HUMAN SELECTION AND TRAIT EVOLUTION IN THE CHICKEN

A CONCENTRATION ON THE DEVELOPMENTAL ANATOMY OF THE SMALL INTESTINE

by

Alicia Greenwalt

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Bachelor of Science in Animal Science with Distinction.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Since the early 20th century, the poultry industry has influenced the growth of broiler chickens significantly through the implementation of highly selective breeding programs which focused primarily on breast muscle deposition, feed efficiency, and growth rate. In this study, the morphological differences between a modern broiler line (Ross708) and a line maintained from the early 20th century (Heritage) were compared. The purpose of the study was to determine if the selection for breast muscle, feed efficiency, and growth rate resulted in an increase in intestinal mass, length, cross-sectional area and villus length to allow for more surface area in order to increase absorption and therefore increase efficiency. Both lines were grown in identical batteries with ad libitum access to feed and water and continuous lighting. Samples were taken of the duodenum, jejunum and ileum intermittently over a 35-day period. The data showed a significant increase in overall jejunum and ileum masses, lengths, cross-sectional areas, and villus lengths from the Heritage to the Ross708 line. The differences within the duodenums of the two lines were not as drastic as seen in the aforementioned sections. The data from this study suggested that the selective breeding in the poultry industry targeted the growth patterns of the small intestine, specifically the jejunum and ileum, by increasing the overall surface area.

Chapter 1

INTRODUCTION

The modern chicken is believed to have been domesticated nearly 8,000 years ago in Asia from its wild ancestor, the red junglefowl.[3] Since then, farmers have been breeding these birds to meet the demand for meat and eggs production. It was not until the early 20th century, however; that the demand resulted in a bifurcation within the poultry industry, leading to extensive genetic selection for either high yield egg- or meat-producing chickens. [4] The two resulting groups of chickens are known as layers and broilers, respectively.

The broiler industry focused specifically on improving muscle deposition, feed efficiency and growth rate.[6] The drive for this focus on muscle growth originated from market demand. The National Chicken Council reported an increase in per capita consumption of poultry over the past 30 years. [9] Not only was the market demanding more chicken but larger chickens as well.[5] Broilers are marketed in three main categories: whole chicken, parts, or further processing, such as deboning.[5] Whole broiler chickens and parts are normally marketed up to 6.25 pounds, while chickens used in restaurants and trade are generally the smallest of three, around 4.5 pounds or less. The chickens used in processing and deboning, however, require a higher meat-to-bone ratio and generally weigh greater than 6.25 pounds.[7] Since 1962, the percentage of broilers sold as whole birds dropped from 83% to only 11% in 2007, while the percentage for chickens being processed increased from 2% to 46%, a 23-fold increase.[5] This drastic shift in demand for larger birds placed considerable pressure on the industry to produce more broilers, faster and larger than before. Today the modern broiler weighs an average of 5.60 pounds, over 3 pounds more than its 1925 counterpart.[11] In addition, that extra weight is gained during a shorter time period of only 48 days as opposed to the 112 day average in 1925.[11]

While it is known that chickens have been bred to grow larger, more rapidly, the mechanisms which allow for that physiological shift are poorly understood. The purpose of this study was to determine if the selective pressures for muscle deposition, feed efficiency and growth rate were associated with an increase in surface area within the small intestine of these birds.

The small intestine is the major site of nutrient absorption in the avian digestion system. The duodenum makes up the proximal end. The jejunum is distal to the duodenum and proximal to the ileum which is the distal-most aspect of the small intestine. All three segments are active in nutrient absorption in the chicken. Within the small intestine there are numerous folds which allow for an increase in surface area. In addition to the folds, villi are present which also increase the surface area significantly.[2] An increase in this surface area would provide more area available for nutrient absorption, therefore increasing absorption rate and possibly growth rate. This concept was supported with work by Mitjans et al (1997) who found that the increase in surface area "…may contribute significantly in satisfying the functional requirements of the animal during development."

The study analyzed the differences in intestinal mass and length, cross-sectional area and villus length between a line of chickens comparable to those grown in the early 20th century, not selected for rapid growth, and a modern line which exhibited the increase in growth rate and overall mass seen in the poultry industry today. The line representing the modern broiler was the Ross708 and was obtained from a local hatchery in Delaware. The UIUC line from the University of Illinois represented the heritage broilers in this study. The line was developed by H.M. Scott and is the result of the cross of a New Hampshire male line and Columbian female line, both inbred since the late 1940s.[10] The Heritage line was compared to the Ross708 as a baseline to understand the characteristics selected for in the small intestine since the early 20th century.

Chapter 2

METHODS AND MATERIALS

Raising the Chickens

Two lines of broiler chickens were chosen for this study. The first line was the Ross708, which was obtained from a local hatchery. This is a popular breed in the broiler production industry currently and represented the modern broiler line. The University of Illinois (UIUC) strain has been maintained since the 1940's and was chosen as our Heritage line to represent the broilers utilized in early 20th century poultry production.

Both lines were raised from age day 2 post hatch(PH) and placed in identical starter batteries with 20 chicks to each battery for the first 3 weeks, whereupon they were transferred into grower batteries. A total of 120 chicks was raised for each line. They received continuous lighting as well as ad libitum access to water and feed. Their diet consisted of a commercial starter feed until they reached 4 weeks of age, where they were then given a commercial finishing feed. The focus on the study was aimed at the differences in development and growth of the two lines; therefore, to prevent a second variable, an effort was made to select only males.

Specimen Sampling

Samples were collected on day 7, 10, 14, 17, 21, 35, and 42 post-hatch (PH). Ten chicks from each line were selected, weighed (live), and euthanized by cervical dislocation. The birds were then dissected and the intestinal segments were separated into the duodenum, jejunum, and ileum. It was at this time that the sex of each bird was confirmed.

The three segments were determined by gross anatomical landmarks. The duodenum was determined to begin at the caudal end of the gizzard and included the length of intestine comprising the duodenual loop. The jejunum began at the end of the duodenum and continued to Meckel's diverticulum. The ileum then continued from that point until it reached the junction with the ceca and the cloaca.[2]

Segments were emptied and cleaned, the mesentery was removed and they were weighed and then measured for length. A smaller section was cut from the middle of each segment and preserved in a formalin solution for further processing.

Preparation of Slides

Two methods were used in the preparation of slides. Hematoxylin and Eosin staining was implemented in order to obtain villus lengths and cross-sectional areas. The specimen were removed from their formalin solutions, embedded in paraffin wax, mounted to slides and were then stained through standard H&E staining protocol and preserved in a xylene-based mounting material.

The second method of preparation was immunohistochemical staining for Proliferating Cell Nuclear Antigen (PCNA) to identify areas of proliferation within the intestine. The kit used was a mouse monoclonal [PC10] to PCNA kit from Abcam Inc. The antibody was visualized using rabbit anti-mouse monoclonal secondary antibody in addition to 3,3' Diaminobenzidine (DAB) with Horseradish peroxidase (HRP).

The manufacturer's protocol was improved upon with an additional rinse with TBS after the peroxide incubation prior to application of the secondary antibody.

This prevented uneven staining and unwanted saturation of the secondary antibody, otherwise seen in the slides. Also, the suggested dilution of the primary antibody at 1:6000 was adapted to 1:2000 for more consistent results. The slides were then counter-stained with eosin in order to detect the remaining tissue. Finally, the slides were dehydrated using a standard ethanol and xylene dehydration protocol and mounted in xylene-based mounting material.

Data Collection Techniques

Data collection occurred at different times during the study. The first occurred during the dissection of the chicks, including the mass and length of each intestinal segment. Measurements later taken after processing of the slides was completed included cross-sectional area, as well as, villus length measurements.

Intestinal Mass and Length

After cervical dislocation, the chicks were dissected for various organs of interest including the three segments of the small intestine. The small intestines were removed from the abdominal cavity and the three sections were separated, the contents removed and the sections were weighed individually. Each segment was then measured for length before being preserved in a formalin solution. The measurements were taken in centimeters using a metric ruler.

Cross-Sectional Area

Cross-sectional area was collected using the H&E stained slides. Because the samples were too large to capture under a microscope, measurements were taken manually with an average standard deviation of +/- 0.0052 cm. Length and width of transverse sections were measured using an Imperial system-based vernier scale and were converted into metric. The length and width of each sample was multiplied to find the area of the surrounding rectangle.

Villus Length

The techniques for the villus length measurements and the PCNA analysis required Nikon Imaging Software. Images were taken at a magnification of 40 in order to capture the entire villus length. Image-Pro Plus software was then used to take the measurements of the villi.

Villus length was defined as the distance from the basement membrane of a crypt to the tip of the corresponding villus. Abnormal villus structures, such as damaged or degraded tissue, were excluded from measuring.

Proliferating Cells

The areas of proliferation were visible by microscope after the staining process. Using the Nikon software, images were captured and assessed based on location of concentrated PCNA positive areas. Some issues did occur with this process due to the age of the specimen. The antigen was difficult to retrieve consistently due to the age of the samples. It is recommended that immunohistochemical staining of PCNA be completed as soon as possible after dissection; otherwise the antigen is difficult to retrieve and therefore produces an unreliable stain.

Statistical Analysis

The statistical software JMP was used to analyze all data collected throughout the study. There were two main effects studied between samples. The first was the analysis of the sample days within each line. The second method of analysis was a comparison of the two lines at each sampling day. Statistical significance was determined by a p value less than 0.05. Only sampling days with three or more different samples per segment were used. Days with less than three reliable samples were not considered. Examples of unreliable samples included segments that were damaged, degraded or obtained from a female bird.

Chapter 3

RESULTS

Intestinal Mass

Duodenum Mass

HERITAGE. The mass of the duodenum continually increased starting with a mean of 1.979 g (S.E. 0.24) at day 7 PH to 11.354 g (S.E. 0.211) at day 42 PH. It was a total increase of 9.374 g or 5.74 fold over the entire growth period (Table 1, Figure 1).

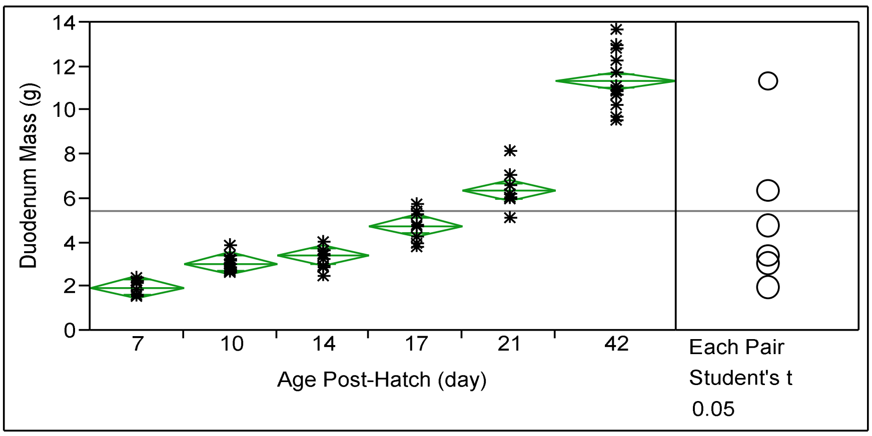


Figure 1 Duodenum Masses of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 1 Comparison of Mean Duodenum Masses of the Heritage Line by Age Post-Hatch Letters represent significant difference between ages. If an age has the same letter as another, they are not significantly different.

Age PH						Mean Mass (g)
42	A					11.353846
21		B				6.356000
17			C			4.771000
14				D		3.403000
10				D		3.059000
7					E	1.979000

Each day showed significant growth from the previous sampling day. The only exception to this was the period between days 10 and 14 PH where no significant growth was observed (Table 1). Average daily gain of the duodenal mass varied throughout the study. Days 7 through 10 PH had a significant increase in rate of gain, followed by a sharp decrease during days 10 to 14 PH. During the decline there was only an average of 0.086 g/day (S.E. 0.085). Afterwards the rate increased and surpassed its previous rate to reach 0.456g/day (S.E. 0.113) during days 14 to 17 PH. There was a gradual decrease from days 17 to 42 PH where a final rate of 0.238g/day (S.E. 0.214) was reached (Figure 2).

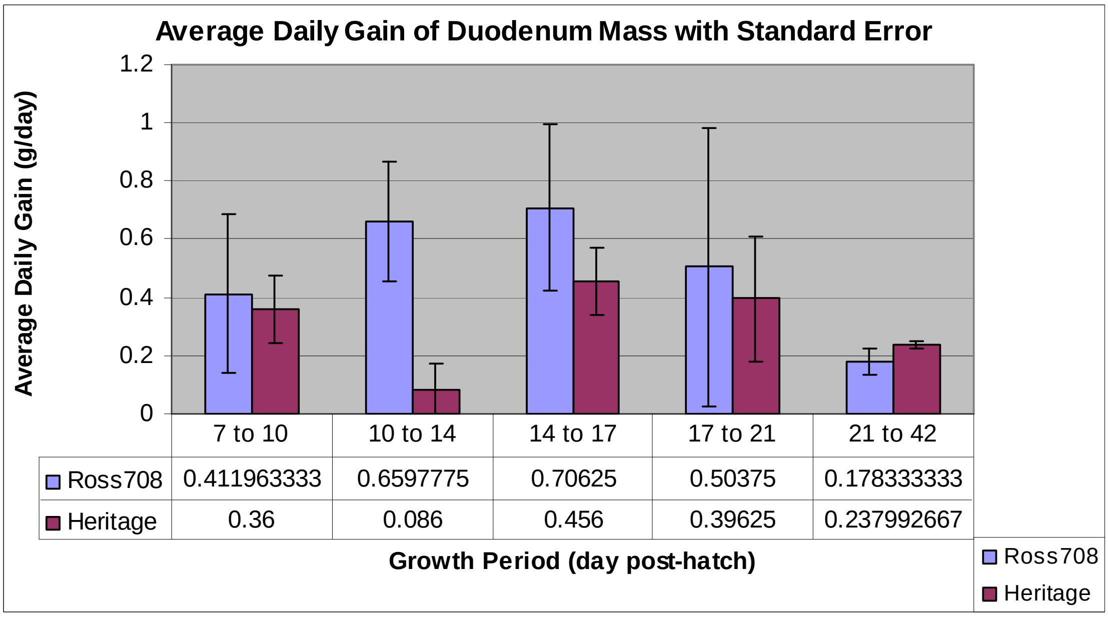


Figure 2 Average Daily Gain (ADG) of Duodenal Mass of Heritage and Ross708 lines with Standard Error: ADG was calculated by the difference in mean mass divided by number of days

<u>ROSS708.</u> The Ross708 line also showed an increase in the duodenal mass over the entire 35-day study. Every sampling period was significantly larger than the previous. The period from Days 7 to 10 PH were not statistically different (Table 2). Starting at Day 7 PH with 3.491 g (S.E. 0.561) the mass of the Ileum reached 15.245g (S.E. 0.648) at its highest on Day 42 PH (Figure 3). There was a total increase of 11.754g or 4.37 fold over the course of the study.

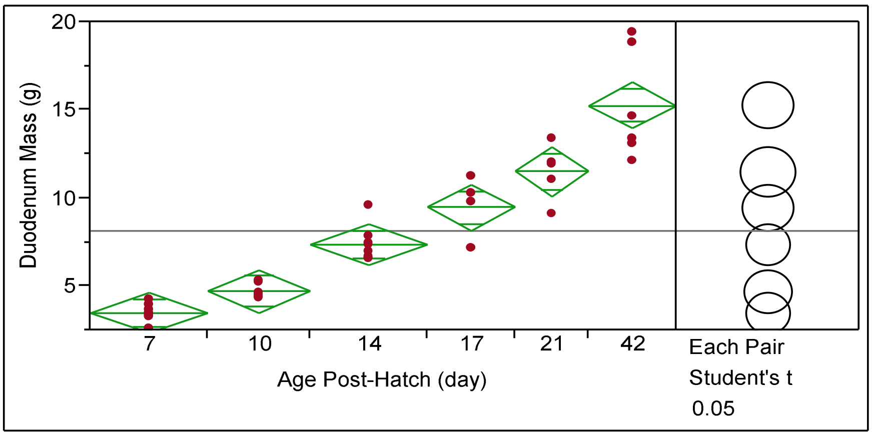


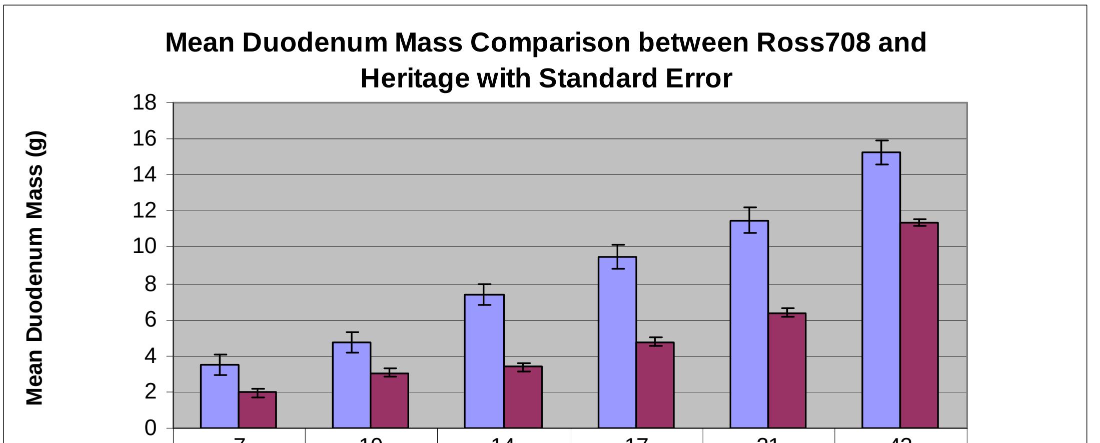
Figure 3 Duodenum Masses of Ross708 Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 2 Comparison of Mean Duodenum Masses of the Ross708 Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH						Mean Mass (g)
42	A					15.245000
21		B				11.500000
17			C			9.485000
14				D		7.366250
10					E	4.727143
7					E	3.491250

Average daily gain increased from Day 7 to 17 PH. There were significant standard errors during the period between Day 17 and 21 PH. In addition, there was a drastic decline in rate of gain by Day 42 PH. The highest rate of gain during the study was observed during the period between Day 14 and 17 PH with an average of 0.70625g/day (S.E. 0.286) and the lowest was at the end with only 0.17833g/day (S.E. 0.046) (Figure 2).

ROSS708 AND HERITAGE. Throughout the entire study the Ross708 line maintained a significantly higher mass than the Heritage line. There was a difference of 1.512 g at day 7 PH and a final difference of 3.891 g at day 42 PH. The day with the greatest difference was day 21 PH with 5.144 g difference between the two lines. Ross708 and Heritage were closest in mass to each other at day 7 PH and there was an observed increase in the difference through day 42 PH (Figure 4).



	7	10	14	17	21	42
Ross	3.4913	4.7271	7.3663	9.485	11.5	15.245
Heritage	1.979	3.059	3.403	4.771	6.356	11.3538

Figure 4 A Comparison of Mean Duodenum Mass by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

Jejunum Mass

HERITAGE. The jejunum of the Heritage line had a starting average mass of 2.056 g (S.E. 0.48872) at day 7 PH. By day 42 PH the mass had increased by 12.584g to reach a final mass of 14.64g (S.E. 0.446). There was a 7.12 fold increase of mass of the jejunum from day 7 PH (Figure 5, Table 3).

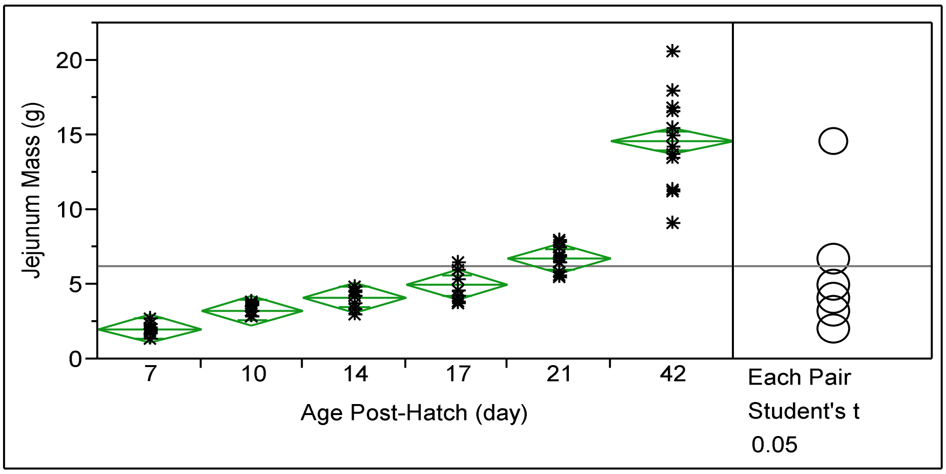


Figure 5 Jejunum Masses of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 3 Comparison of Mean Jejunum Masses of the Heritage Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Level						Mean
42	A					14.640000
21		B				6.734000
17			C			4.976000
14			C	D		4.143000
10				D	E	3.285000
7					E	2.056000

There was significant overall increase in the mass of the jejunum, however; the consecutive days had some instances of statistically insignificant growth. The growth rate during the period from day 7 to 10 PH was insignificant, as were days 10 to 14 PH and days 14 to 17 PH (Table 3). Average daily gain of the Heritage jejunum varied throughout the study with a noticeable decrease in rate of gain during the period between days 10 to 14 PH. The final daily gain seen during days 21 to 42 PH was 0.3765 g/day (S.E. 0.032) (Figure 6).

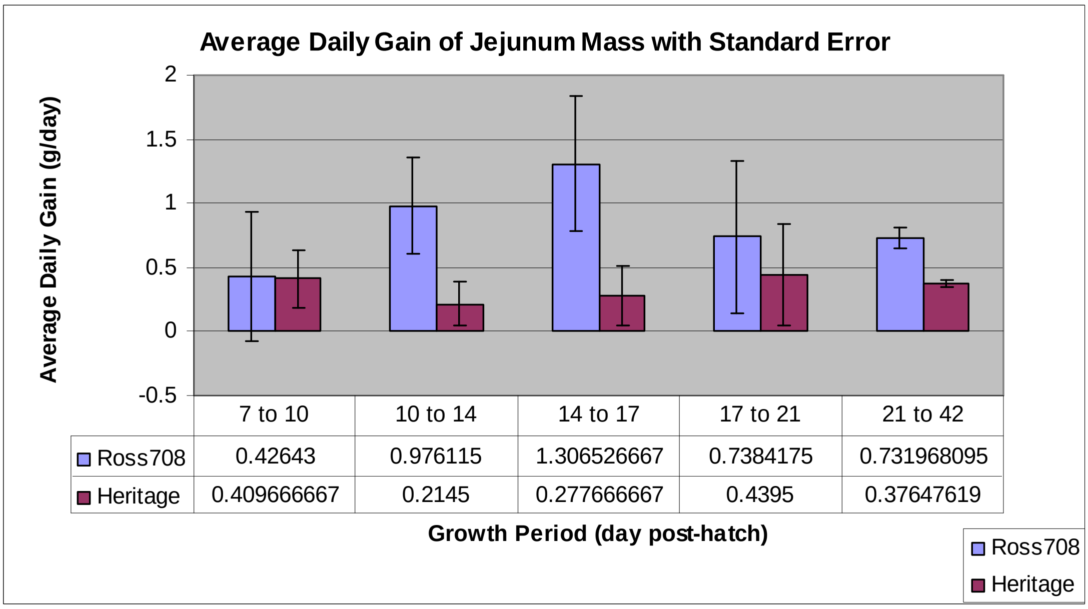


Figure 6 Average Daily Gain (ADG) of Jejunum Mass of Heritage and Ross708 lines with Standard Error: ADG was calculated by the difference in mean mass divided by number of days

<u>ROSS708.</u> The mass of the jejunum of the Ross708 line increased from 5.565g (S.E. 0.4887) at day 7 PH to 32.993g (S.E.0.4461) at day 42 PH. This was an increase of 27.428g or 5.93 fold over the entire study (Figure 7, Table 4).

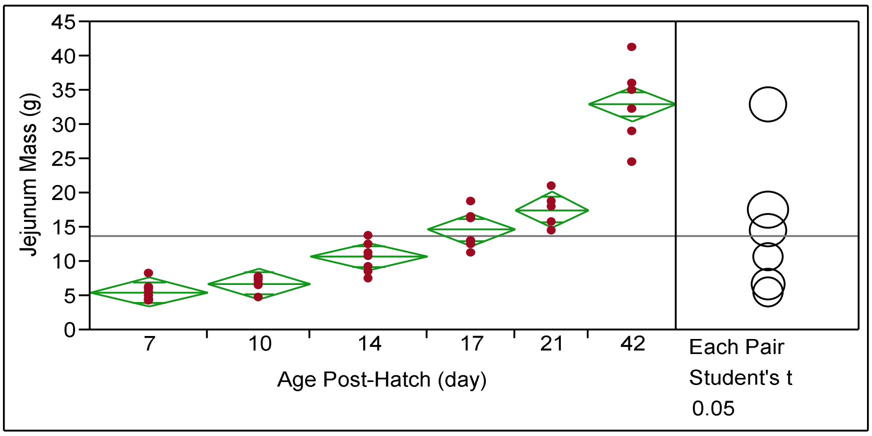


Figure 7 Jejunum Masses of Ross708 Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

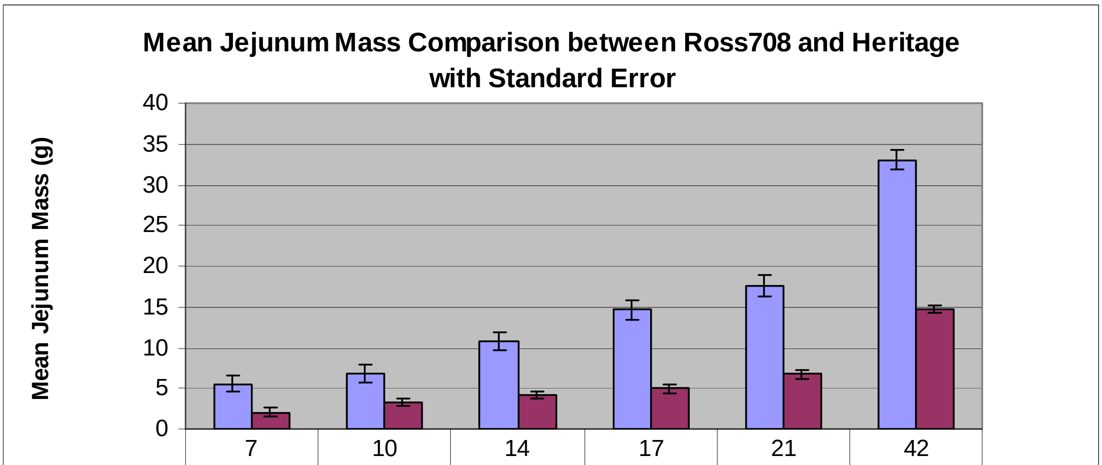
Table 4 Comparison of Mean Jejunum Masses of the Ross708 Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH					Mean Mass (g)
42	A				32.993333
21		B			17.622000
17		B			14.668333
14			C		10.748750
10				D	6.844286
7				D	5.565000

There was a significant increase in mass in the jejunum during the study overall, however; days 7 and 10 PH did not display a statistical difference in mass, nor did days 17 and 21 PH (Table 4). Average daily gain of the jejunal mass increased from days 7 to 17 PH, reaching a peak between days 14 and 17 PH of 1.307g/day (S.E. 0.532). After day 17 PH there was a decrease in the rate of gain by 0.5681 g/day. From days 21 to 42 PH there was only a slight decrease in rate to 0.7320 g/day (S.E. 0.084)

(Figure 6). In the case of Ross708, the lowest rate of gain was seen during days 7 to 10 PH and not at the end of the study.

ROSS708 AND HERITAGE. The mass of the Ross708 jejunum was significantly greater than the Heritage line on every day throughout the study. The difference between the lines at day 7 PH was 3.509 g and it only increased as the study continued. By day 42 PH there was a 2.25 fold difference between the two lines (Figure 8).



	7	10	14	17	21	42
Ross	5.565	6.8443	10.7487	14.6683	17.622	32.9933
Heritage	2.056	3.285	4.143	4.976	6.734	14.64

Figure 8 A Comparison of Mean Jejunum Mass by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

Ileum Mass

HERITAGE. The mean ileal mass increased significantly from 1.724g (S.E. 0.2560) on day 7 PH to 8.910g (S.E. 0.2337) on day 42 PH. This was a total increase of 7.186g or 5.17 fold. By day 42 there was a wider range of values than seen in the previous sampling days (Figure 9, Table 5).

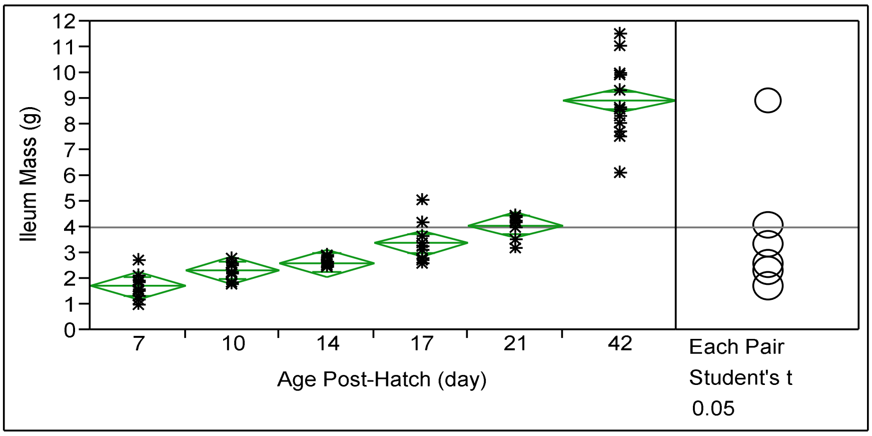


Figure 9 Ileum Masses of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 5 Comparison of Mean Ileum Masses of the Heritage Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH					Mean Mass (g)
42	A				8.9100000
21		B			4.0820000
17		B			3.3720000
14			C		2.6080000
10			C	D	2.3300000
7				D	1.7240000

There was a significant increase in ileal mass over the entire study. There were periods of time where there was not a statistically significant difference between masses. These periods of time included days 7 to 10, days 10 to 14, days 17 to 21 PH. While these days were not statistically different from their specific counterparts, they were, however; significantly different from the other sampling days in the study (Table 5). Average daily gain of ileal mass saw a drastic decrease from 0.202 g/day (S.E. 0.12) between day 7 and 10 PH to 0.0695 g/day (S.E. 0.090) during the period of day 10 to 14 PH. From days 14 to 17 PH there was an increase to the maximum rate of 0.2547 g/day (S.E.0.121) and it was quickly followed by a decline during days 17 to 21 PH and a slight increase at the end of the study from days 21 to 42 PH (Figure 10).

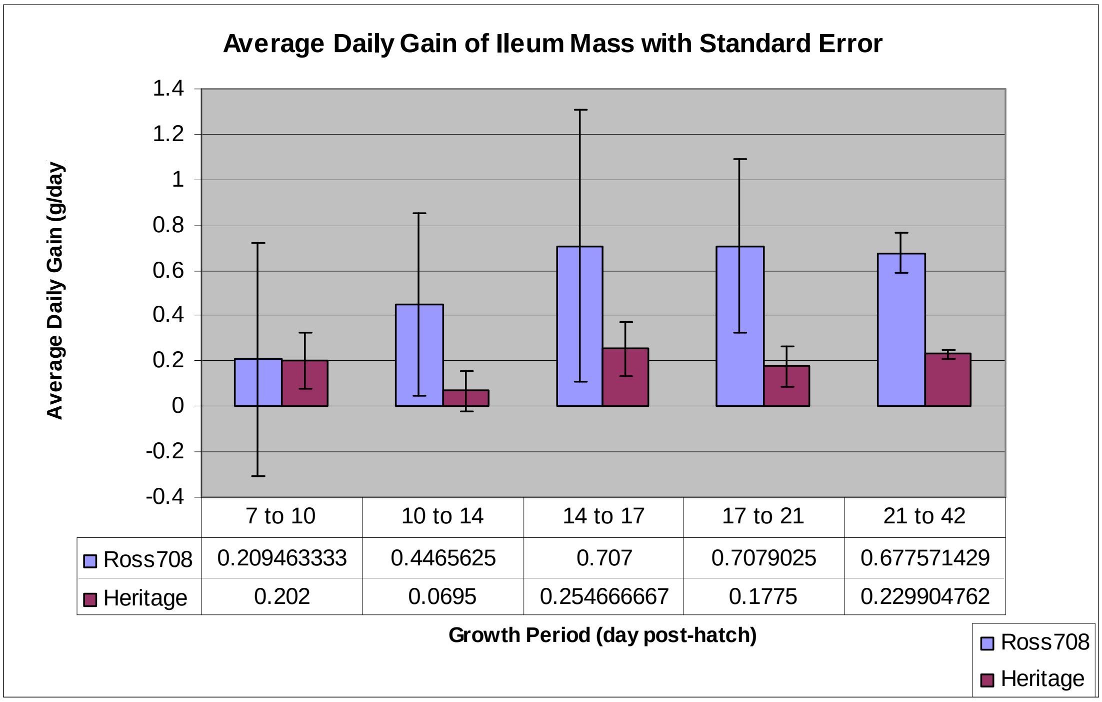


Figure 10 Average Daily Gain (ADG) of Ileal Mass of Heritage and Ross708 lines with Standard Error: ADG was calculated by the difference in mean mass divided by number of days

<u>ROSS708.</u> There was a significant increase in ileal mass for Ross708 over the entire study. The starting average of the ileal mass was 4.448g (S.E. 1.0512) on day 7 PH and the final average mass was 26.045g (S.E.1.2138) on day 42 PH. The total increase in mass was 21.596 g or 5.85 fold (Figure 11, Table 6).

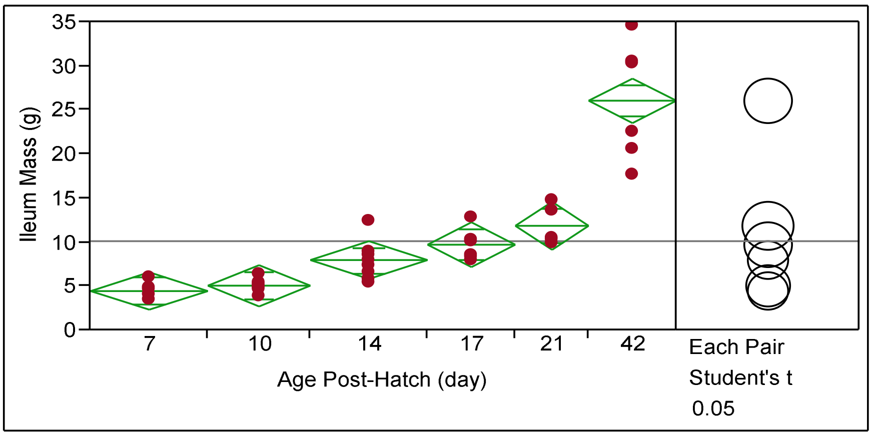


Figure 11 Ileal Masses of Ross708 Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 6 Comparison of Mean Ileum Masses of the Ross708 Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH						Mean Mass (g
42	A					26.045000
21		B				11.816000
17		B	C			9.695000
14			C	D		7.908750
10				D	E	5.077143
7					E	4.448750

The ileal mass of Ross708 increased significantly from day 7 to 42 PH however there were multiple groupings of sampling days which did not show significant growth between them. Days 7 to 10, 10 to 14, 14 to 17, and 17 to 21 PH were pairs of days between which, there was no statistically significant growth. On the other hand, all of these groups were significantly different from each other (Table 6). Average daily gain saw a trend of an increase from 0.2095 g/day (S.E. 0.513) during day 7 to 10 PH to a maximum of 0.7079 g/day (S.E.0.385) during days 17 to 21 PH.

Standard errors were very high during these growth periods, especially from days 7 to 10 PH. After this time period, the average daily gain decreased slightly to 0.6776 g/day (S.E. 0.086) during days 21 and 42 PH. The minimum rate of gain was seen during the beginning of the study between days 7 and 10 PH (Figure 10).

ROSS708 AND HERITAGE. The Ross708 ileum had significantly higher masses than that of the Heritage line through the entire study. The difference between the two lines continued to increase from day 7 to day 42 PH. At the beginning of the study the difference was 2.72 g by day 42 PH however, it rose to 17.14g. By the end of the study the Ross708 ileum mass was 17.135g or 2.90 fold greater than the Heritage line (Figure 12).

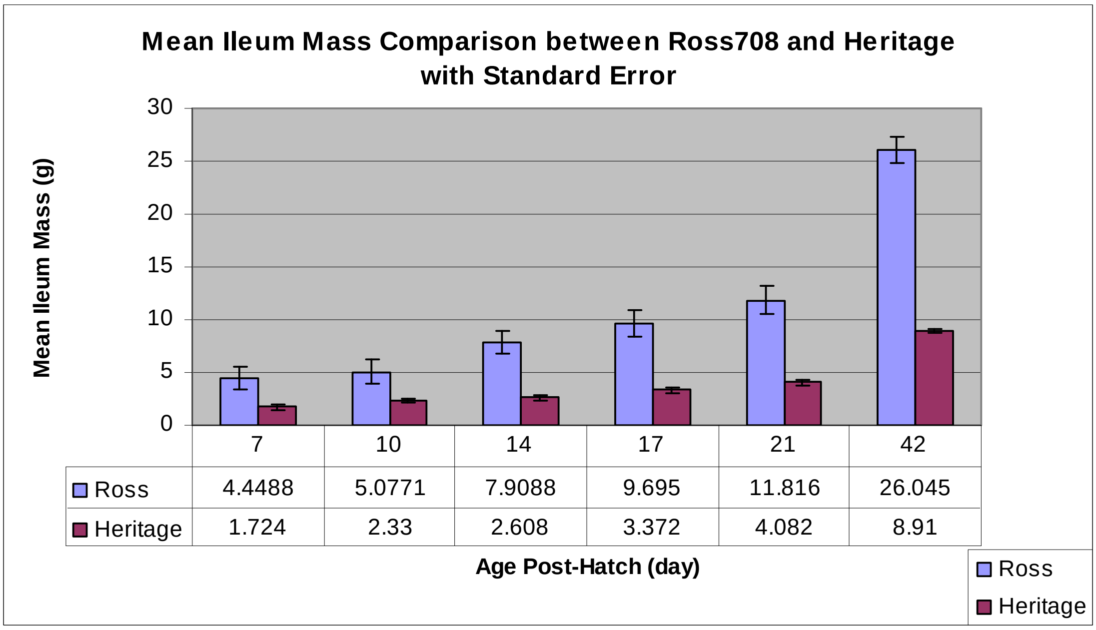


Figure 12 A Comparison of the Mean Ileum Mass by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

Intestinal Length

Duodenum Length

HERITAGE. The growth of the duodenum was significant between day 7 and 42 PH with a starting length of 15.450 cm (S.E. 0.84324) and a final length of 27.369 cm (S.E. 0.73957). It increased by 11.919 cm or 77%. Days 10 to 17 PH had very little growth and were statistically very similar in size (Figure 13, Table 7).

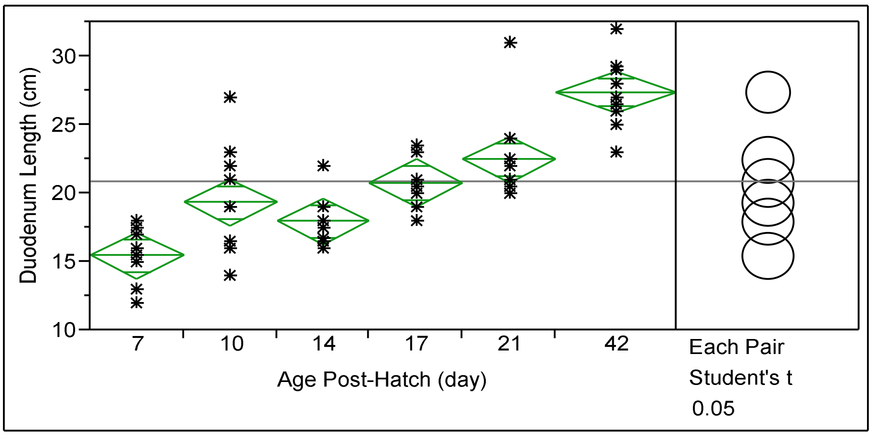


Figure 13 Mean Duodenum Length of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 7 Comparison of Mean Duodenum Length of the Heritage Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH						Mean Length (cm)
42	A					27.369231
21		B				22.450000
17		B	C			20.750000
14				D		17.980000
10			C	D		19.350000
7					E	15.450000

There were certain sampling days which did not show significant growth between them. This occurred in the middle of the study, between days 10 and 14, 14 and 17, and 17 and 10 PH. Days 42 and 7 PH were significantly different in size from any of the other sampling days (Table 7). Average daily growth had an overall decrease in rate during the study. The original rate of 1.30 cm/day (S.E. 0.396) from days 7 to 10 PH dropped significantly during days 10 to 14 PH to 0.345 cm/day (S.E. 0.398). There was a larger standard error during this time period. The rate then increased during days 14 to 17 PH only to decrease in the following growth periods. The final average daily growth was also the lowest rate observed during the study, with 0.2342 cm/day (S.E. 0.053) (Figure 14).

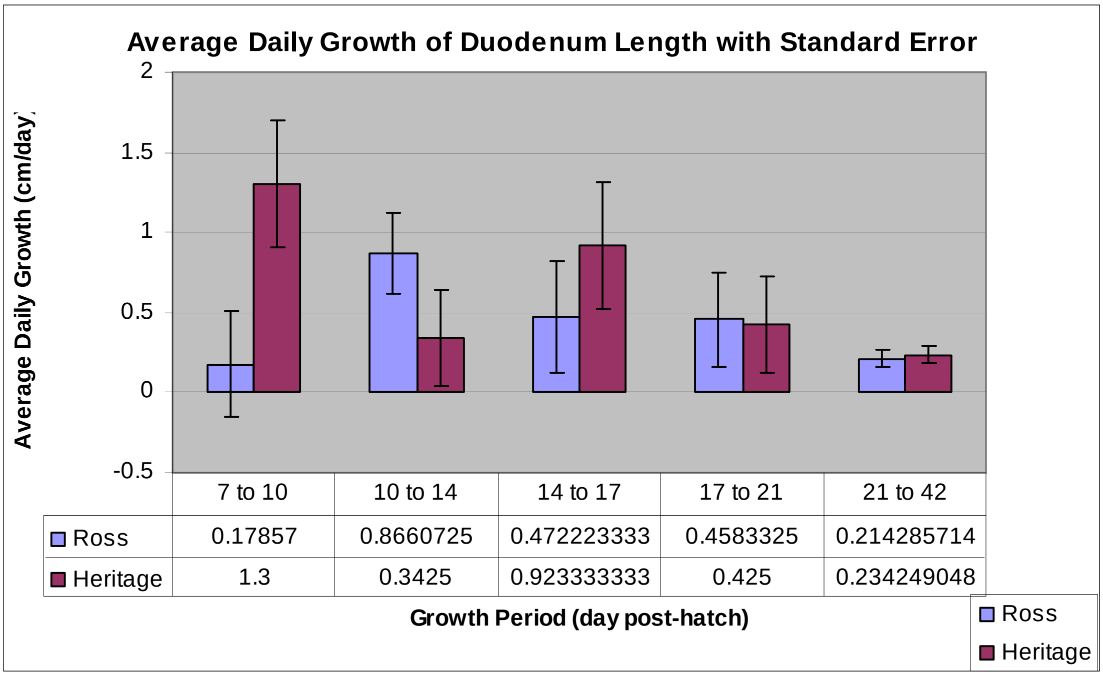


Figure 14 Average Daily Growth (ADG) of Duodenum Length of Heritage and Ross708 Lines with Standard Error: ADG was calculated by the difference in mean mass divided by number of days

<u>ROSS708.</u> The Ross708 line saw a significant increase in overall duodenal length from an average of 18.75 cm (S.E. 0.68536) for day 7 PH to 30.5 cm (S.E. 0.79138) at day 42 PH. This is a total increase of 11.75 cm or 63% (Figure 15, Table 8).

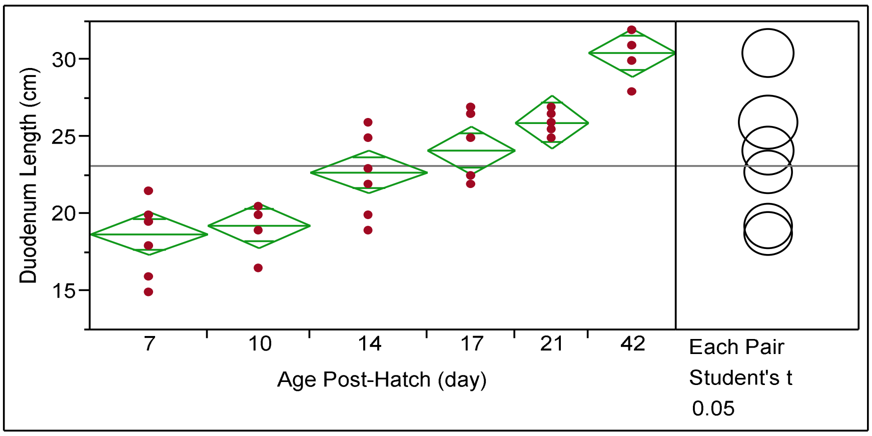


Figure 15 Mean Duodenum Length of Ross708 Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 8 Comparison of Mean Duodenum Length of the Ross708 Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH					Mean Length (cm)
42	A				30.500000
21		B			26.000000
17		B	C		24.166667
14			C		22.750000
10				D	19.285714
7				D	18.750000

While there was significant overall growth, there were days that were not statistically different in size from one another. These days include days 7 and 10 PH, 14 and 17, and 17 and 21 (Table 8). Average daily growth showed a spike in growth rate during days 10 and 14 PH. There was no significant daily gain from days 7 to 10 PH. Following that time period the rate rose to 0.866 cm/day (S.E. 0.251) between days 10 and 14 PH. After that period, the rate began to decline until it reached 0.214 cm/day (S.E. 0.056) during days 21 to 42 PH.

<u>ROSS708 AND HERITAGE.</u> The length of the Ross708 and Heritage lines were very similar in size from day 7 to day 42 PH. There was a significant difference between the lengths of the two lines for every day except for day 10 where $p > 0.05$. The difference between Ross708 and Heritage at day 7 PH was 3.30 cm. The largest difference was seen on day 14 PH and was 4.77 cm. The difference between the lengths then continued to hover around 3.4 cm until day 42 PH which lessened to the smallest observed difference of only 3.13 cm (Figure 16).

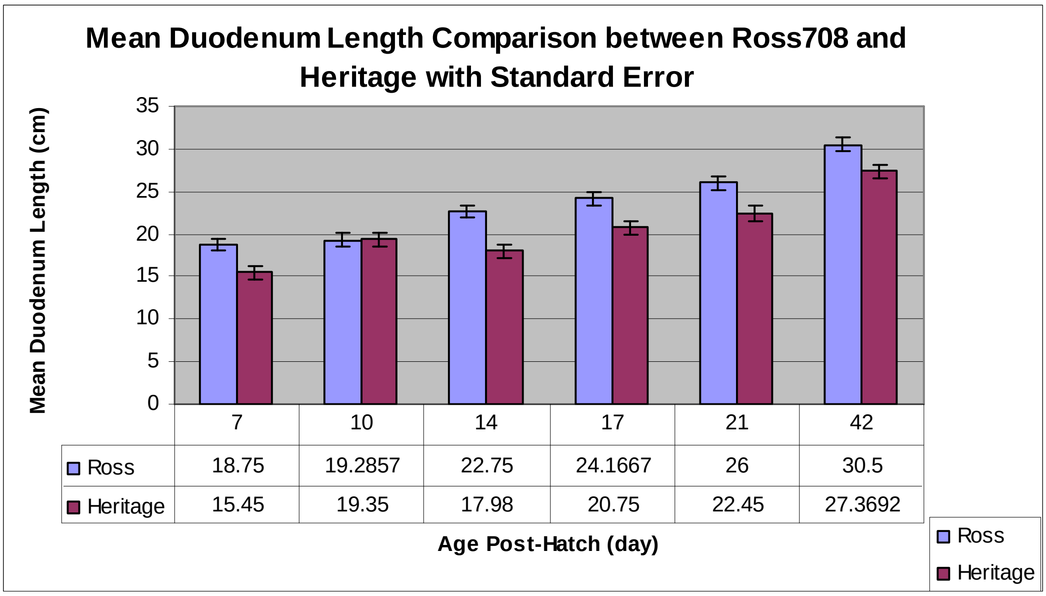


Figure 16 A Comparison of Mean Duodenum Length by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

Jejunum Length

HERITAGE. There was significant growth in the length of the jejunum of the Heritage Line. Day 7 PH had an average length of 26.75 cm (S.E. 1.124) and had a final length of 51.591 cm (S.E. 1.072) on day 42 PH. It was a total increase in length of 24.841 cm or 93% over the entire study (Figure 17, Table 9).

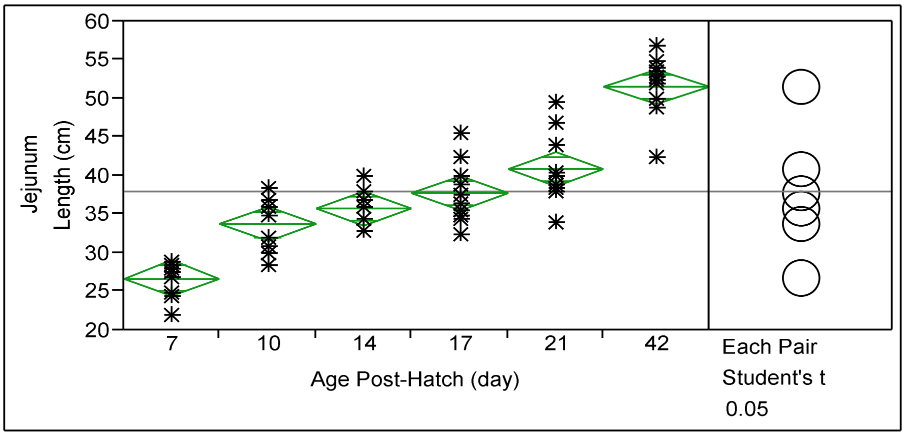


Figure 17 Mean Jejunum Length of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 9 Comparison of Mean Jejunum Length of the Heritage Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH						Mean Length (cm)
42	A					51.590909
21		B				40.850000
17		B	C			37.850000
14			C	D		35.850000
10				D		33.700000
7					E	26.750000

While there was significant growth from day 7 to 42 PH there were some days which did not show a difference in length from one another. These days included, days 10 and 14, 14 and 17, 17 and 21 PH (Table 9). Average daily growth of the jejunum showed the highest rate from days 7 to 10 PH of 2.317 cm/day (S.E. 0.530). There was a drop in growth rate during days 10 to 14 PH because these two days were found have no significant difference in size. After days 10 to 14 PH there was a slight increase in daily growth from day 14 to 17 PH, only to decrease, finally to 0.511 cm/day (S.E. 0.074) at days 21 to 42 PH (Figure 18).

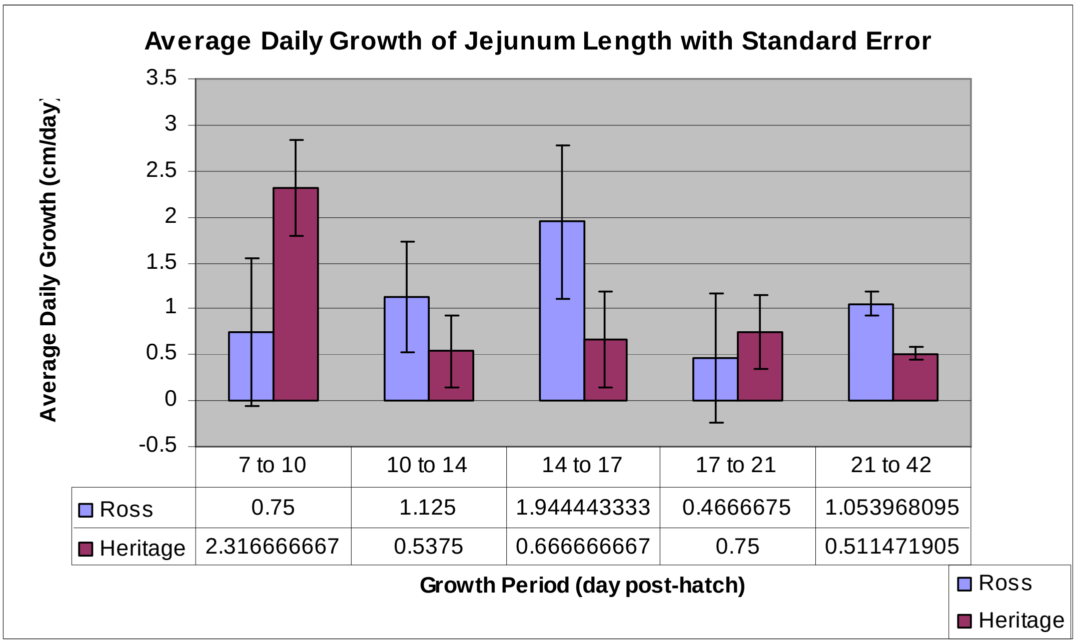


Figure 18 Average Daily Growth (ADG) of Jejunum Length of Heritage and Ross708 Lines with Standard Error: ADG was calculated by the difference in mean mass divided by number of days of growth period.

ROSS708. There was a significant increase in jejunum length for the Ross708 line from day 7 to 42 PH. The mean length at day 7 PH was 42.75 cm (S.E. 1.63) and the final length was 79.33cm (S.E. 1.89) on day 42 PH. It was a total growth of 36.58 cm or 86% (Figure 19, Table 10).

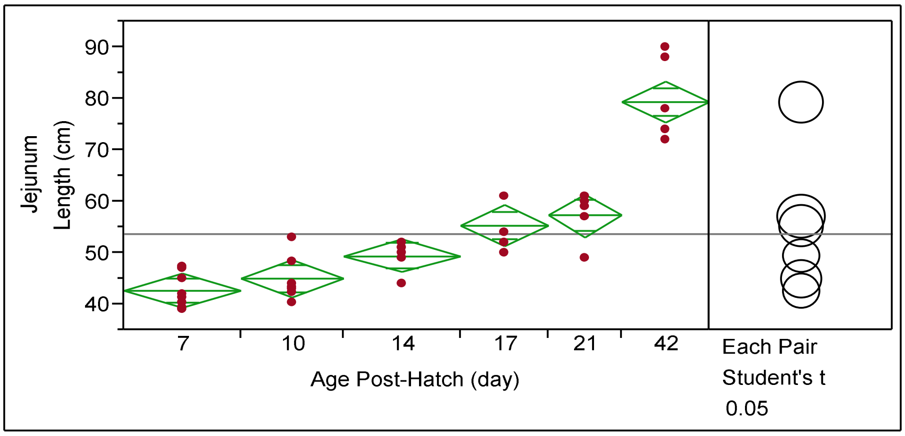


Figure 19 Mean Jejunum Length of Ross708 Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 10 Comparison of Mean Jejunum Length of the Ross708 Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH					Mean Length (cm)
42	A				79.333333
21		B			57.200000
17		B			55.333333
14			C		49.500000
10			C	D	45.000000
7				D	42.750000

While there was a significant difference between day 7 PH and day 42 PH, there were multiples days which did not show significant growth from the previous. These days include day 7 to 10, 10 to 14, and 17 to 21 PH (Table 10). Average daily growth of the Ross708 jejunum had an increase from day 7 to 10 PH to a maximum of 1.944 cm/day (S.E. 0.835) during day 14 to 17 PH. Day 17 and 21 PH were not statistically different from one another and therefore showed a dramatic decrease in growth rate. The final average growth rate was 1.053 cm/day (S.E. 0.134) during day 21 to 42 PH (Figure 18).

ROSS708 AND HERITAGE. Throughout the study the Ross708 jejunum was significantly longer than the Heritage. At day 7 PH Ross708 was 16 cm greater than that of the Heritage line. This difference decreased on day 10 by 4.7 cm. The difference then began to increase until Ross708 was 27.74 cm or 54% greater than Heritage at day 42 PH (Figure 20).

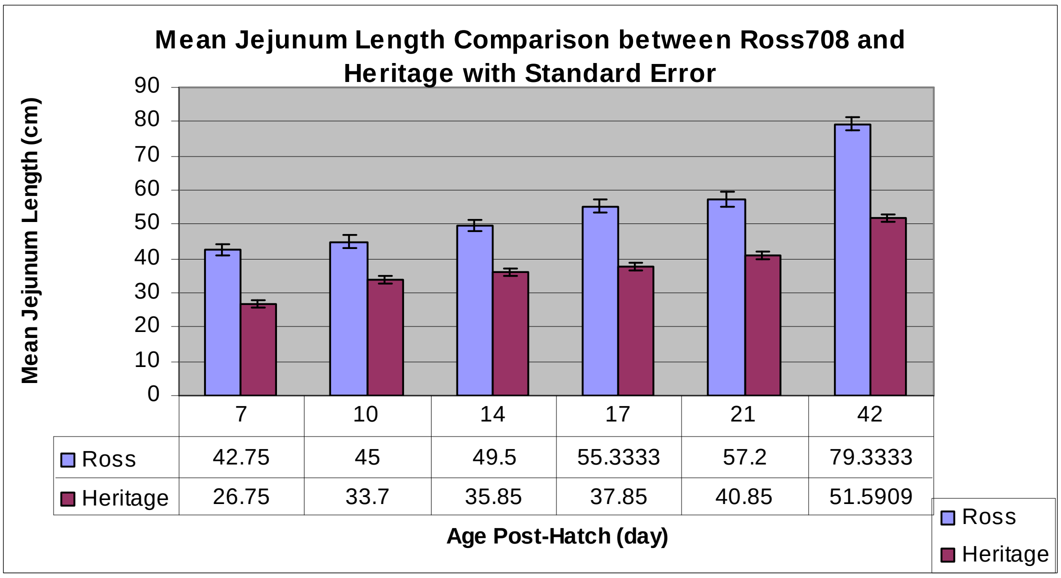


Figure 20 A Comparison of Mean Jejunum Length by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

Ileum Length

HERITAGE. The ileum of the Heritage line increased in length significantly over the 35 day study. At the first sampling day, day 7 PH the average length was 25.70 cm (S.E. 1.123). By day 42 the average length of the ileum in the Heritage line reached a maximum of 27.409 cm (S.E. 1.061). This is an increase of 21.809 cm or 85% (Figure 21, Table 11).

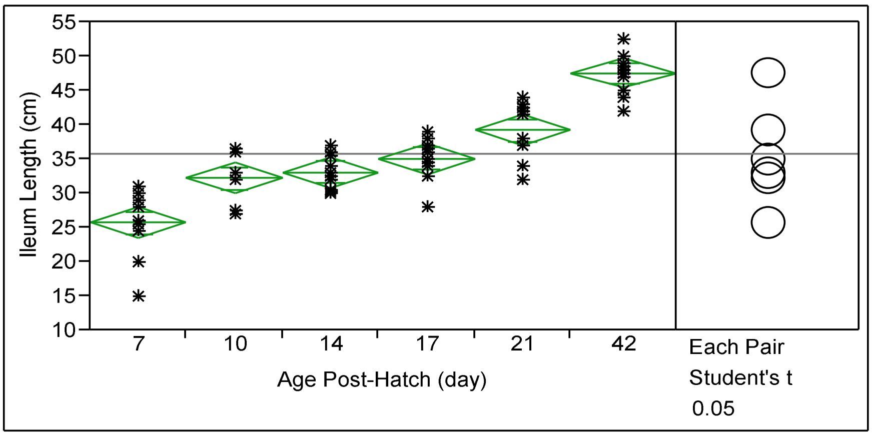


Figure 21 Mean Ileum Length of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 11 Comparison of Mean Ileum Length of the Heritage Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH					Mean Length (cm)
42	A				47.509091
21		B			39.200000
17			C		35.050000
14			C		33.070000
10			C		32.200000
7				D	25.700000

While there was significant growth from day 7 to 42 PH, there was a period between day 10 and 17 where little growth was observed. This stall in growth hovered around 32 to 35 cm in length for the 7 day span (Table 11). Average daily growth of the Heritage ileum showed a high rate of 2.167 cm/day (S.E. 0.525) from day 7 to 10 PH. During days 10 to 17 PH, where no significant growth occurred, these rates were much lower. By days 17 to 21 PH there was still a low rate of 1.034 cm/day

(S.E. 0.393). It declined again between days 21 and 42 PH to only 0.396 cm/day (S.E. 0.073) (Figure 22).

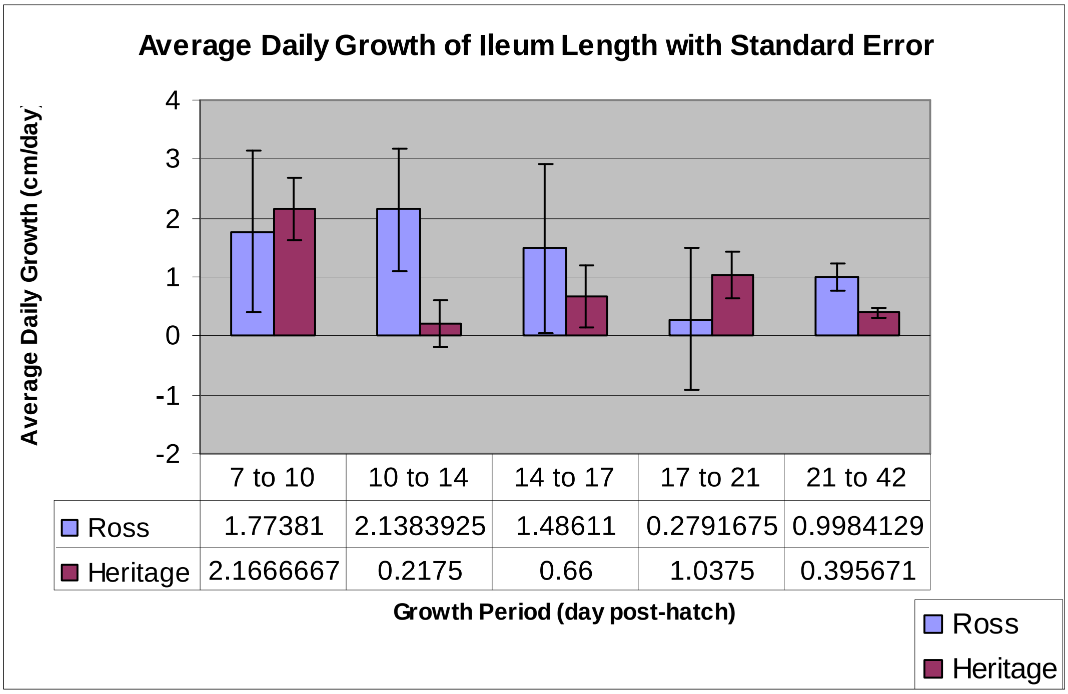


Figure 22 Average Daily Growth (ADG) of Ileum Length of Heritage and Ross708 Lines with Standard Error: ADG was calculated by the difference in mean mass divided by number of days of growth period.

ROSS708. The ileum of the Ross708 line had significant growth from day 7 to 42 PH. On day 7PH the average length was 38.750 cm (S.E. 2.816). By day 42 PH the ileum had reached 79.167 cm (S.E. 3.251). This was an increase of 40.417 cm or 2.04 fold (Figure 23, Table 12).

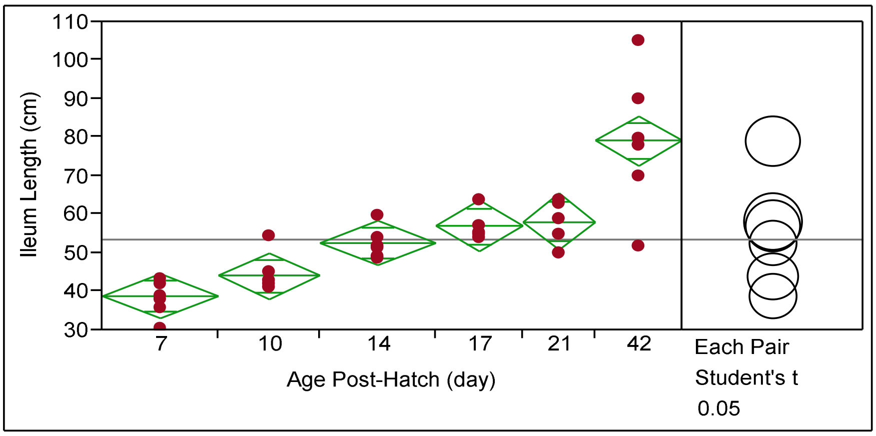


Figure 23 Mean Ileum Length of Ross708 Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 12 Comparison of Mean Ileum Length of the Ross708 Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH				Mean Length (cm)
42	A			79.166667
21		B		58.200000
17		B		57.083333
14		B		52.625000
10			C	44.071429
7			C	38.750000

Significant growth was seen overall however there was no significant growth observed between days 7 to 10 PH, nor during days 14 through 21 PH (Table 12). The average daily growth depicted these decreases in growth rate. Days 10 to 14 PH however, show a significant growth rate of 2.138 cm/day (S.E. 0.103) and then another drop during days 14 through 21 PH. At the end of the study days 21 to 42 PH had an increase in rate to 0.998 cm/day (S.E. 0.230) (Figure 22).

ROSS708 AND HERITAGE. From day 7 PH until day 42PH the Ross708 ileum was longer than the Heritage. During the period from day 7 to 10 PH the differences between the two lengths were 13.05 and 11.87 cm respectively. Afterwards the difference grew to 19.555 cm on day 14 PH and was somewhat maintained during that time. By day 42 PH however, the difference had increased to 31.658 cm with a final length of the Ross708 ileum being 79.167 cm (S.E. 3.251) and the Heritage being 47.409 cm (S.E. 1.061) (Figure 24).

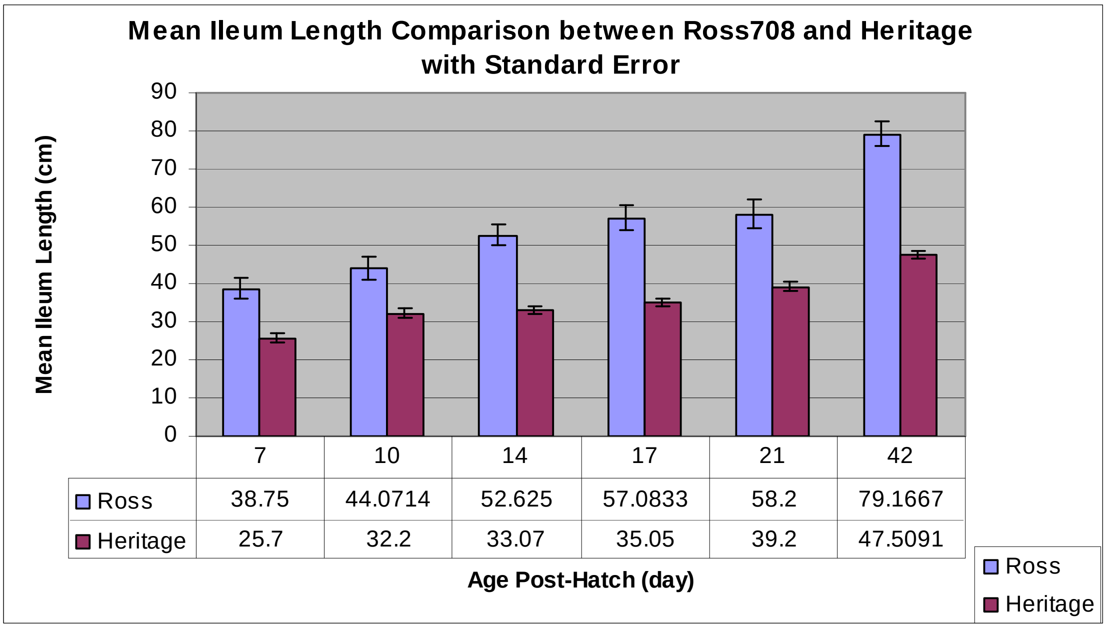


Figure 24 A Comparison of Mean Ileum Length by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

Cross-Sectional Area

Duodenum Cross-Sectional Area

HERITAGE. Due to a limiting number of measureable samples, only days 7 through 21 PH provided a sufficient number of measurements. There was a significant increase in average cross-sectional area in the Heritage duodenum from day 7 to 21 PH. Day 7 PH had an average of 0.106 cm^2 (S.E. 0.0123). By day 21 PH the area increased to 0.225 cm^2 (S.E. 0.011). This was an increase of 0.119 cm^2 or 2.12 fold (Figure 25, Table 13).

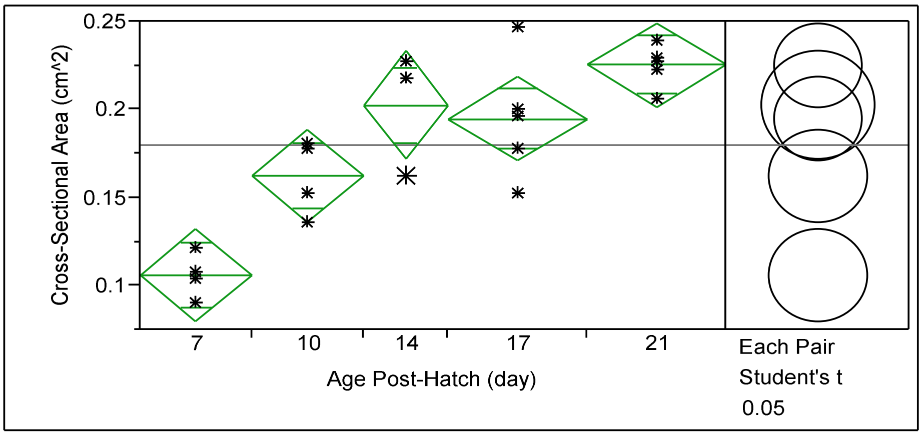


Figure 25 Duodenum Cross-Sectional Area of Heritage Line by Age Post-Hatch
Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 13 Comparison of Mean Duodenum Cross-Sectional Area of the Heritage Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH				Mean Cross-Sectional Area (cm^2)
21	A			0.22526000
17	A	B		0.19504000
14	A			0.20283684
10		B		0.16215000
7			C	0.10613936

While there was significant growth overall there were days that did not show an increase to the next sampling day. These days included days 10 to 17 and 14 to 21 PH (Table 13). The average daily growth of the cross-sectional area of the Heritage duodenum had a maximum rate of 0.0187 cm^2/day (S.E. 0.006). The rate then decreased during days 10 to 14 PH and decreased further from day 14 to 21 PH because these days had no significant growth (Figure 26).

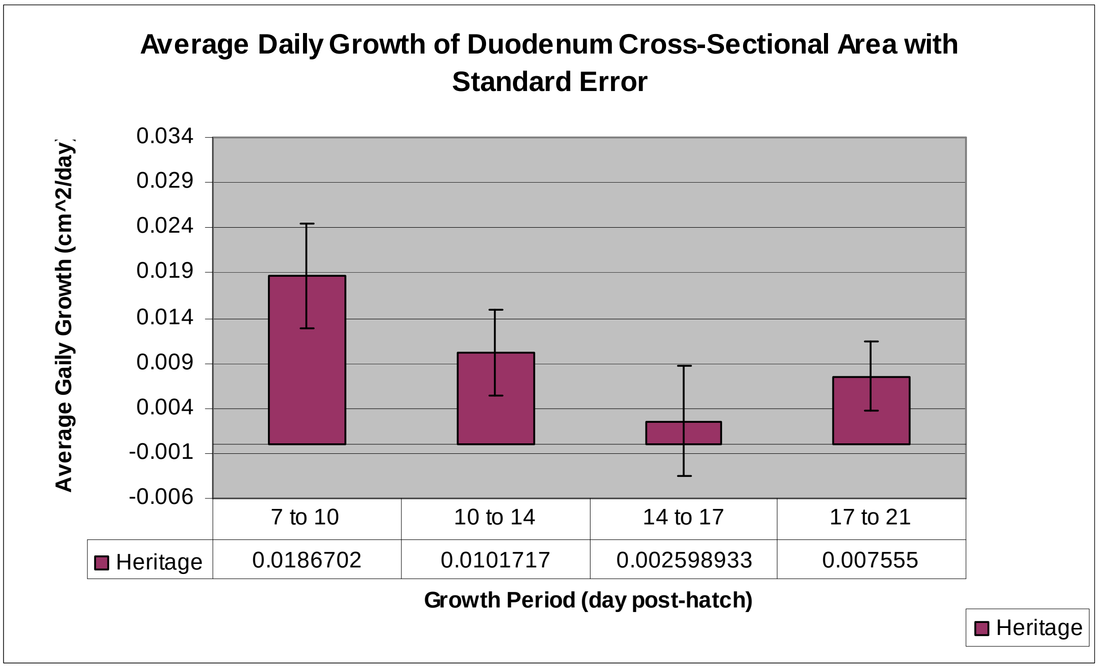


Figure 26 Average Daily Gain (ADG) of Duodenal Cross-Sectional Area of Heritage Line with Standard Error: ADG was calculated by the difference in mean mass divided by number of days

ROSS708. There was a problem with a sufficient amount of measurable samples with the Ross708 duodenum cross-sectional area, therefore, only days 7 and 14 PH were available for comparison. From day 7 to 14 PH there was significant growth in the cross-sectional area. Day 7 PH had an average area of 0.168 cm^2 (S.E. 0.013), while day 14PH had an average of 0.386 cm^2 (S.E. 0.016). This was an overall increase of 0.218 cm^2 or 2.30 fold (Figure 27, Table 14).

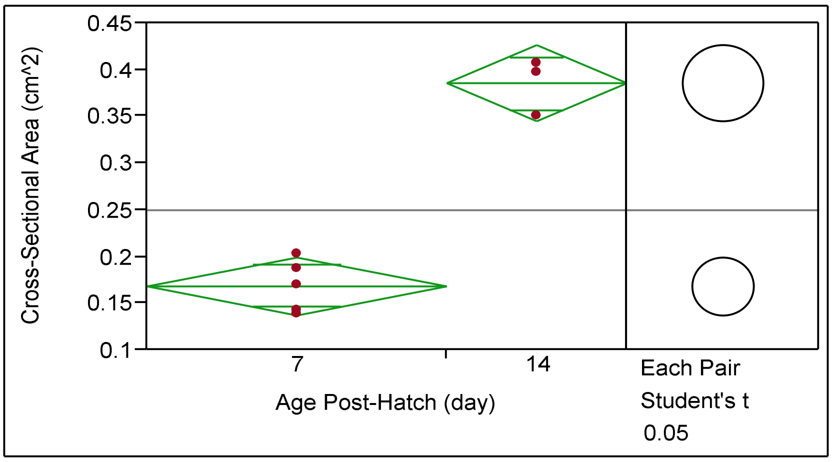
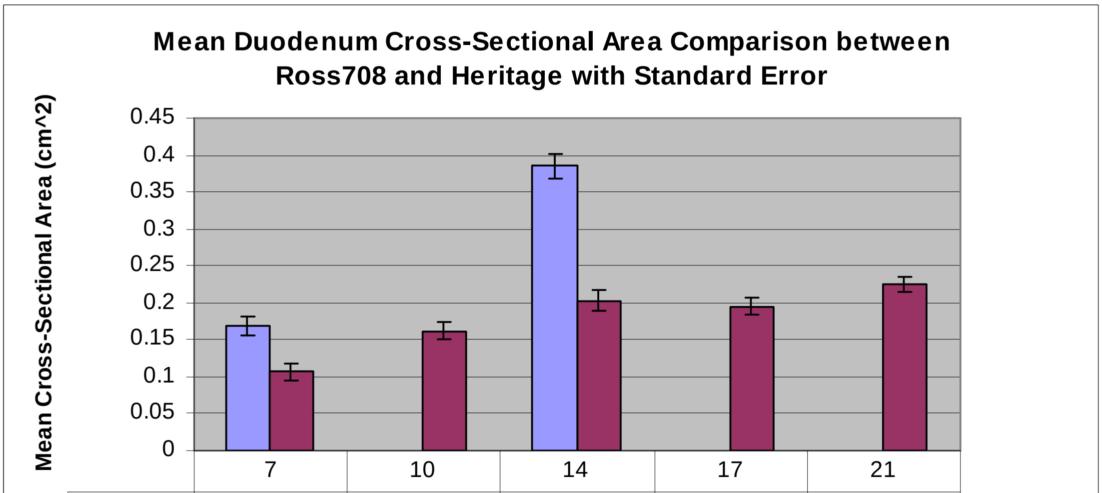


Figure 27 Duodenum Cross-Sectional Area of Ross708 Line by Age Post-Hatch
Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 14 Mean Duodenum Cross-Sectional Area of the Ross708 Line by Age Post-Hatch

Age PH	Number of Samples	Mean Cross-Sectional Area (cm^2)	Standard Error
7	5	0.168458	0.01276
14	3	0.385514	0.01647

ROSS708 AND HERITAGE. When comparing Ross708 and Heritage lines the former was significantly larger than the latter. On day 7 PH the Ross708 cross-sectional area was 0.062 cm^2 greater than its counterpart. By day 14 PH the difference between the two lines increased to 0.183 cm^2. On day 14 PH the Ross708 line was 90% larger than the Heritage line (Figure 28).



	7	10	14	17	21
Ross	0.168458		0.385514		
Heritage	0.106139	0.16215	0.202837	0.19504	0.22526

Figure 28 A Comparison of Mean Duodenum Cross-Sectional Area by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

Jejunum Cross-Sectional Area

HERITAGE. There was significant growth in the Heritage cross-sectional area of the jejunum from day 7 to 21 PH. On day 7 PH the average area was 0.082 cm^2 (S.E. 0.013). By day 21 the average cross-sectional area had increased to 0.169 cm^2 (S.E. 0.013). This was an increase of 0.087 cm^2 or 2.06 fold (Figure 29, Table 15).

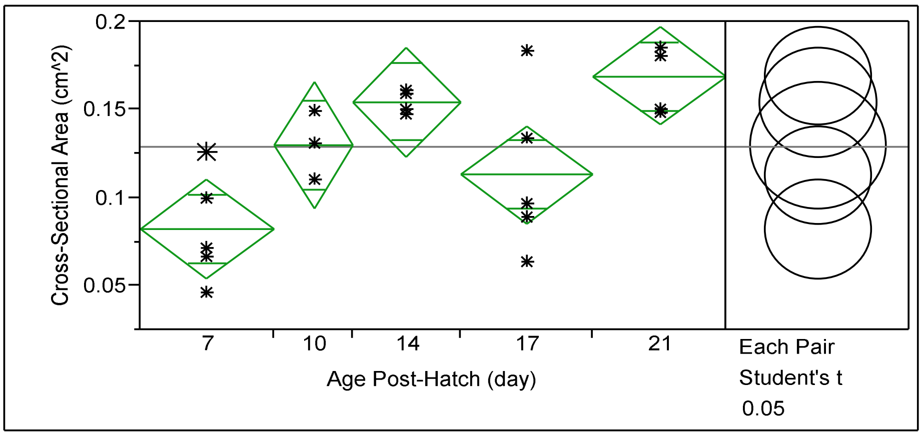


Figure 29 Jejunum Cross-Sectional Area of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 15 Comparison of Mean Jejunum Cross-Sectional Area of the Heritage Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH				Mean Cross-Sectional Area (cm^2)
21	A			0.16910000
17		B	C	0.11336000
14	A	B		0.15449735
10	A	B		0.13016667
7			C	0.08234633

There were multiple groups of sampling days which were not significantly different from each other. Days 7 and 17 and 10 to 17 PH did not show significant growth. Days 10, 14, and 21 PH were also not significantly different from one another. Day 17 PH was unlike any of the other sampling days. It showed a decreased average cross-sectional area of 0.113 cm^2 (S.E. 0.013) (Table 15).

ROSS708. In the Ross708 line there was significant growth of the cross-sectional area from day 7 to 14 PH. Day 7 PH had an average area of 0.138 cm^2 (S.E. 0.017). Day 14 had an average of 0.247 cm^2 (S.E. 0.021). This was an increase of 0.109 cm^2 or 79%. Day 17 PH was not significantly different from either day 14 or day 7 PH, therefore, it may be possible that there was a decrease in the cross-sectional area of the jejunum at that time (Figure 30, Table 16).

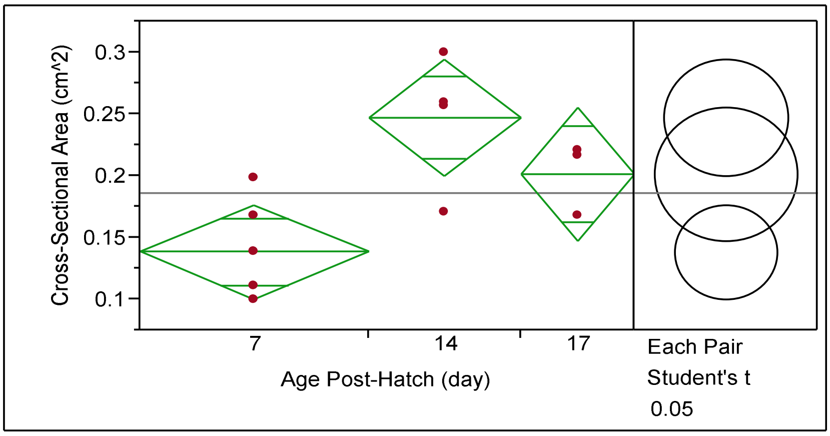


Figure 30 Jejunum Cross-Sectional Area of Ross708 Line by Age Post-Hatch
Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 16 Comparison of Mean Jejunum Cross-Sectional Area of the Ross708 Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH			Mean Cross-Sectional Area (cm^2)
17	A	B	0.20163333
14	A		0.24713822
7		B	0.13821666

ROSS708 AND HERITAGE. All three days available for comparison in the Ross708 line were significantly larger than the Heritage line. On day 7 PH the Ross708 cross-sectional area was greater by 0.685 cm^2. By day 14 PH that difference decreased to only 0.093 cm^2. The difference between the two lines on day 17 PH was 0.088 cm^2 (Figure 31).

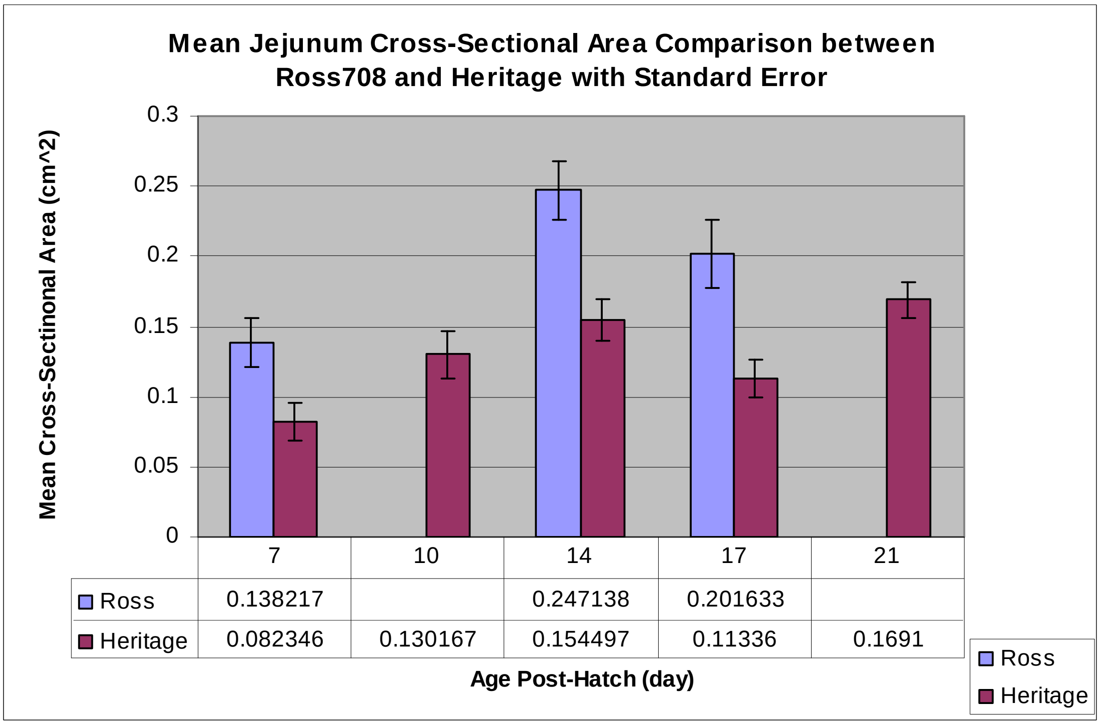


Figure 31 A Comparison of Mean Jejunum Cross-Sectional Area by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

Ileum Cross-Sectional Area

HERITAGE. There was growth seen in the cross-sectional area of the Heritage ileum from day 7 to 21 PH. Day 7 PH had an average of 0.0614 cm^2 (S.E. 0.013). Day 21 PH increased to an average of 0.113 cm^2 (S.E. 0.013). The growth of the cross-sectional area was very slow over the 14 days and the majority of the days were not significantly different from each other. Days 7 through 14 and 10 through 21 PH were not significantly different within the respective group (Figure 32, Table 17).

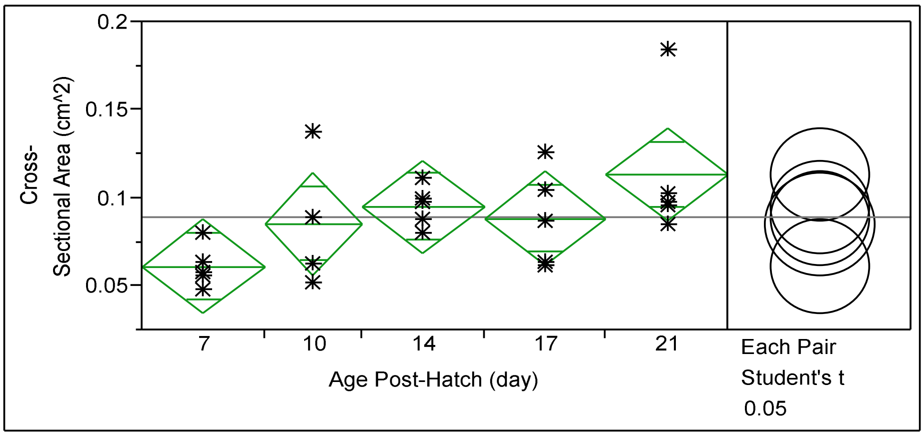


Figure 32 Ileum Cross-Sectional Area of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 17 Comparison of Mean Ileum Cross-Sectional Area of the Heritage Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH			**Mean Cross-Sectional Area (cm^2)**
21	A		0.11336000
17	A	B	0.08863400
14	A	B	0.09531340
10	A	B	0.08542500
7		B	0.06136506

ROSS708. The Ross708 line had significant growth from day 7 to 14PH. After day 14 PH there was no significant growth. Day 7 PH had an average cross-sectional area of 0.102 cm^2 (S.E 0.010) and increased to an average of 0.157 cm^2 (S.E. 0.013) by day 17 PH. This is an overall increase of 0.055 cm^2 or 54% (Figure 33, Table 18).

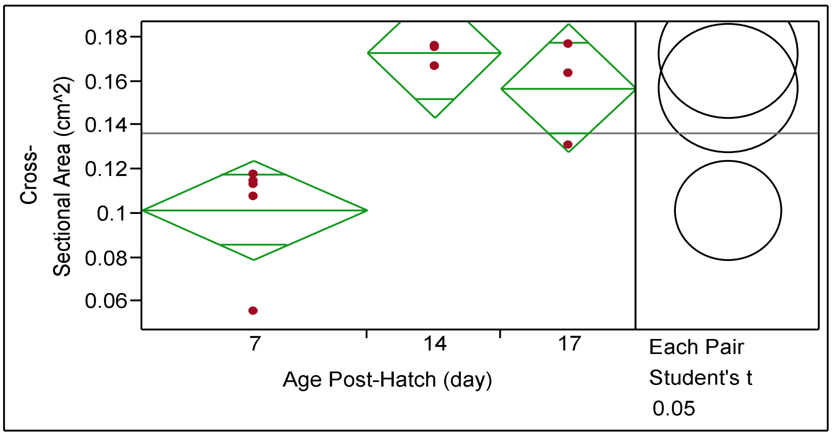


Figure 33 Ileum Cross-Sectional Area of Ross708 Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 18 Comparison of Mean Ileum Cross-Sectional Area of the Ross708 Line by Age Post-Hatch Letters represent significant difference (p value < 0.05) between ages. If an age has the same letter as another, they are not significantly different.

Age PH			Mean Cross-Sectional Area (cm^2)
17	A		0.15703333
14	A		0.17276094
7		B	0.10158367

ROSS708 AND HERITAGE. From day 7 to 17 PH , the Ross708 line had a larger cross-sectional area for the ileum. On day 7 PH the Ross708 line was 0.040 cm^2 greater than the Heritage. By day 17 PH the difference between the two lines increased to 0.068 cm^2. At day 17 PH the Ross708 ileum was 77% greater than the Heritage line (Figure 34).

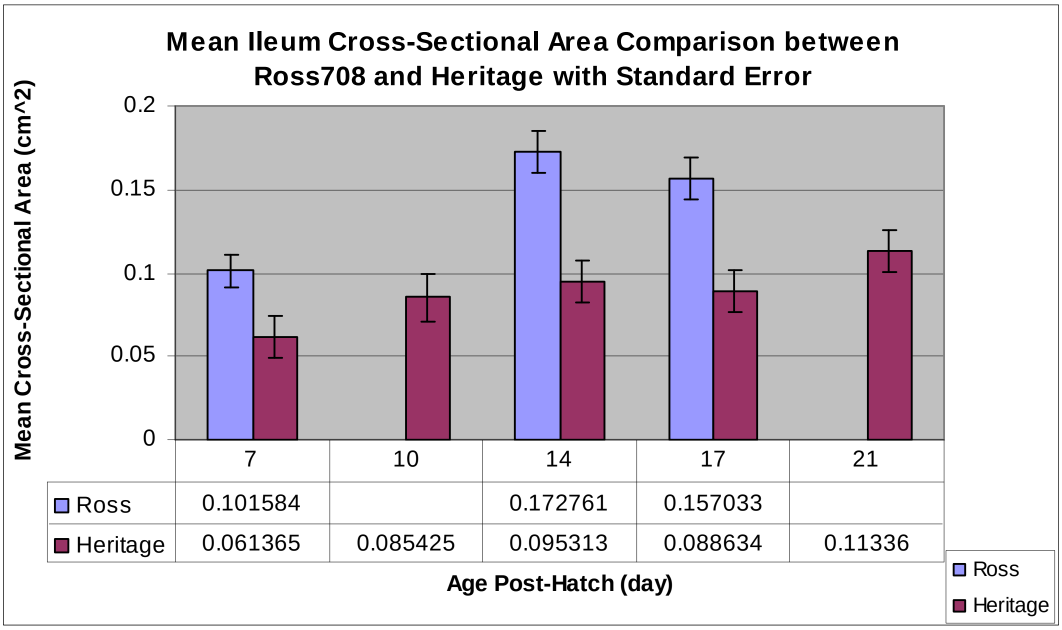


Figure 34 A Comparison of Mean Ileum Cross-Sectional Area by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

Villus Length

Duodenum Villus Length

HERITAGE. There was no significant difference in villus length between day 7 and day 14 PH. The average villus length from day 7 PH was 892.32 microns (S.E. 68.033) and 1027.31 microns (S.E. 33.329) for day 14 PH (Figure 35, Table 19).

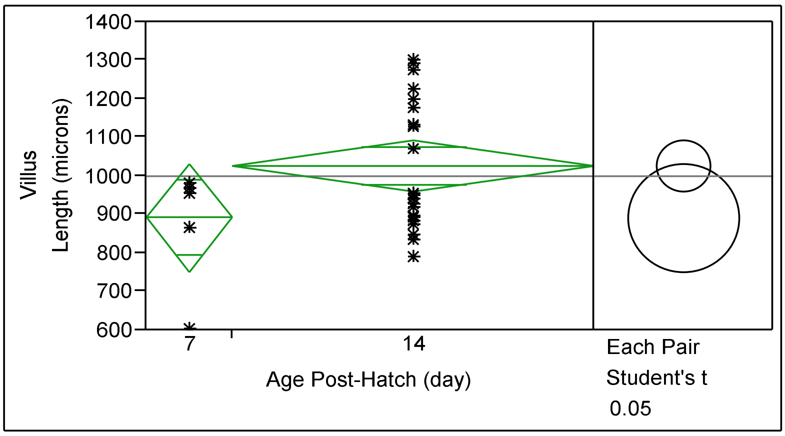


Figure 35 Duodenum Villus Length of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 19 Mean Duodenum Villus Length of the Heritage Line by Age Post-Hatch with Standard Error Standard error uses a pooled estimate of error variance.

Age PH	Number of Samples	Mean Villus Length (microns)	Standard Error
7	6	892.32	68.033
14	25	1027.31	33.329

<u>ROSS708.</u> The Ross708 line had no significant growth from day 7 to 14 PH. The average villus length of day 7 PH was 1220.56 microns (S.E. 75.182) with a length of 1184.24 microns (S.E. 75.182) on day 14 PH (Figure 36, Table 20).

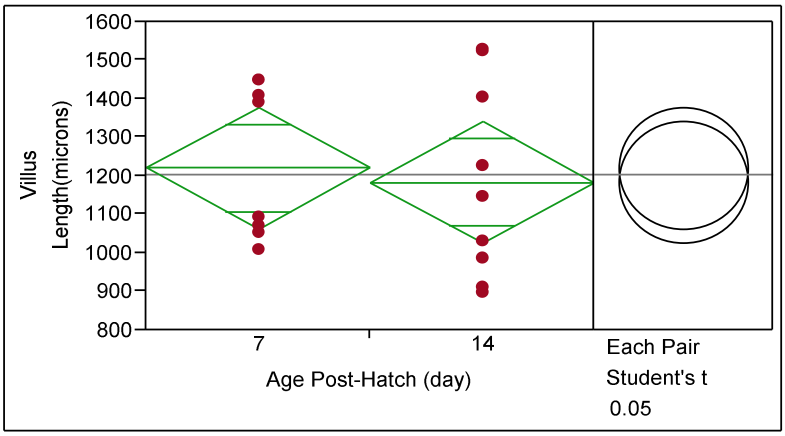


Figure 36 Duodenum Villus Length of Ross708 Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 20 Mean Duodenum Villus Length of the Ross708 Line by Age Post-Hatch with Standard Error Standard error uses a pooled estimate of error variance.

Age PH	Number of Samples	Mean Villus Length (microns)	Standard Error
7	9	1220.56	75.182
14	9	1184.24	75.182

<u>ROSS708 AND HERITAGE.</u> There was a significant difference between the Ross708 and Heritage lines for both day 7 and 14 PH individually. This difference did not increase because of the lack of growth between the two sampling days (Figure 37).

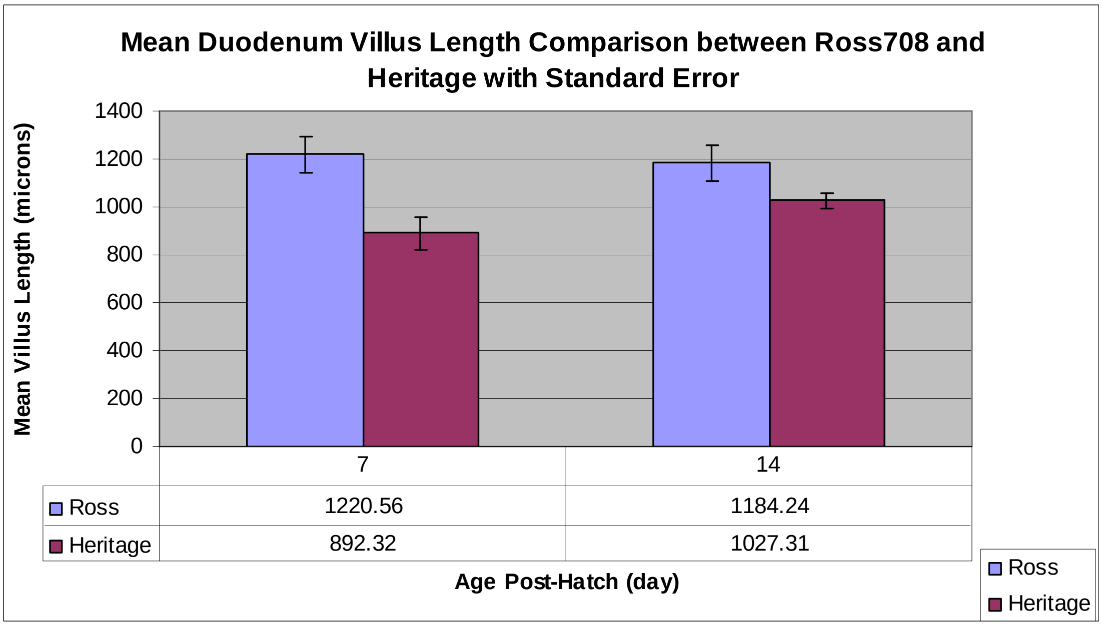


Figure 37 A Comparison of Mean Duodenum Villus Length by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

<u>Jejunum Villus Length</u>

<u>HERITAGE.</u> During days 7 to 14 PH there was significant growth seen in the Heritage jejunum villus length. Day 7 PH had an average of 450.526 microns (S.E. 42.132). After 7 days, villus length increased by 299.033 cm to reach 749.559 cm (S.E. 39.970) on day 14 PH. This is an increase of 66% over 7 days (Figure 38, Table 21).

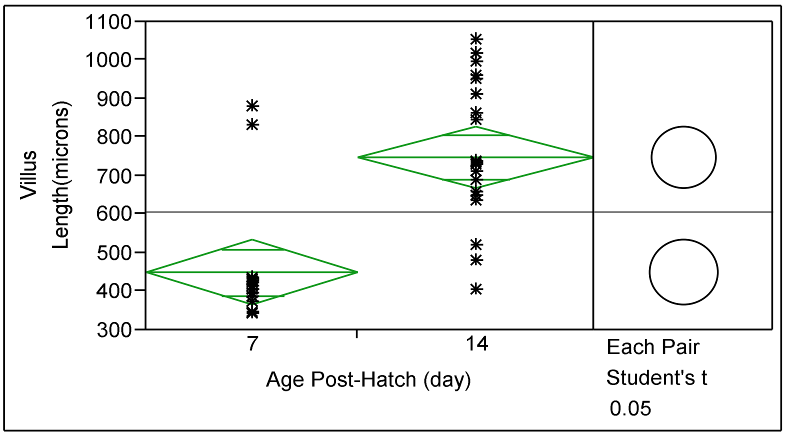


Figure 38 Jejunum Villus Length of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 21 Mean Jejunum Villus Length of the Heritage Line by Age Post-Hatch with Standard Error Standard error uses a pooled estimate of error variance.

Age PH	Number of Samples	Mean Villus Length (microns)	Standard Error
7	18	450.526	42.132
14	20	749.559	39.970

ROSS708. There was significant growth in villus length in the Ross708 jejunum during days 7 to 14 PH. Day 7 PH had an average length of 741.51 microns (S.E.33.667). Day 14 PH had an average villus length of 1207.220 microns (S.E. 29.783). This was an increase of 465.71 microns or 63% (Figure 39, Table 22).

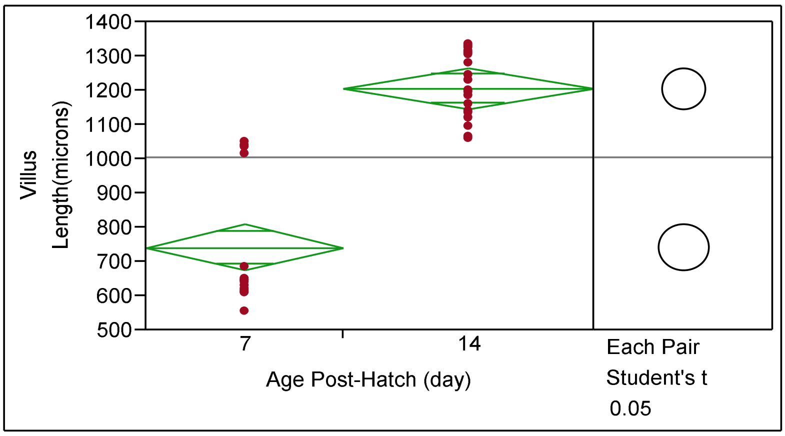


Figure 39 Jejunum Villus Length of Ross708 Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 22 Mean Jejunum Villus Length of the Ross708 Line by Age Post-Hatch with Standard Error Standard error uses a pooled estimate of error variance.

Age PH	Number of Samples	Mean Villus Length (microns)	Standard Error
7	18	741.51	33.667
14	23	1207.22	29.783

ROSS708 AND HERITAGE. On both, day 7 and day 14 PH the Ross708 line had significantly greater villus length. Day 7 PH showed a difference of 290.986 microns. The difference at day 14 PH was even greater, at 457.66 microns. By day 14 PH the Ross708 villus length was 61% longer than the Heritage line (Figure 40).

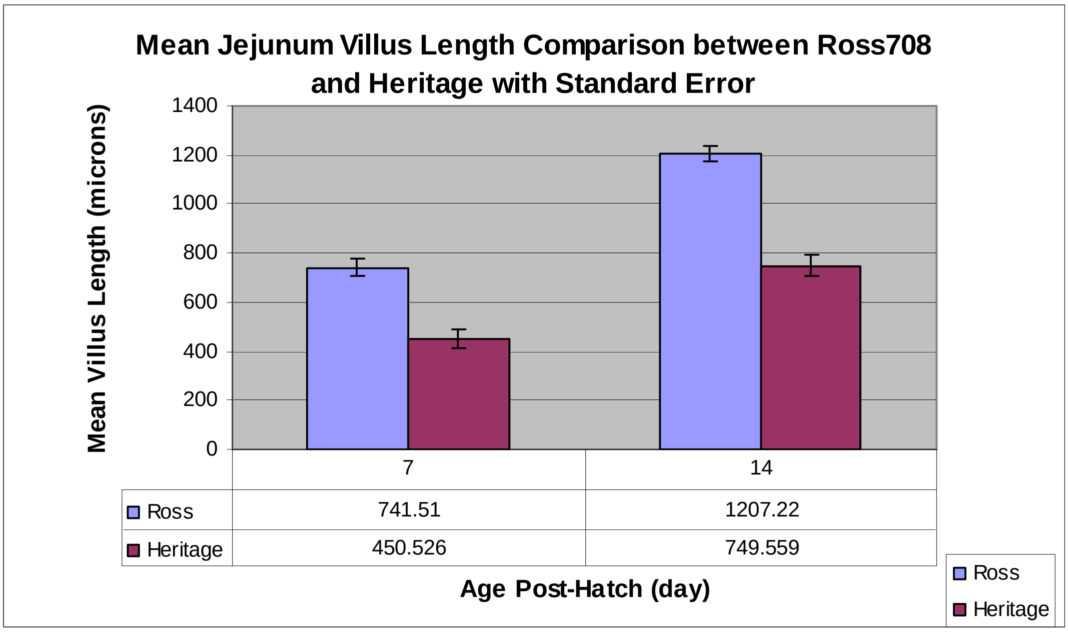


Figure 40 A Comparison of Mean Jejunum Villus Length by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

<u>Ileum Villus Length</u>

<u>HERITAGE.</u> There was significant villus length growth from days 7 to 14 PH in the Heritage ileum. The average length at day 7 PH was 336.613 microns (S.E. 30.953). The average increased to 486.406 microns (S.E. 27.068) on day 14 PH. This was an increase of 150.276 microns or 45% (Figure 41, Table 23).

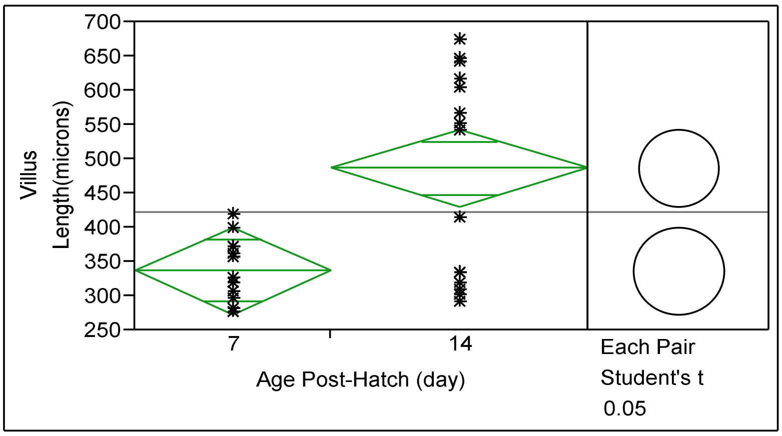


Figure 41 Ileum Villus Length of Heritage Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 23 Mean Ileum Villus Length of the Heritage Line by Age Post-Hatch with Standard Error Standard error uses a pooled estimate of error variance.

Age PH	Number of Samples	Mean Villus Length (microns)	Standard Error
7	13	336.613	30.953
14	17	486.406	27.068

ROSS708. The Ross708 line also showed an increase in villus length in the ileum over the 7 day sampling period. Day 7 PH had an average length of 541.263 microns (S.E. 37.238). Day 14 PH had an average of 675.323 microns (S.E. 42.224). This was an increase of 134.06 microns or 25% (Figure 42, Table 24).

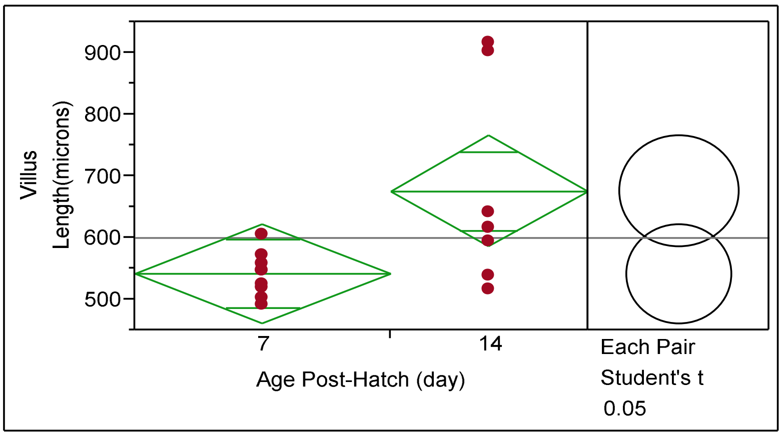


Figure 42 Ileum Villus Length of Ross708 Line by Age Post-Hatch Statistical significance with a p value < 0.05 was presented on the right side of the graph.

Table 24 Mean Ileum Villus Length of the Ross708 Line by Age Post-Hatch with Standard Error Standard error uses a pooled estimate of error variance.

Age PH	Number of Samples	Mean Villus Length (microns)	Standard Error
7	9	541.263	37.238
14	7	675.323	42.224

<u>**ROSS708 AND HERITAGE.**</u> For both day 7 and day 14 PH, Ross708 had a significantly longer ileum villus length. On day 7 PH the difference between the two lines was 204.65 microns. By day 14 PH the difference was 188.917 microns. The average villus length in the Ross708 line was 39% longer than that of the Heritage line by day 14 PH (Figure 43).

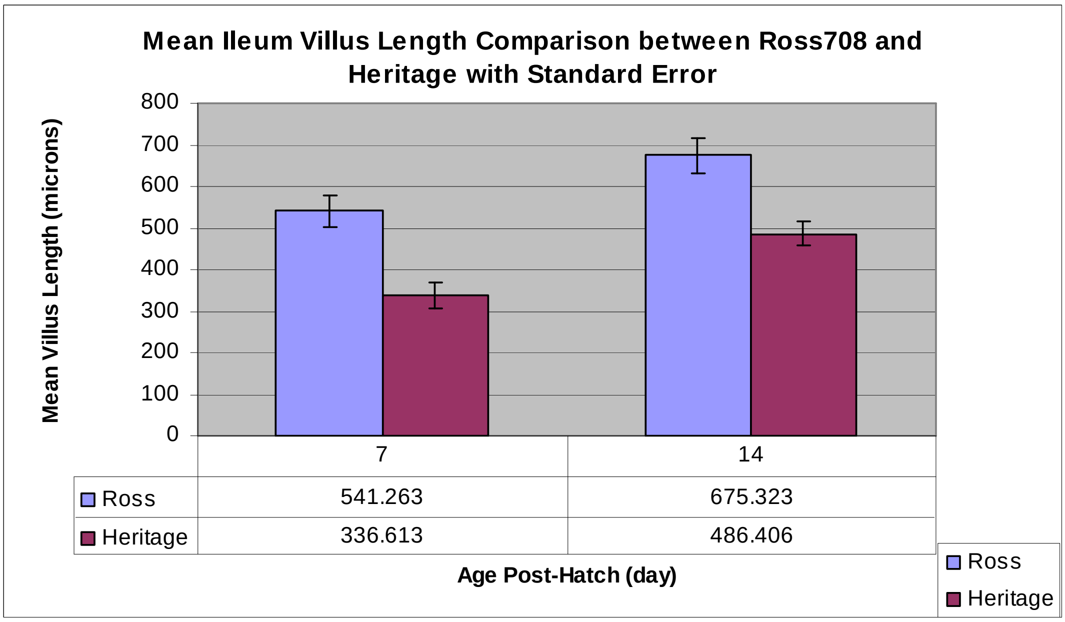


Figure 43 A Comparison of Mean Ileum Villus Length by Age Post-Hatch between Ross708 and Heritage lines with Standard Error Mean values for each line are displayed in the table below the associated age

Proliferating Cells

Proliferating cells were identified by immunohistochemical staining with Proliferating Cell Nuclear Antigen (PCNA). All sections of the small intestine displayed the same staining patterns. Also, there was no observable difference in staining between the two lines. The highest concentration of PCNA positive cells was located along the crypt of the villi (Figure 44A). In some samples there were positive cells along the length of the villus but there was no noticeable trend for this staining (Figure 45). Also, there were numerous samples where there was a high concentration of PCNA positive staining within the lamina propria but, again, there was no noticeable trend to explain this occurrence (Figure 44B).

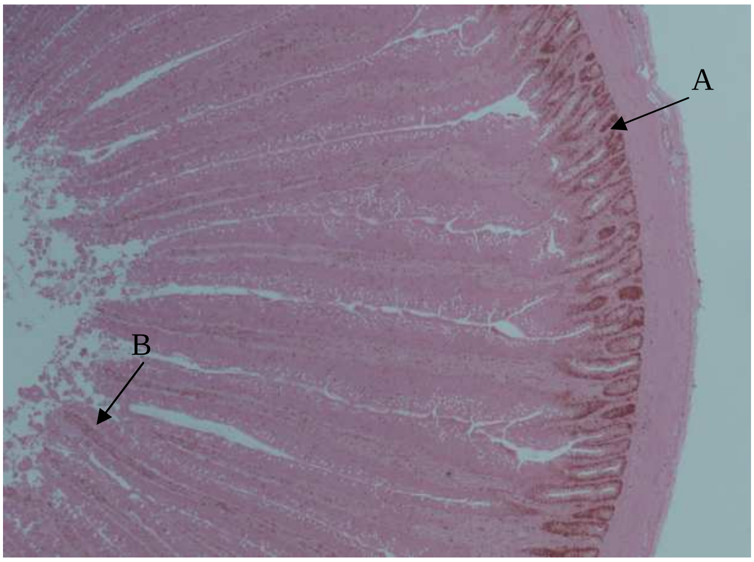


Figure 44 Small Intestine Tissue Stained for Proliferating Cell Nuclear Antigen (PCNA) with Eosin counterstain with Positive Cells in Crypt and Lamina Propria
Arrow (A) depicts the PCNA positive cells concentrated along the crypts of the villi. Arrow (B) depicts the PCNA positive cells found in the lamina propria of some samples

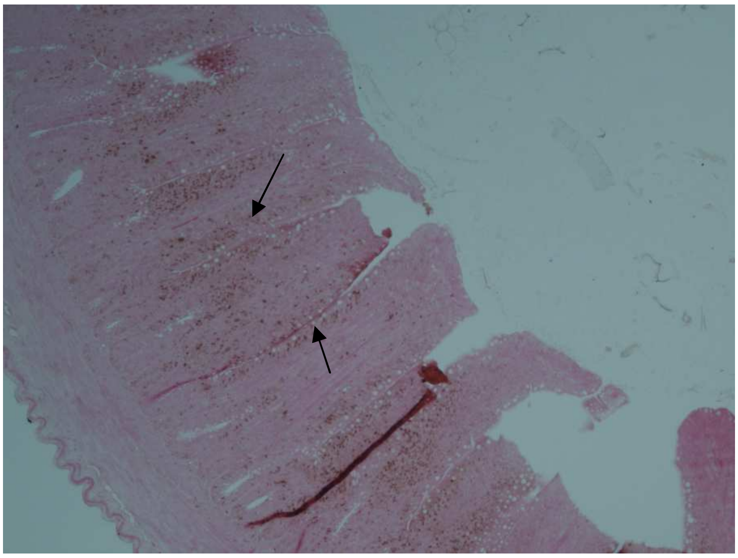


Figure 45 Small Intestine Tissue Stained for Proliferating Cell Nuclear Antigen (PCNA) with Eosin Counterstain with Positive Cells along Villus Length The two arrows are identifying areas where PCNA positive cells were concentrated along the sides of the villi.

Chapter 5

DISCUSSION

Intestinal Mass

The intestinal mass does not directly relate to the surface area active in absorption in the small intestine; rather, it can be used to support the claim that areas within the small intestine were increasing in size. Therefore, when both Ross708 and Heritage had a significant increase in mass overall, both lines should have reflected this in the growth of specific aspects of the small intestine, such as the villus length or intestinal length.

Both the Ross708 and Heritage lines saw significant increases in mass throughout the small intestine, however the modern line maintained a higher mass during the entire study. By day 42 PH the mass of the Ross708 duodenum was 34% greater, the jejunum was 2.25 fold greater, and the ileum was 2.92 fold greater than that of the Heritage line. Because the duodenum of the Ross708 did not show as large a difference in mass as the other two segments, it is possible that the duodenum does not have as much of an impact on the rapid growth rate of modern broilers. A previous study (Schmidt, unpublished) has shown that the duodenum of Heritage line is significantly larger than the comparably sized Red Jungle Fowl. Conceivably, selection prior to the early 20th century may have led to the maximum contribution to growth that can be made by the duodenum.

The average daily gain had a trend throughout all of the intestinal segments. From days 10 to 14 PH the Heritage line had a decrease in average daily gain while the Ross708 showed a significant increase during that time. Following that, days 14 to 17 PH had a slight recovery of the rate for the Heritage line while Ross708 continued to increase and reached its highest rate during this time. The Ross708 then decreased in rate and the two lines became nearly the same by days 21 to 42 PH. This significant spike in the mass of the Ross708 line during the decrease in the Heritage shows a specific change in the physiology and growth of the modern line. The selective breeding in the industry has altered the growth pattern in the broiler lines to increase intestinal mass at a much faster rate. This may be due to a change in regulation in gene expression or in the ability of the Ross708 line to recognize a certain growth factor. Further research is necessary to identify the causative agent.

Intestinal Length

The length of each intestinal segment was directly related to the available surface area in the small intestine. The duodenum of both lines were not noticeably different in length and therefore, most likely insignificant in causing the drastic change in growth rate seen in the modern broiler. The jejunum and ileum however, were significantly longer than the Heritage line from day 7 through day 42 PH. By day 42 PH the Ross708 segments were 54% and 66% longer, respectively. This increase in intestinal length supported the trend seen in the intestinal mass analysis, that the selective breeding specifically targeted these two caudal segments of the small intestine in order to increase absorption rate.

The average daily growth of the small intestine was similar among all three segments but did not exactly reflect the pattern seen in intestinal mass. From day

7 to 10 PH the Heritage duodenum and jejunum were increasing significantly faster than the respective Ross708 segments. In the ileum during that time, Ross708 had an extremely large standard error, making the difference between two lines insignificant. Day 10 to 14 PH had the same pattern in growth rates as before. All three segments of the Ross708 had increased in daily growth rate, while the Heritage drastically decreased. During this time the Heritage line was not allocating its energy into the growth of the small intestine. It must have been directed elsewhere while the Ross708 line continued to increase its growth rate and length throughout this period, increasing its surface area significantly.

Cross-sectional Area

The cross-sectional area of the small intestine was associated with the increase in surface area in a dimension other than that of the intestinal length, by allowing for an increase in the number of villi. The Ross708 line had a significantly larger cross-sectional area for all segments in all days available for comparison. By day 14 PH the duodenal cross-sectional area was 90% larger. By day 17 PH the Ross708 jejunum was 78% larger and the ileum was 77% larger than the Heritage line. These were all significant size differences and contributed greatly to the increase in surface area within the small intestine.

Average daily growth was not possible to analyze between the two lines because there was not sufficient data from certain sampling days. This made it difficult to compare the growth rates directly.

Villus Length

The average length of the villi in the small intestine related directly to the surface area available within that segment of intestine. An increase in average villus length lended to a significant increase in overall surface area. There was no significant growth seen between days 7 to 14 PH in the duodenum in either line; however the Ross708 line was 15% longer than the Heritage during that time. The jejunum and ileum did have significant growth during that time period and for those segments the Ross708 was 61% and 39% longer, respectively.

Proliferating Cells

During the qualitative analysis for proliferating cells in the small intestine there was no notable difference in quantity of cells between lines, ages, or segments. The majority of the proliferating cells were located within the crypts of the villi. There were some samples which depicted an increase in staining in the lamina propria but no trend was seen for these results. Further research is required to understand the cause of this unexpected staining.

Chapter 5

CONCLUSION

Selective breeding of the broiler chicken during the mid-twentieth century targeted the growth rate and breast muscle size of the poultry lines during that time. While the two aforementioned changes were obvious in the industry, it was unclear physiologically, what was altered to allow for this drastic change. The data from this study suggests that the selective breeding targeted the growth patterns of the small intestine, specifically the jejunum and ileum. These two segments of the small intestine were increased in overall length, mass, cross-sectional area, as well as villus length from the baseline, Heritage line. All of these increases provided a significantly larger surface area within the small intestine to promote efficient absorption and utilization of nutrients, therefore, increasing growth rate and tissue deposition.

Further research is necessary to determine the exact cause of this dramatic increase in size of the small intestine. A study in mice found that the Wnt5a gene was essential for intestinal elongation.[1] While this gene has not be identified in the chicken, it is possible that there is a similar gene controlling the phenotype. In addition, because the majority of measurements showed the Ross708 line surpassing the Heritage line by day 7 PH, the first sampling day, it may be worthwhile to investigate the growth of the small intestine *in ovo* and immediately after hatch to determine if there is a specific age at which the two lines diverge.

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