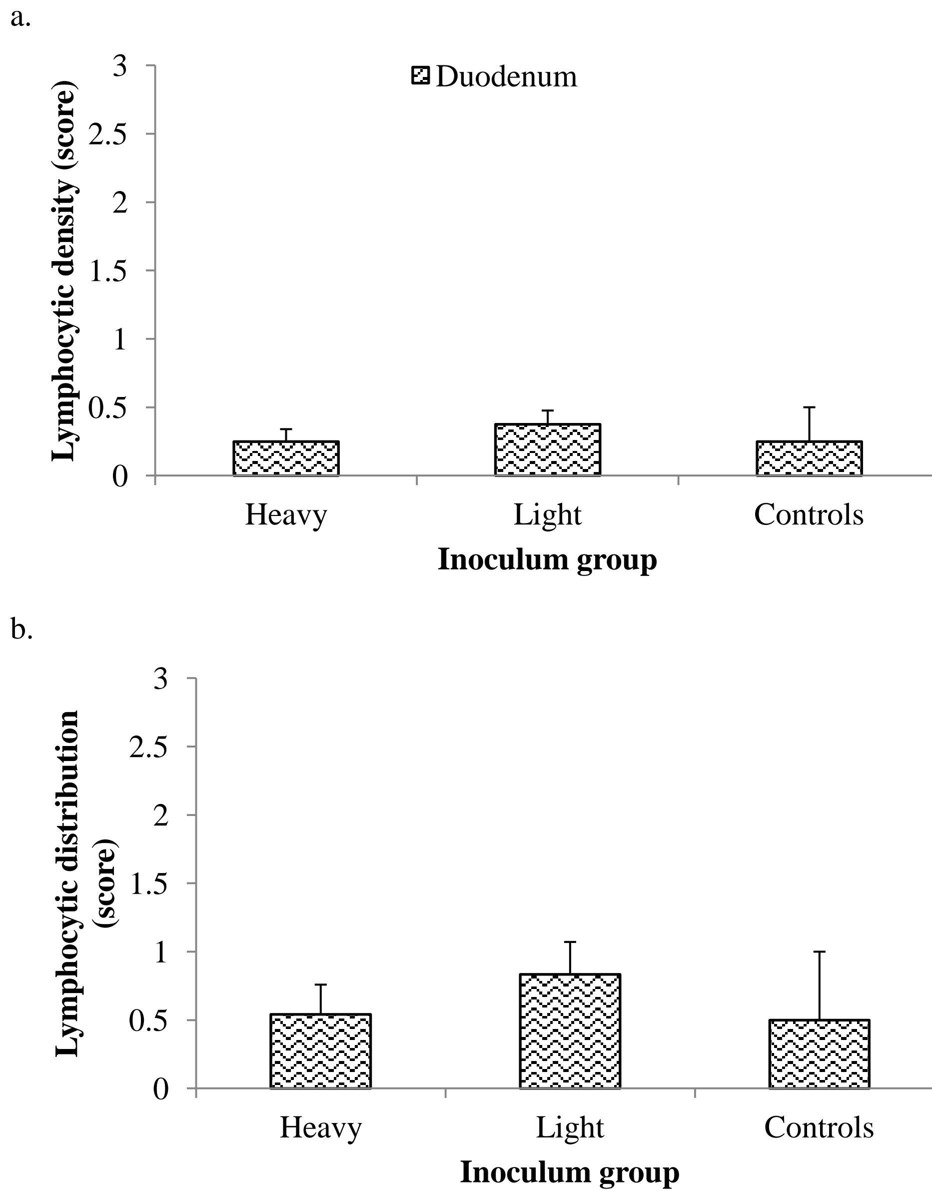


Figure 4.18 Lymphocytic density and distribution in the duodenum by weight group
Results are means with standard error bars, N = 24 per treatment group and N = 4 for controls
a. Light weight poults had significantly higher scores than heavy weight. Heavy weight poults had significantly higher scores than controls ($P < 0.0470$)
b. Light weight poults had significantly higher scores than heavy weight. Heavy weight poults had significantly higher scores than controls ($P < 0.0083$)

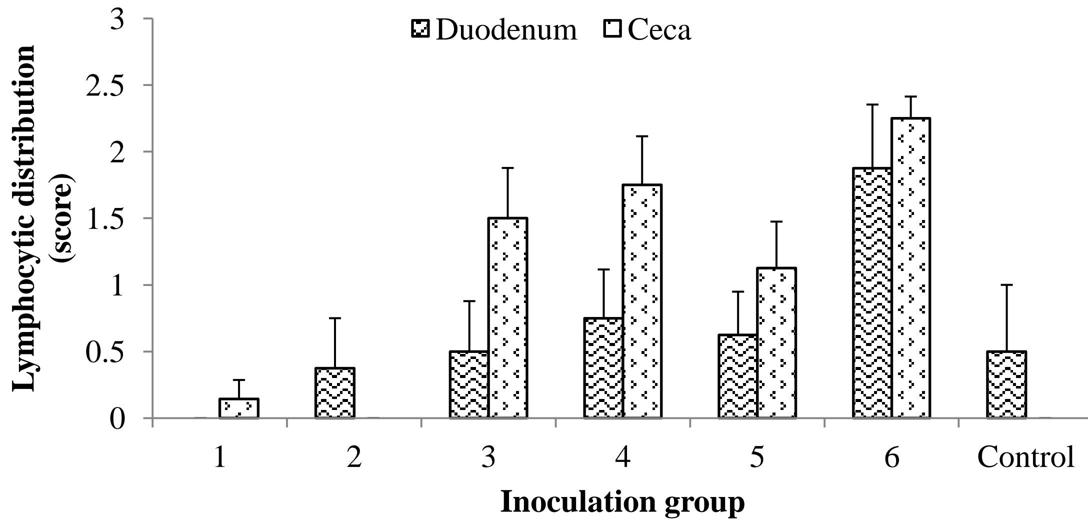


Figure 4.19 Lymphocytic distribution in the duodenum and ceca by flock
Results are means with standard error bars, N = 8 per treatment group and N = 4 for controls
Differences were seen among flocks in the duodenum and ceca ($P < 0.0587$, $P < 0.0123$)

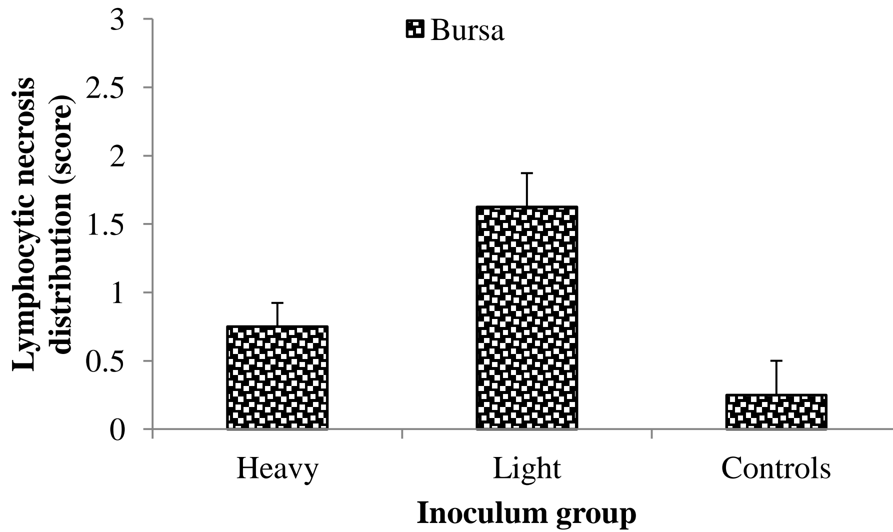


Figure 4.20 Distribution of lymphocytic necrosis in the bursa by weight group
Results are means with standard error bars, N = 24 per treatment group and N = 4 for controls
Light weight poults had significantly higher scores than heavy weight. Heavy and light weight poults were not different from controls ($P < 0.0040$)

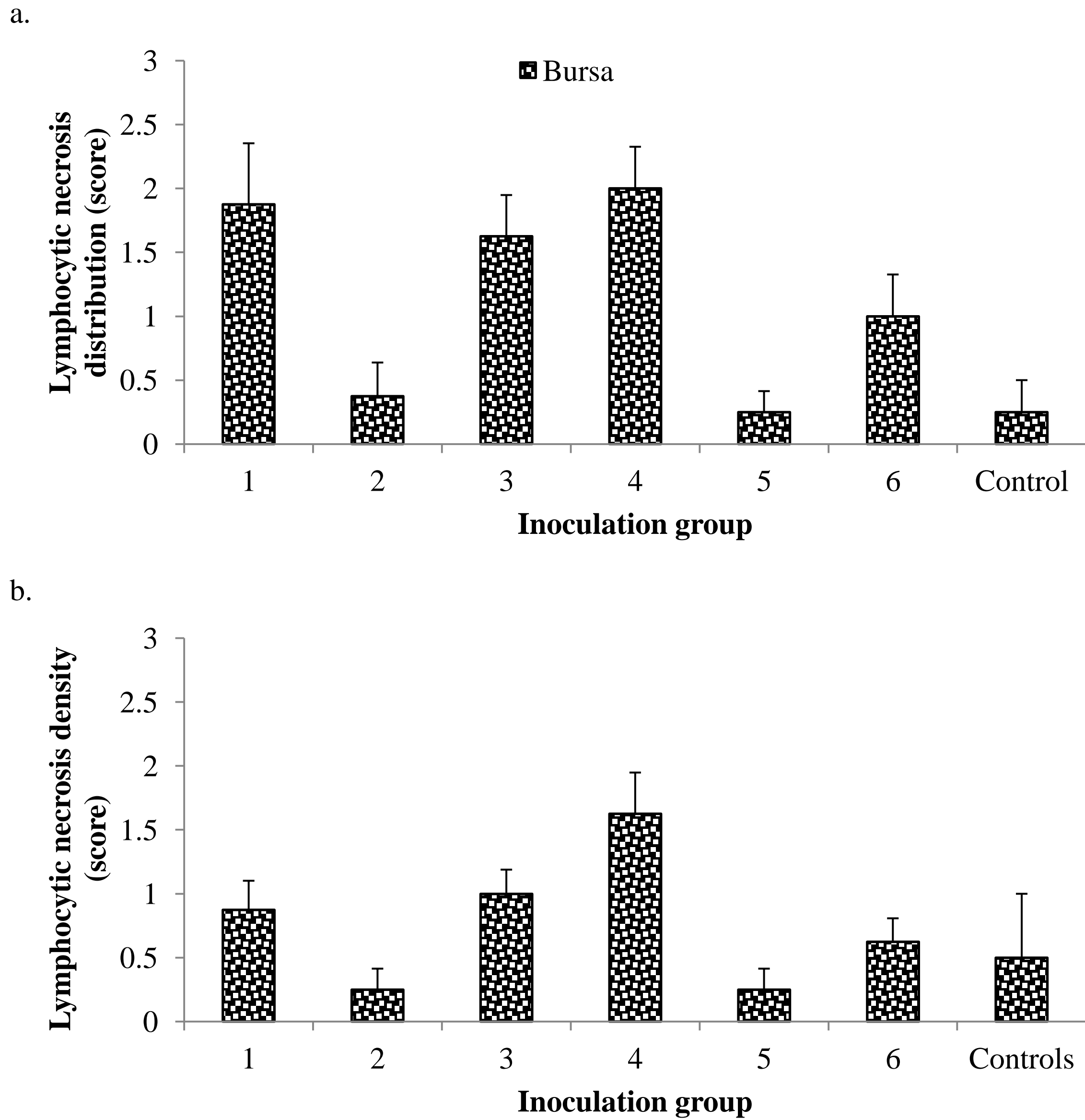


Figure 4.21 Distribution and density of lymphocytic necrosis in the bursa by flock

Results are means with standard error bars, N = 8 per treatment group and N = 4 for controls

a. Differences were seen among flocks for distribution ($P < 0.0009$)

b. Differences were seen among flocks for density ($P < 0.0025$)

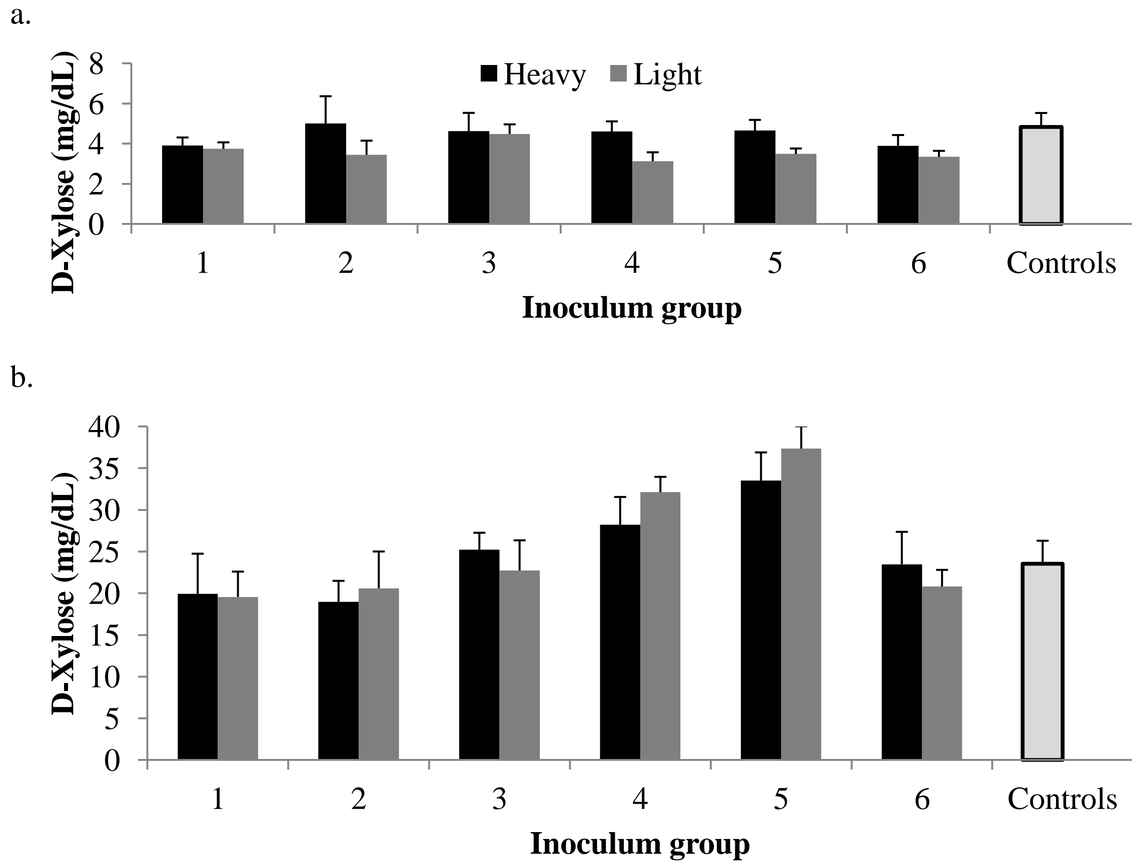


Figure 4.22 Xylose concentrations from 15 day old poults
Results are means with standard error bars, N = 8 to 12
a. Xylose levels prior to gavaging
b. Xylose levels 60 minutes after gavaging

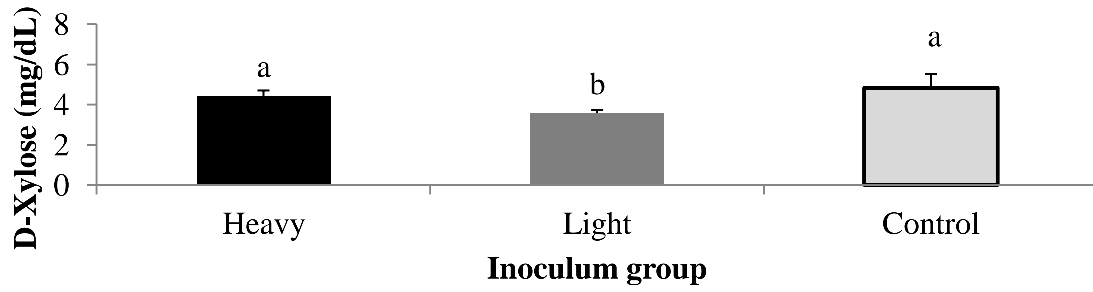


Figure 4.23 Xylose concentrations prior to gavaging by weight group
Results are means with standard error bars, N = 11 to 61
a,b,c: Means with different letters are significantly different ($P < 0.05$)
Light group had less xylose in their plasma prior to gavaging than heavy or control groups ($P < 0.0088$)

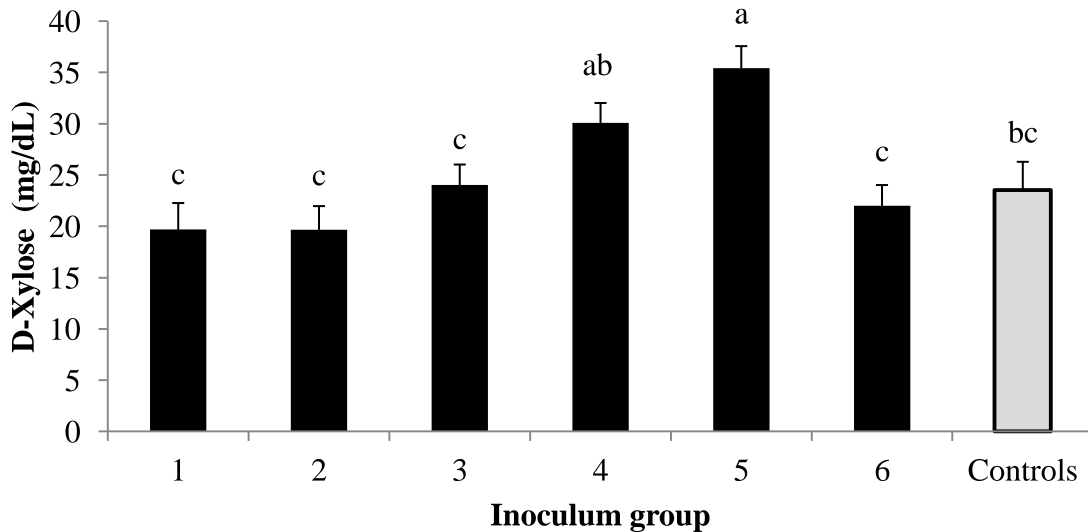


Figure 4.24 Xylose concentrations 60 minutes after gavaging by flock
Results are means with standard error bars, N = 11 to 23
a,b,c: Means with different letters are significantly different ($P < 0.05$)
Xylose concentrations differed among flock groups ($P < 0.0001$)

Chapter 5

SUMMARY

The field trial and inoculum trial were run as complements to each other. The field trial looked at the commercially raised poult to gain a better understanding of what the poults were experiencing. The inoculation trial looked at whether the traits were reproducible and if there were differences in feed consumption. Body weights for the 2 week old poults were on average higher for the field trial than the inoculation trial by 85 grams. A higher percentage of pools (pens) were positive for astrovirus and rotavirus in the inoculation trial than had been in the field trial pools. These differences may be explained by low levels in the field trial samples. *Campylobacter* and *Salmonella* were not found in gut contents from the inoculated poults even though they had previously been found in at least one pool in all of the field trial flocks except Flock 6. This is easier to explain in that the poults may not have been currently shedding the bacteria even if they were infected with it. Heterophilic infiltrates in the inoculation trial were seen in higher amounts in the control poults than both weight groups compared to more heterophilic infiltrates being seen in the light weight poults in Flock 3 in the field trial. Lymphocytes were found in higher amounts in the light weight poults in the inoculation trial and in more heavy weight poults in the field trial. The field trial scores are an average of three sampling times in contrast to the one sampling time for the inoculation trial. The inoculation trial results are giving a snap shot of what is going on in the poults where as the field trial results are giving us a more general look at the immune response during brooding. The bursal results for lymphocellular necrosis were similar between both studies and showed an increase of necrosis in the light weight poults. For xylose absorption, weight groups were not different at T60 for the inoculation trial. In the field trial some flocks showed higher absorption in the heavy weight poults and some in the light weight poults. From our study it does not appear that nutrient absorption is a major factor in LTS at least not at 2 and 3 weeks of age.

REFERENCES

Ackerman, G. A. and R. A. Knouff. 1959. Lymphocytopoiesis in the bursa of Fabricius. Am. J. Anat. 104:163-205.

Arakawa, H., K. Kuma, M. Yasuda, S. Ekino, A. Shimizu, and H. Yamagishi. 2002. Effect of Environmental Antigens on the Ig Diversification and the Selection of Productive V-J Joints in the Bursa. The Journal of Immunology 169:818-828.

Atkinson, J. C. 1954. Studies of Ascites in the Turkey Poult. Ph.D. The Pennsylvania State University.

Awad, W. A., K. Ghareeb, S. Abdel-Raheem, and J. Böhm. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Science 88:49-56.

Barnes, H. J., J. S. Guy, and J. P. Vaillancourt. 2000. Poult enteritis complex. Revue scientifique 19:565.

Bar-Shira, E., D. Sklan, and A. Friedman. 2002. Establishment of immune competence in the avian GALT during the immediate post-hatch period. Developmental comparative immunology 27:147-157.

Butterworth, C. E., E. Perez-Santiago, J. M. de Jesus, and R. Santini. 1959. Studies on the Oral and Parenteral Administration of D(+)xylose. N. Engl. J. Med. 261:157-164.

Davison, T. F., B. Kaspers, and K. A. Schat. 2008. Avian immunology. Academic Press, Amsterdam, Boston.

Doerfler, R. E., L. D. Cain, F. W. Edens, C. R. Parkhurst, M. A. Qureshi, and G. B. Havenstein. 2000. D-xylose absorption as a measurement of malabsorption in poult enteritis and mortality syndrome. Poultry Science 79:656-660.

Eberts, T., R. Sample, M. Glick, and G. Ellis. 1979. A simplified, colorimetric micromethod for xylose in serum or urine, with phloroglucinol. Clin. Chem. 25:1440-1443.

Fowweather, F. S. 1953. The detection of galactose in urine. Biochem. J. 55:718-720.

Francisco, A. 1967. D-Xylose transport in the chicken small intestine. Comp. Biochem. Physiol. 20:461-470.

Gast, R. K. and C. W. Beard. 1990. Production of Salmonella enteritidis-Contaminated Eggs by Experimentally Infected Hens. Avian Dis. 34:438-446.

Glick, B. 1956. Normal Growth of the Bursa of Fabricius in Chickens. Poultry Science 35:843-851.

Goodwin, M. A., M. A. Dekich, K. S. Latimer, and O. J. Fletcher. 1985. Quantitation of Intestinal D-Xylose Absorption in Normal Broilers and in Broilers with Pale-Bird Syndrome. Avian Dis. 29:630-639.

Goodwin, M. A., B. N. Nersessian, J. Brown, and O. J. Fletcher. 1985. Gastrointestinal Transit Times in Normal and Reovirus-Inoculated Turkeys. Avian Dis. 29:920-928.

Goodwin, M. A., K. S. Latimer, and O. J. Fletcher. 1984. Quantitation of Intestinal D-Xylose Absorption in Normal Turkeys. Poultry Science 63:1742-1747.

Harmon, B. 1998. Avian heterophils in inflammation and disease resistance. Poultry Science 77:972-977.

Hayhow, C. S. and Y. M. Saif. 1993. Experimental Infection of Specific-Pathogen-Free Turkey Poults with Single and Combined Enterovirus and Group A Rotavirus. Avian Dis. 37:546-557.

Hodges, R. D. 1974. The histology of the fowl. Academic Press, London, New York.

Jindal, N., D. P. Patnayak, Y. Chander, A. F. Ziegler, and S. M. Goyal. 2011. Comparison of capsid gene sequences of turkey astrovirus-2 from poult-enteritis-syndrome-affected and apparently healthy turkeys. Arch. Virol. 156:969-977.

Jindal, N., D. P. Patnayak, Y. Chander, A. F. Ziegler, and S. M. Goyal. 2010. Detection and molecular characterization of enteric viruses from poult enteritis syndrome in turkeys. Poult. Sci. 89:217-226.

Jindal, N., D. P. Patnayak, A. F. Ziegler, A. Lago, and S. M. Goyal. 2009a. Duration of growth depression and pathogen shedding in experimentally reproduced poult enteritis syndrome. Avian Dis. 53:517-522.

Jindal, N., D. P. Patnayak, A. F. Ziegler, A. Lago, and S. M. Goyal. 2009b. A retrospective study on poult enteritis syndrome in Minnesota. Avian Dis. 53:268-275.

Jindal, N., D. P. Patnayak, A. F. Ziegler, A. Lago, and S. M. Goyal. 2009c. Experimental reproduction of poult enteritis syndrome: clinical findings, growth response, and microbiology. Poult. Sci. 88:949-958.

Joklik, W. K. 1981. Structure and Function of the Reovirus Genome. Micro. Rev. 45:483-501.

Klasing, K. C. 2007. Nutrition and the immune system. Br. Poult. Sci. 48:525-537.

Levitt, D. G., A. A. Hakim, and N. Lifson. 1969. Evaluation of components of transport of sugars by dog jejunum in vivo. American Journal of Physiology 217:777-783.

Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. Blakiston Division, McGraw-Hill, New York.

Malewitz, T. D. and M. L. Calhoun. 1958. The gross and microscopic anatomy of the digestive tract, spleen, kidney, lungs and heart of the turkey. Poult. Sci. 37:388-398.

Masteller, E. L. and C. B. Thompson. 1994. B Cell Development in the Chicken. Poultry Science 73:998-1011.

McNulty, M.S., G.M. Allan, D. Todd, J.B. McFerran, E.R. McKillop, D.S. Collins, R.M. McCracken. 1980. Isolation of rotavirus from turkeys and chickens: demonstration of distinct serotypes and RNA electropherotypes. Avian Pathol. 9:363-375.

Metz, A. L. 1986. The Interaction of Dietary Vitamin a and Vitamin D in the Turkey Poult (Hypervitaminosis D, Rickets). Ph.D. University of Minnesota.

Norton, S. and H. R. Wolfe. 1949. The growth of the spleen in the chicken. Anat. Rec. 105:83-93.

Oláh, I. and B. Glick. 1978. The Number and Size of the Follicular Epithelium (FE) and Follicles in the Bursa of Fabricius. Poultry Science 57:1445-1450.

Oláh, I. and B. Glick. 1982. Splenic white pulp and associated vascular channels in chicken spleen. Am. J. Anat. 165:445-480.

Paramithiotis, E. and M. J. H. Ratcliffe. 1994. Survivors of Bursal B Cell Production and Emigration. Poultry Science 73:991-997.

Rautenschlein, S. and J. M. Sharma. 2000. Developmental Comparative Immunology 24:237-246.

Reynolds, D. L. and Y. M. Saif. 1986. Astrovirus: A Cause of an Enteric Disease in Turkey Poults. Avian Dis. 30:728-735.

Roe, J. H. and E. W. Rice. 1948. A photometric method for the determination of free pentoses in animal tissues. Journal of Biological Chemistry 173:507-512.

Saif, Y. M., A. M. Fadly, and J. R. Glisson. 2008. Diseases of poultry. 12th ed. Blackwell Pub., Ames, Iowa.

Saif, Y. M. 2003. Diseases of poultry. 11th ed. Iowa State Press, Ames.

Sloss, M. W., R. L. Kemp, and A. M. Zajac. 1994. Veterinary clinical parasitology. Iowa State University Press, Ames.

Spackman, E., J. M. Day, M. J. Pantin-Jackwood. 2010. Avian Diseases 54:16-21.

Sturkie, P. D. and C. A. Benzo. 1986. Avian physiology. 4th ed. Springer-Verlag, New York.

Torres-Rodriguez, A., A. M. Donoghue, D. J. Donoghue, J. T. Barton, G. Tellez, and B. M. Hargis. 2007. Performance and Condemnation Rate Analysis of Commercial Turkey Flocks Treated with a Lactobacillus spp.-Based Probiotic. Poultry Science 86:444-446.

Trinder, P. 1975. Micro-determination of xylose in plasma. Analyst 100:12-15.

Wagh, P. V. and P. E. Waibel. 1966. Metabolizability and Nutritional Implications of L-Arabinose and D-Xylose for Chicks. The Journal of Nutrition 90:207-211.

Whittow, G. C. 2000. Sturkie's avian physiology. 5th ed. Academic, San Diego.

Yegani, M. and D. R. Korver. 2008. Factors Affecting Intestinal Health in Poultry. Poultry Science 87:2052-2063.

Yersin, A. G. 1990. Observations on physiological alterations in the turkey poult during Bordetella avium infection. Ph.D. ed. North Carolina State University, North Carolina.

APPENDIX

Table 1 Presence of coccidia in duodenum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	25.0%	25.0%
	4	0	0	25.0%	25.0%
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
2	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	50.0%	28.9%	0	0
3	1	0	0	0	0
	2	0	0	0	0
	3	50.0%	28.9%	50.0%	28.9%
	4	75.0%	25.0%	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	50.0%	28.9%	25.0%	25.0%

See Table 3.21 for significant differences
N = 4 samples
Flocks 1, 2, 5, 6 and 7 were removed from the statistical data

Table 2 Presence of coccidia in jejunum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
2	1	0	0	0	0
	2	0	0	0	0
	3	25.0%	16.4%	0	0
	4	25.0%	16.4%	50.0%	18.9%
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	12.5%	12.5%	25.0%	16.4%
3	1	0	0	0	0
	2	0	0	0	0
	3	1	0	1	0
	4	87.5%	12.5%	87.5%	12.5%
	5	0	0	0	0
	6	0	0	0	0
	7	12.5%	12.5%	0	0
	8	37.5%	18.3%	62.5%	18.3%

See Table 3.21 for significant differences
N = 8 samples
Flocks 1, 2, 5 and 6 and week one were removed from the statistical data

Table 3 Presence of coccidia in ileum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	25.0%	25.0%
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
2	1	0	0	0	0
	2	0	0	0	0
	3	50.0%	28.9%	50.0%	28.9%
	4	0	0	50.0%	28.9%
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
3	1	0	0	0	0
	2	0	0	0	0
	3	1	0	1	0
	4	1	0	1	0
	5	0	0	0	0
	6	0	0	0	0
	7	50.0%	28.9%	0	0
	8	75.0%	25.0%	50.0%	28.9%

See Table 3.21 for significant differences
N = 4 samples
Flocks 1, 2, 5 and 6 were removed from the statistical data

Table 4 Presence of coccidia in ceca from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	.[1]	.	.	.
	2	.	.	.	.
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	25.0%	25.0%
	8	0	0	0	0
2	1	.	.	.	.
	2	.	.	.	.
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
3	1	0	0	0	0
	2	0	0	0	0
	3	75.0%	25.0%	75.0%	25.0%
	4	50.0%	28.9%	50.0%	28.9%
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	25.0%	25.0%
	8	25.0%	25.0%	0	0

See Table 3.21 for significant differences
N = 4 samples
Flocks 1, 2, 5 and 6 and week two were removed from the statistical data
[1] Ceca samples were not collected from Flocks 1 and 2 at one and two weeks of age

Table 5 Heterophilic density in the duodenum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0.25	0.25
2	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
3	1	0	0	0.25	0.25
	2	0	0	0.25	0.25
	3	0	0	0.5	0.289
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0

See Table 3.23 for significant differences
N = 4 samples
Flocks 4, 5, 6, 7 and week 2 were removed from the statistical data

Table 6 Heterophilic density in the jejunum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0.375	0.183
	5	0	0	0	0
	6	0.125	0.125	0	0
	7	0	0	0	0
	8	0	0	0	0
2	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0.125	0.125
	4	0	0	0	0
	5	0.25	0.164	0.125	0.125
	6	0.125	0.125	0	0
	7	0	0	0	0
	8	0	0	0	0
3	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0.125	0.125	0.25	0.164
	7	0	0	0	0
	8	0	0	0	0

See Table 3.23 for significant differences
N = 8 samples
Flocks 1, 2, 7 and 8 were removed from the statistical data

Table 7 Heterophilic density in the ileum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0.25	0.25	0	0
	3	0	0	0	0
	4	0	0	0.25	0.25
	5	0	0	0	0
	6	0.5	0.289	0.25	0.25
	7	0	0	0	0
	8	0	0	0.25	0.25
2	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0.5	0.289	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
3	1	0	0	0	0
	2	0.25	0.25	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0.25	0.25	0.25	0.25
	7	0	0	0	0
	8	0	0	0	0

See Table 3.23 for significant differences
N = 4 samples
Flocks 1, 3 and 7 were removed from the statistical data

Table 8 Heterophilic density in the ceca from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	.	.	.	.
	2	.	.	.	.
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0.25	0.25	0	0
2	1	.	.	.	.
	2	.	.	.	.
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0.5	0.289
3	1	0.5	0.289	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0.25	0.25	0.25	0.25
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0

See Table 3.23 for significant differences
N = 4 samples
Flocks 2, 3, 4, 6 and 7 were removed from the statistical data and Flocks 1 and 2 only had samples collected at three weeks of age

Table 9 Heterophilic distribution in the duodenum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0.25	0.25	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0.25	0.25
	5	0.25	0.25	0	0
	6	0	0	0.25	0.25
	7	0	0	0	0
	8	0.75	0.25	0.5	0.289
2	1	0	0	0	0
	2	0.5	0.289	0.5	0.289
	3	0	0	0	0
	4	0	0	0.25	0.25
	5	0.75	0.25	1	0
	6	0	0	0	0
	7	0	0	0	0
	8	0.25	0.25	0.25	0.25
3	1	0.25	0.25	0.25	0.25
	2	0.5	0.289	0.5	0.289
	3	0	0	0.5	0.289
	4	0	0	0.25	0.25
	5	0.5	0.289	0.25	0.25
	6	0	0	0.5	0.289
	7	0.25	0.25	0	0
	8	0	0	0	0

See Table 3.24 for significant differences
N = 4 samples

Table 10 Heterophilic distribution in the jejunum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0.125	0.125	0	0
	2	0.125	0.125	0.25	0.164
	3	0.375	0.183	0.75	0.164
	4	0.25	0.164	0.875	0.125
	5	0.25	0.164	0	0
	6	0.5	0.189	0.375	0.183
	7	0.375	0.183	0.5	0.189
	8	0.375	0.183	0.5	0.189
2	1	0.125	0.125	0.375	0.183
	2	0.5	0.189	0.25	0.164
	3	0.125	0.125	0.625	0.183
	4	0.625	0.183	0.375	0.183
	5	0.875	0.125	0.75	0.164
	6	0.625	0.183	0.625	0.183
	7	0.25	0.164	0.375	0.183
	8	0	0	0.75	0.164
3	1	0.25	0.164	0.25	0.164
	2	0	0	0.375	0.183
	3	0.25	0.164	0	0
	4	0.375	0.183	0.5	0.189
	5	0.625	0.183	0.5	0.189
	6	0.875	0.125	1	0
	7	0.5	0.189	0.375	0.183
	8	0.125	0.125	0	0

See Table 3.24 for significant differences
N = 8 samples

Table 11 Heterophilic distribution in the ileum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0.25	0.25	0.25	0.25
	2	0.25	0.25	0	0
	3	0.75	0.25	0.5	0.289
	4	0.5	0.289	0.75	0.25
	5	0.5	0.289	0.25	0.25
	6	1	0	0.75	0.25
	7	0.25	0.25	0.25	0.25
	8	0	0	1	0
2	1	0.25	0.25	0	0
	2	0.5	0.289	0.25	0.25
	3	0.75	0.25	0.5	0.289
	4	0.75	0.25	0.75	0.25
	5	1	0	0.75	0.25
	6	0.5	0.289	1	0
	7	0.5	0.289	0.5	0.289
	8	0.25	0.25	0.75	0.25
3	1	0	0	0	0
	2	0.75	0.25	0.5	0.289
	3	0.25	0.25	0	0
	4	0	0	0.5	0.289
	5	0.25	0.25	0.75	0.25
	6	1	0	1	0
	7	0.5	0.289	0.5	0.289
	8	0	0	0	0

See Table 3.24 for significant differences
N = 4 samples

Table 12 Heterophilic distribution in the ceca from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	.	.	.	.
	2	.	.	.	.
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0.25	0.25	0.25	0.25
	8	0.5	0.289	0	0
2	1	.	.	.	.
	2	.	.	.	.
	3	0.25	0.25	0.25	0.25
	4	0.5	0.289	0	0
	5	0.25	0.25	1	0
	6	0.25	0.25	0	0
	7	0	0	0	0
	8	0	0	1	0
3	1	0	0	0	0
	2	0.5	0.289	0	0
	3	0	0	0	0
	4	0.25	0.25	0.25	0.25
	5	0.75	0.25	0.25	0.25
	6	0.5	0.289	0.75	0.25
	7	0.5	0.289	0.75	0.25
	8	0	0	0.5	0.289

See Table 3.24 for significant differences
N = 4 samples
Flock 1 was removed from the statistical data and Flocks 1 and 2 only had samples collected at three weeks of age

Table 13 Lymphocytic density in the duodenum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0.25	0.25
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0.25	0.25	0	0
	8	0.25	0.25	0	0
2	1	0.25	0.25	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0.25	0.25	0	0
	5	0.5	0.289	0	0
	6	0.25	0.25	0	0
	7	0	0	0	0
	8	0	0	0.75	0.25
3	1	0	0	0.25	0.25
	2	0.25	0.25	0.5	0.289
	3	0.25	0.25	0.5	0.289
	4	0	0	0	0
	5	0	0	0.25	0.25
	6	0	0	0.25	0.25
	7	0	0	0	0
	8	0	0	0.5	0.289

See Table 3.25 for significant differences
N = 4 samples

Table 14 Lymphocytic density in the jejunum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0.125	0.125	0	0
	8	0	0	0	0
2	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0.25	0.164	0.375	0.183
	8	0	0	0	0
3	1	0.25	0.164	0.25	0.164
	2	0	0	0.25	0.164
	3	0	0	0.125	0.125
	4	0	0	0	0
	5	0	0	0.125	0.125
	6	0.25	0.164	0.125	0.125
	7	0.25	0.164	0.125	0.125
	8	0.25	0.164	0	0

See Table 3.25 for significant differences
N = 8 samples
Flock 4 was removed from the statistical data

Table 15 Lymphocytic density in the ileum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0.5	0.289	0	0
	2	0.5	0.289	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
2	1	0.25	0.25	0	0
	2	0	0	0.25	0.25
	3	0	0	0	0
	4	0	0	0	0
	5	0.25	0.25	0	0
	6	0	0	0	0
	7	0	0	0.25	0.25
	8	0.25	0.25	0.25	0.25
3	1	0	0	0.25	0.25
	2	0.25	0.25	0	0
	3	0.5	0.289	0	0
	4	0.25	0.25	0	0
	5	0	0	0.25	0.25
	6	0.5	0.289	0.25	0.25
	7	0.25	0.25	0	0
	8	0	0	0.25	0.25

See Table 3.25 for significant differences
N = 4 samples

Table 16 Lymphocytic density in the ceca from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	.	.	.	.
	2	.	.	.	.
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0.5	0.289	0	0
2	1	.	.	.	.
	2	.	.	.	.
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0.25	0.25	0	0
	8	0.25	0.25	0	0
3	1	0.25	0.25	0.5	0.289
	2	0.5	0.289	0	0
	3	0.5	0.289	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0.5	0.289	0	0
	7	0.5	0.289	0.5	0.289
	8	0.75	0.25	0.25	0.25

See Table 3.25 for significant differences
N = 4 samples
Flocks 4 and 5 were removed from the statistical data and Flocks 1 and 2 only had samples collected at three weeks of age

Table 17 Lymphocytic distribution in the duodenum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0.25	0.25	0	0
	3	0	0	0	0
	4	0	0	0.25	0.25
	5	0	0	0	0
	6	0.25	0.25	0	0
	7	0.75	0.25	0.5	0.289
	8	0.25	0.25	0	0
2	1	0.5	0.289	0.5	0.289
	2	0.5	0.289	0.5	0.289
	3	0.5	0.289	0.5	0.289
	4	0.25	0.25	0.5	0.289
	5	1	0	1	0
	6	0.75	0.25	0.75	0.25
	7	1	0	0.75	0.25
	8	0.75	0.25	0.75	0.25
3	1	0	0	0.75	0.25
	2	0.75	0.25	0.75	0.25
	3	0.5	0.289	0.75	0.25
	4	1	0	1	0
	5	0.5	0.289	0.75	0.25
	6	1	0	1	0
	7	1	0	1	0
	8	0.5	0.289	0.75	0.25

See Table 3.26 for significant differences
N = 4 samples

Table 18 Lymphocytic distribution in the jejunum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0.25	0.164	0	0
	7	0.375	0.183	0.625	0.183
	8	0.625	0.183	0.75	0.164
2	1	0.75	0.164	0.5	0.189
	2	0.125	0.125	0.125	0.125
	3	0.25	0.164	0	0
	4	0.375	0.183	0.125	0.125
	5	1	0	0.5	0.189
	6	1	0	0.5	0.189
	7	1	0	1	0
	8	1	0	1	0
3	1	0.75	0.164	0.75	0.164
	2	0.75	0.164	0.625	0.183
	3	0.875	0.125	0.875	0.125
	4	1	0	1	0
	5	0.875	0.125	1	0
	6	0.875	0.125	0.875	0.125
	7	1	0	1	0
	8	0.75	0.164	0.875	0.125

See Table 3.26 for significant differences
N = 8 samples

Table 19 Lymphocytic distribution in the ileum from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0.25	0.25	0	0
	2	0	0	0	0
	3	0.25	0.25	0.25	0.25
	4	0.5	0.289	0.5	0.289
	5	0	0	0	0
	6	0	0	0	0
	7	0.5	0.289	0.25	0.25
	8	1	0	0.5	0.289
2	1	0	0	0	0
	2	0.5	0.289	0.75	0.25
	3	1	0	0.5	0.289
	4	0.25	0.25	0.25	0.25
	5	0.25	0.25	0.75	0.25
	6	1	0	1	0
	7	1	0	1	0
	8	1	0	1	0
3	1	1	0	0.75	0.25
	2	0.75	0.25	0	0
	3	1	0	1	0
	4	1	0	1	0
	5	0.5	0.289	0.5	0.289
	6	1	0	0.75	0.25
	7	1	0	1	0
	8	1	0	0.75	0.25

See Table 3.26 for significant differences
N = 4 samples

Table 20 Heterophilic density in the bursa from the field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0.25	0.25	0	0
	4	0	0	0	0
	5	0.25	0.25	0.25	0.25
	6	0.25	0.25	0	0
	7	0	0	0	0
	8	0	0	0	0
2	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0.25	0.25	0.5	0.289
	7	0	0	0	0
	8	0	0	0	0
3	1	0.25	0.25	0.25	0.25
	2	0.25	0.25	0	0
	3	0	0	0	0
	4	0.25	0.25	0	0
	5	0.25	0.25	0.25	0.25
	6	0.5	0.289	0.25	0.25
	7	0	0	0	0
	8	0.25	0.25	0	0

See Table 3.27 for significant differences
N = 4 samples
Flock 7 was removed from the statistical data

Table 21 Heterophilic distribution in the bursa from the field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0.75	0.25	0.25	0.25
	4	0	0	0	0
	5	0.5	0.289	0.5	0.289
	6	0.75	0.25	0	0
	7	0	0	0	0
	8	0	0	0	0
2	1	0	0	0	0
	2	0	0	0	0
	3	0.25	0.25	0.25	0.25
	4	0.25	0.25	0	0
	5	0.25	0.25	0	0
	6	0.5	0.289	0.75	0.25
	7	0.25	0.25	0.25	0.25
	8	0.5	0.289	0	0
3	1	0	0	0.25	0.25
	2	0	0	0.5	0.289
	3	0.25	0.25	0	0
	4	0.25	0.25	0.5	0.289
	5	0	0	0.75	0.25
	6	1	0	0.5	0.289
	7	0.5	0.289	0.25	0.25
	8	0	0	0.25	0.25

See Table 3.27 for significant differences
N = 4 samples
Flock 8 was removed from the statistical data
Flocks 1 and 8 were removed from the statistical data

Table 22 Lymphocellular depletion, density in the bursa from the field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0.5	0.289	0.5	0.289
	5	0.75	0.25	0.25	0.25
	6	0.5	0.289	0	0
	7	0	0	0.75	0.25
	8	0	0	0	0
2	1	0	0	0	0
	2	0	0	0	0
	3	0.5	0.289	1	0
	4	0.5	0.289	0.75	0.25
	5	0	0	0	0
	6	0.25	0.25	0.75	0.25
	7	0	0	0.5	0.289
	8	0	0	0	0
3	1	0	0	0	0
	2	0	0	0.5	0.289
	3	0.25	0.25	0.5	0.289
	4	0.25	0.25	0.25	0.25
	5	0.25	0.25	0	0
	6	0.25	0.25	0.25	0.25
	7	0	0	0	0
	8	0	0	0	0

See Table 3.27 for significant differences
N = 4 samples
Flocks 1 and 8 were removed from the statistical data

Table 23 Lymphocellular depletion, distribution in the bursa from the field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0.75	0.25
	2	0	0	0.25	0.25
	3	0.25	0.25	0.25	0.25
	4	0.25	0.25	1	0
	5	0.75	0.25	0.5	0.289
	6	0.75	0.25	0.75	0.25
	7	0.75	0.25	0.75	0.25
	8	0	0	0	0
2	1	0.75	0.25	0	0
	2	0	0	0	0
	3	0.5	0.289	1	0
	4	0.5	0.289	0.5	0.289
	5	0.25	0.25	0	0
	6	0.25	0.25	0.75	0.25
	7	0	0	1	0
	8	0	0	0	0
3	1	0.25	0.25	0.25	0.25
	2	0	0	0	0
	3	0.25	0.25	0.5	0.289
	4	0	0	0.25	0.25
	5	0	0	0	0
	6	0.25	0.25	0.25	0.25
	7	0	0	0	0
	8	0	0	0	0

See Table 3.27 for significant differences
N = 4 samples
Flock 8 was removed from the statistical data

Table 24 Atrophy of the bursa from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0.25	0.25	0	0
	3	0.75	0.25	0	0
	4	0.75	0.25	1	0
	5	0.25	0.25	0.25	0.25
	6	1	0	0.25	0.25
	7	0	0	0	0
	8	0	0	0.5	0.289
2	1	0.25	0.25	0	0
	2	0.25	0.25	0	0
	3	0	0	0	0
	4	0.75	0.25	0.25	0.25
	5	0	0	0.75	0.25
	6	0.75	0.25	0.25	0.25
	7	0	0	0	0
	8	0	0	0	0
3	1	0	0	0.25	0.25
	2	0	0	0.5	0.289
	3	0.25	0.25	0	0
	4	0	0	0.5	0.289
	5	0	0	0.25	0.25
	6	0.5	0.289	0.75	0.25
	7	0.25	0.25	0.75	0.25
	8	0.5	0.289	0.75	0.25

See Table 3.27 for significant differences
N = 4 samples

Table 25 Lymphocellular depletion of the spleen from the field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0.25	0.25
	8	0	0	0	0
2	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0.25	0.25	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0.5	0.289	0	0
	8	0	0	0	0
3	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0.5	0.289	0	0
	8	0	0	0	0

See Table 3.28 for significant differences
N = 4 samples
Flocks 1, 2, 3, 5, 6 and 8 were removed from the statistical data

Table 26 Germinal center hyperplasia in the spleen from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0.25	0.25
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0	0	0	0
2	1	0.25	0.25	0.25	0.25
	2	0	0	0.25	0.25
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0.25	0.25	0	0
	7	0	0	0	0
	8	0	0	0	0
3	1	0	0	0	0
	2	0	0	0.25	0.25
	3	0	0	0	0
	4	0	0	0	0
	5	0.5	0.289	1	0
	6	1	0	0.5	0.289
	7	0	0	0	0
	8	0	0	0	0

See Table 3.28 for significant differences
N = 4 samples
Flocks 3, 4, 7 and 8 were removed from the statistical data

Table 27 Ellipsoid necrosis in the spleen from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0.5	0.289	0.25	0.25
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0	0	0	0
	8	0.25	0.25	0.25	0.25
2	1	0	0	0	0
	2	0	0	0	0
	3	0.25	0.25	0.5	0.289
	4	0	0	0	0
	5	0	0	0.25	0.25
	6	0	0	0	0
	7	0	0	0	0
	8	1	0	0.25	0.25
3	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
	7	0.5	0.289	0	0
	8	1	0	0.75	0.25

See Table 3.28 for significant differences
N = 4 samples
Flocks 1, 2, 4 and 6 were removed from the statistical data

Table 28 Abnormal red pulp in the spleen from field study

Age (weeks)	Flock	Heavy Weight Group		Light Weight Group	
		Average	SE	Average	SE
1	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0.25	0.25
	4	0	0	0	0
	5	0.25	0.25	0	0
	6	0.25	0.25	0	0
	7	0	0	0	0
	8	0.25	0.25	0	0
2	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0.25	0.25
	4	0	0	0.25	0.25
	5	0	0	0	0
	6	0	0	0	0
	7	0.5	0.289	0	0
	8	0	0	0	0
3	1	0	0	0	0
	2	0	0	0	0
	3	0	0	0	0
	4	0	0	0	0
	5	1	0	1	0
	6	0	0	0	0
	7	0.25	0.25	0.25	0.25
	8	0	0	0	0

See Table 3.28 for significant differences
N = 4 samples
Flocks 1 and 2 were removed from the statistical data

Table 29 Individual poult body weights per pen at 13 days of age

Group	Pen 1		Pen 2		Pen 3		Pen 4	
	Weight (g)	CV	Weight (g)	CV	Weight (g)	CV	Weight (g)	CV
1 Heavy	163.8	7.8	178.9	7.5	179.6	9.7	172.5	17.6
1 Light	178.4	9.6	175.9	13.4	181.2	9.8	181.7	9.4
2 Heavy	170.5	9.4	186.9	8.7	181.4	4.3	186.5	12.7
2 Light	178.8	10.9	175.9	10.3	169.8	6.7	168.2	6.8
3 Heavy	181.1	9.9	188.9	10.2	193.3	8.6	178.8	8.8
3 Light	198.4	9.6	172.7	12.1	187.4	14.5	169.7	22.7
4 Heavy	172.6	7.8	202.7	6.4	194.3	18.5	179.3	6.8
4 Light	183.6	6.0	171.6	10.2	181.9	7.4	180.8	16.5
5 Heavy	193.6	6.8	194.0	14.0	175.6	27.5	199.3	10.3
5 Light	208.0	7.1	206.4	13.9	197.7	9.0	203.6	9.4
6 Heavy	194.5	9.7	195.3	8.7	193.0	6.8	204.9	11.1
6 Light	193.9	9.3	195.7	10.4	198.1	12.1	186.6	9.4
Controls	218.1	10.0	222.7	14.2	208.0	9.1	205.1	14.5

N = 6 poults per pen

Table 30 Percent of pools positive for rotavirus and astrovirus in the inoculation trial

Group		Rotavirus		Astrovirus	
		Mean	SE	Mean	SE
Heavy	1	0	0	100%	0%
	2	50%	58%	100%	0%
	3	50%	58%	100%	0%
	4	25%	50%	100%	0%
	5	100%	0	100%	0%
	6	0	0	100%	0%
Light	1	75%	50%	100%	0%
	2	25%	50%	100%	0%
	3	75%	50%	100%	0%
	4	100%	0	100%	0%
	5	25%	50%	100%	0%
	6	0	0	100%	0%
0 days of age		0	0	0	0
7 days of age		0	0	0	0
Controls		0	0	100%	0%

N = 4 pools of 3 poults

Table 30 Percent of pools positive for *E. coli.*

Group		*E. coli.*	
		Mean	SE
Heavy	1	100%	0%
	2	100%	0%
	3	100%	0%
	4	100%	0%
	5	100%	0%
	6	100%	0%
Light	1	100%	0%
	2	100%	0%
	3	0	0
	4	100%	0%
	5	100%	0%
	6	100%	0%
7 days of age		100%	0
Controls		100%	0

N = 4 pools of 3 poults

Table 31 Average counts for bacteria (log_{10} cfu/g)

Group		Aerobic	Anaerobic	Lactobacilli	Heterofermentative Lactobacilli
Heavy	1	7.18	7.53	8.11	8.02
	2	6.79	6.86	7.83	4.62
	3	5.41	5.45	7.35	8.34
	4	6.86	6.62	7.96	7.93
	5	7.43	8.00	8.30	8.34
	6	7.12	7.20	7.86	7.99
Light	1	7.50	7.57	8.10	8.11
	2	7.45	7.92	8.14	8.19
	3	6.49	6.59	8.26	8.24
	4	5.56	6.22	8.26	8.17
	5	8.19	8.28	8.38	8.42
	6	7.62	7.75	8.15	8.13
7 days of age		6.29	6.29	6.45	6.29
Controls		7.87	7.86	8.18	8.14

N = 1 except for day 7 where N = 2

Table 32 Heterophilic density in the inoculation trial

Group		Duodenum		Jejunum		Ileum		Ceca	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Heavy	1	0	0	0.63	0.263	1	0.408	0	0
	2	0.75	0.25	0.63	0.183	0.5	0.289	0.5	0.289
	3	0.25	0.25	0.5	0.189	0.75	0.25	0.25	0.25
	4	0	0	0.75	0.164	1	0	0.75	0.479
	5	0	0	0.5	0.189	0.5	0.289	0.25	0.25
	6	0.75	0.25	0.88	0.125	1	0	0.75	0.25
Light	1	0.75	0.25	0.38	0.183	1	0	0.25	0.25
	2	0.5	0.289	0.38	0.183	0	0	1	0.408
	3	0.75	0.25	0.75	0.164	1	0	0.25	0.25
	4	0	0	0.38	0.183	1	0	0.5	0.289
	5	0	0	0.88	0.125	0.75	0.25	0	0
	6	0.25	0.25	1.13	0.227	1	0	0.75	0.25
7 days of age		0.5	0.289	0.5	0.289	0.75	0.25	0.25	0.25
Controls		0.5	0.289	1	0	1	0	0.25	0.25

N = 4 for the duodenum, ileum and ceca
N = 8 for the jejunum

Table 33 Heterophilic distribution in the inoculation trial

Group		Duodenum		Jejunum		Ileum		Ceca	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Heavy	1	0	0	1	0.423	2.25	0.75	0	0
	2	1.5	0.5	1.38	0.46	1.25	0.75	1.25	0.75
	3	0.75	0.75	1.25	0.526	1.75	0.629	0.25	0.25
	4	0	0	2.13	0.479	2	0.408	0.75	0.479
	5	0	0	1.25	0.491	1.25	0.75	0.25	0.25
	6	1	0.408	2.38	0.375	2.75	0.25	1.5	0.5
Light	1	1.5	0.645	1	0.5	2.75	0.25	0.5	0.5
	2	1	0.707	1	0.5	0	0	1.5	0.5
	3	1.75	0.629	1.88	0.479	2.5	0.5	0.5	0.5
	4	0	0	1	0.5	2.5	0.289	1	0.707
	5	0	0	2.38	0.375	2.25	0.75	0	0
	6	0.75	0.75	2.38	0.375	3	0	1.25	0.479
7 days of age		1	0.577	1	0.577	2	0.707	0.5	0.5
Controls		0.75	0.479	2.75	0.164	2.75	0.25	0.75	0.75

N = 4 for the duodenum, ileum and ceca
N = 8 for the jejunum

Table 34 Lymphocytic density in the inoculation trial

Group		Duodenum		Jejunum		Ileum		Ceca	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Heavy	1	0	0	0	0	0	0	0.33	0.333
	2	0.25	0.25	0.25	0.164	0.75	0.479	0	0
	3	0.5	0.289	0.13	0.125	0.25	0.25	0.75	0.25
	4	0.25	0.25	0	0	0.25	0.25	0.75	0.25
	5	0	0	0.38	0.183	1	0	0.75	0.25
	6	0.5	0.289	0	0	0.25	0.25	1	0
Light	1	0	0	0.13	0.125	0	0	0	0
	2	0	0	0.13	0.125	0	0	0	0
	3	0	0	0	0	0	0	0.75	0.25
	4	0.5	0.289	0.25	0.164	0.25	0.25	1	0
	5	0.75	0.25	0	0	0	0	0.5	0.289
	6	1	0	0.13	0.125	0.5	0.289	1	0
7 days of age		0	0	0	0	0	0	0	0
Controls		0.25	0.25	0	0	0.75	0.75	0	0

N = 4 for the duodenum, ileum and ceca
N = 8 for the jejunum

Table 35 Lymphocytic distribution in the inoculation trial

Group		Duodenum		Jejunum		Ileum		Ceca	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Heavy	1	0	0	0	0	0	0	0.33	0.333
	2	0.75	0.75	0.63	0.42	1	0.577	0	0
	3	1	0.707	0.38	0.375	0.5	0.5	1.75	0.629
	4	0.5	0.5	0	0	0.5	0.5	1.25	0.479
	5	0	0	1.13	0.549	2.75	0.25	1.25	0.479
	6	1	0.707	0	0	0.5	0.5	2.25	0.25
Light	1	0	0	0.25	0.25	0	0	0	0
	2	0	0	0.38	0.375	0	0	0	0
	3	0	0	0	0	0	0	1.25	0.479
	4	1	0.577	0.63	0.42	0.75	0.75	2.25	0.479
	5	1.25	0.479	0	0	0	0	1	0.577
	6	2.75	0.25	0.38	0.375	1.5	0.866	2.25	0.25
7 days of age		0	0	0	0	0	0	0	0
Controls		0.5	0.5	0	0	0.75	0.75	0	0

N = 4 for the duodenum, ileum and ceca
N = 8 for the jejunum

Table 36 Bursa density and distribution scores in the inoculation trial

Group		Heterophilic				Lymphocytic Necrosis			
		Density		Distribution		Density		Distribution	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Heavy	1	0.5	0.5	0.25	0.25	0.5	0.289	0.75	0.479
	2	0	0	0	0	0.25	0.25	0.25	0.25
	3	0	0	0	0	0.75	0.25	1	0.408
	4	0.25	0.25	0.5	0.5	1.25	0.479	1.5	0.5
	5	0	0	0	0	0.25	0.25	0.25	0.25
	6	0.5	0.289	0.5	0.289	0.5	0.289	0.75	0.479
Light	1	0	0	0	0	1.25	0.25	3	0
	2	0.25	0.25	0.25	0.25	0.25	0.25	0.5	0.5
	3	0.25	0.25	0.5	0.5	1.25	0.25	2.25	0.25
	4	0.5	0.5	0.5	0.5	2	0.408	2.5	0.289
	5	0	0	0	0	0.25	0.25	0.25	0.25
	6	0.25	0.25	0.5	0.5	0.75	0.25	1.25	0.479
7 days of age		1	0	2	0	0	0	0	0
Controls		0.25	0.25	0.5	0.5	0.5	0.5	0.25	0.25

N = 4

Table 37 Bursa atrophy scores in the inoculation trial

Group		Atrophy	
		Mean	SE
Heavy	1	0	0
	2	0.25	0.25
	3	0.75	0.25
	4	0.5	0.289
	5	0	0
	6	1	0
Light	1	1	0.408
	2	0.5	0.289
	3	0.5	0.5
	4	1.5	0.289
	5	0.75	0.25
	6	0	0
7 days of age		0	0
Controls		0	0

N = 4

Table 38 Spleen scores in the inoculation trial

Group		Lymphocellular necrosis		Germinal center hyperplasia		Ellipsoid necrosis		Red pulp	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
Heavy	1	0	0	1	0	0	0	0	0
	2	0	0	0.5	0.289	0	0	0.25	0.25
	3	0	0	0.5	0.289	0	0	0	0
	4	0.25	0.25	1	0.408	0	0	0	0
	5	0	0	1.25	0.25	0	0	0	0
	6	0	0	0.5	0.289	0	0	0	0
Light	1	0	0	0.5	0.5	0	0	0	0
	2	0	0	1	0.408	0	0	0.25	0.25
	3	0	0	0	0	0	0	0	0
	4	0	0	0.5	0.5	0.25	0.25	0	0
	5	0	0	0.25	0.25	0	0	0	0
	6	0	0	0.5	0.289	0	0	0	0
7 days of age		0	0	0	0	0	0	0	0
Controls		0.5	0.289	0	0	0	0	0	0

N = 4