fortune Journals

Research Article

INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
ISSN: 2231-4490

Comparative Intestinal Histological Features Observed in 1940 Leghorn vs. 2016 Leghorn-Based Commercial Laying Hens Fed Representative Diets

Dannica C Wall[1], Ramon D. Malheiros[1], Kenneth (Ken) Anderson[1]*, Nick Anthony[2]

Abstract

Nutrient absorption is essential for all stages of life and production in Leghorn hens. The selection for production traits, specifically linked to promoting digestive utilization of feed, has resulted in improved feed efficiency and ultimately increased egg production. Digestion of ingested feed and nutrient absorption takes place within the small intestine by the crypts and villi of the absorptive epithelium, specifically in the crypts and microvilli. Understanding the absorptive epithelium and its structural changes, related to genetic selection and improved feed efficiency, is important for continued efforts to improve egg production. The objective of this study was to determine and compare the histological changes in the duodenum, jejunum, and ileum of the 1940 Leghorn vs. the 2016 Leghorn-based commercial laying hens fed diets representative of those fed by the industry during the respective years of production.

In order to compare the effect of dietary regimen on intestinal histology, a total of 320 White Leghorn laying hens of two different strains were distributed into a 2 × 2 factorial arrangement. The factors were diet and strain: 1940 diet, 2016 diet, 1940 layer, and 2016 layer. The 4 experimental treatment groups: 1). 2016 hen on 1940 diet, 2). 2016 hen on 2016 diet, 3). 1940 hen on 1940 diet, and 4). 1940 hen on 2016 diet with 8 replicates per treatment. Five villi samples for histological analysis were taken from the three segments of the small intestine: the duodenum, jejunum, and ileum, and analyzed from 12 birds representing each treatment. Histologically the villus height, epithelium height, crypt depth, mucosal enlargement factor, and the tunica muscularis thickness were measured in the duodenum, jejunum, and ileum. The experiment was a completely randomized design, and all data were analyzed with a one-way analysis of variance (ANOVA). Tukey's test was applied to compare the significance of differences between the means. Statistical significance was considered at $P< 0.05$. Significant differences were demonstrated among all treatment groups for the duodenum, jejunum, and ileum. Results from this data suggest that the functionality of the small intestines possibly influences response to dietary manipulations. Further studies are necessary to evaluate the effects of diet on intestinal functionality and nutrient digestibility.

Keywords: Histology; Small intestines; Genetics; Nutrition; Leghorns

Introduction

The avian gastrointestinal tract comprises functional anatomical and histological characteristics that are critical for feed conversion efficiency [1,2]. The roles in the final phase of nutrient digestion and assimilation take place in the small intestine, via the intestinal villi and absorptive epithelial cells [3].

Affiliation:

[1]Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC, USA

[2]University of Arkansas, Land-grant University in Fayetteville, Fayetteville, Arkansas, United States

***Corresponding author:**
Kenneth (Ken) Anderson, Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC, USA.

Citation: Dannica C Wall, Ramon D. Malheiros, Kenneth (Ken) Anderson, Nick Anthony. Comparative Intestinal Histological Features Observed in 1940 Leghorn vs. 2016 Leghorn-Based Commercial Laying Hens Fed Representative Diets. International Journal of Plant, Animal and Environmental Sciences. 13 (2023): 116-125.

Received: October 19, 2023
Accepted: October 30, 2023
Published: November 15, 2023

Wall DC, et al., Int J Plant Anim Environ Sci 2023
DOI:10.26502/ijpaes.4490157

The chicken's gastrointestinal tract consists of 3 components belonging to the glandular stomach (proventriculus), gizzard (ventriculus), and intestine (small and large) [1]. To promote maximal absorption of dietary components the intestinal mucosa is highly convoluted and specialized [4]. The feeding process occurs when the feed material is ingested, moisturized, ground into small particles, acidified, and attacked by endogenous enzymes [3]. The macronutrients are further broken down into monosaccharides, dipeptides and amino acids, free fatty acids, and monoglycerides to be absorbed. Compared to other monogastric animal species, the digestive tract of poultry is similar, therefore, both digestion and absorption of ingested feed occur in the small intestine making it essential for further research.

Small Intestines

The small intestine is subdivided into 3 compartments: duodenum, jejunum, and ileum. Most digestion and almost all absorption of nutrients take place in the small intestine. The duodenal loop is the first segment of the small intestines containing the outlet of the pancreatic and bile ducts. While there is short retention of ingesta occurring in the duodenal loop, the acidic contents from the gizzard are mixed with bile and pancreatic juices via gastroduodenal refluxes [3]. The second segment belongs to the jejunum extending from the ducts to the Meckel's diverticulum. The role of the jejunum is to digest and absorb all the major nutrients. Retention time within this segment is approximately only 40 to 60 minutes, half the time retention time of the ileum due to a larger amount of material entering this segment compared to jejunum and ileum [3]. Absorption of digested products from fat, starch, and protein is completed by the end of the jejunum. The third segment of the small intestines is the ileum ending at the ileo-caeco-colic junction. The length of the ileum has been reported to be the same length as the jejunum however, the weight of this segment is much lower. Some digestion and absorption of fat, protein, and starch take place in the ileum, but the main role of the ileum is to absorb water, minerals, and electrolytes.

Intestinal villi are protrusions of the lamina propria into the intestinal lumen where they enlarge the digestive and absorptive area [5]. The surface of the villus is covered with columnar epithelial cells that contain absorptive, goblet, and entero-endocrine cells. Towards the base of the villi is a cell layer that lines the inside surface of tubular indentations of intestinal crypts that reach the mucosal muscle layer [5]. The epithelial cells which have an apical characteristic covered by a dense matting of microvilli thus forming a brush border is produced by the epithelium being folded into villi [1]. This increases the small intestinal surface area for absorption by about 600-fold resulting in a higher capacity for nutrient absorption [6]. The intestinal function is presumed to determine by measuring the 1) villus height, cell area, and cell mitosis utilizing light microscopy, 2) morphological observations of the villus surface utilizing an electron scan microscope, and 3) observing the ultrastructure of epithelial cells utilizing a transmission electron microscope.

The objective of this study was to determine and compare the histological changes exhibited in the duodenum, jejunum, and ileum of 1940 Leghorn vs. 2016 Leghorn-based commercial laying hens fed on representative diets.

Materials and Methods

Bird Management and Diet

A total of 320 16-week-old laying hens (WL40 and WL36) were transported and housed in a laying facility at North Carolina Chicken Education Unit in Raleigh, NC. The rearing of these birds was carried out in accordance with the NCSU IACUC. All birds were randomly divided into 4 treatment groups with 80 hens per treatment. There were 2 hens per cage consisting of 8 replicates. The 4 experimental treatment groups: 1). 2016 hen on 1940 diet, 2). 2016 hen on 2016 diet, 3). 1940 hen on 1940 diet, and 4). 1940 hen on 2016 diet. Feed and water were provided ad libitum throughout the experimental period of 69 weeks (Table 1). Feed intake and body weight gain were measured on a 28d period resulting in 12 cycles. Hens were given a 2-week acclimation period to adjust to the new environment and diets. All animal management and sampling procedures were in accordance with the NCSU IACUC.

Gastrointestinal Organ Measurements

At the end of the trial, 12 birds from each treatment group were collected, weighed, and euthanized by cervical dislocation. The visceral organs were removed. The lengths of the small intestines were measured and then the contents of those segments, including the gizzard and proventriculus were removed. The length of each intestinal segment was measured, i.e., duodenum (from the gizzard junction to the end of the (pancreatic) duodenal loop, jejunum (from the aboral pancreatic loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to the ileocecal junction), and colorectum (from the ileocecal junction to the cloaca). The weights of the glandular stomach, gizzard, and intestine segments were determined using an electronic laboratory balance (scale specifics) with a measurement accuracy of 0.01g.

Sample Collection and Histology Examination

At the end of the trial, samples (size 5cm) of the mid-segment of the jejunum, duodenum, and ileum (1 cm proximal to the ileocecal junction) were excised and fixed in neutral buffered formalin (10%, pH 7, 72 h, ambient temperature).

Morphometric Analysis

Three cross-sections for each intestinal segment (duodenum, jejunum, and ileum) were fixed in a 70% ethanol

Wall DC, et al., Int J Plant Anim Environ Sci 2023
DOI:10.26502/ijpaes.4490157

Table 1: Feed Ingredients and Mash Diet1 Compositions.

Ingredients	2016 Layer Diet[2]	1940 Layer Diet[2]
Corn	940.5	1146.38
Soybean Meal	718	232.57
Alfalfa Meal		305.97
Limestone, gr.	145.5	124.2
Coarse limestone	50	
Fat	110	
Phosphate Mono/D	17.6	
Salt	6.8	5
D.L. Methionine	2.9	
T-Premix	1	
Sodium Bi-carb	2	
Prop Acid 505	1	
Choline CL 60%	1.3	4
Hy-D 62.5 mg/lb		
Trace Min PMX[3]	1	
L-Vitamin PMX[4]	1	
.06% Selenium[5]	1	
Ronozyme HI P (GT)	0.4	
Total	**2000**	**2000**
Calculated Analysis		
Protein %	20.8	20
ME kcal/kg	2926	1330
Calcium %	4.1	0.9
A. Phos %	0.45	0.42
Lysine %	1.2	0.82
TSAA %	0.81	

[1]Diets were acquired from the North Carolina State University Feed Mill in mash form

[2]Lay diet fed starting no later than 17 weeks

[3]Vitamin premix supplied the following per kilogram of feed: vitamin A, 26,400 IU; cholecalciferol, 8,000 IU; niacin, 220 mg; pantothenic acid, 44 mg; riboflavin, 26.4 mg; pyridoxine, 15.8 mg; menadione, 8 mg; folic acid, 4.4 mg; thiamin, 8 mg; biotin, 0.506 mg; vitamin B12, 0.08 mg; and ethoxyquin, 200 mg. The vitamin E premix provided the necessary amount of vitamin E as DL-α-tocopheryl acetate.

[4]Mineral premix supplied the following per kilogram of feed: 120 mg of Zn as ZnSO4H2O, 120 mg of Mn as MnSO4H2O, 80 mg of Fe as FeSO4H2O, 10 mg of Cu as CuSO4, 2.5 mg of I as Ca(IO3)2, and 1.0 mg of Co as CoSO4.

[5]Selenium premix provided 0.3 ppm Se from sodium selenite.

solution. Each segment was then embedded in paraffin, and a 2-µm section of each sample was placed on a glass slide and stained with hematoxylin and eosin for examination with a light microscope. A total of 10 intact, well-oriented crypt-villus units were measured in each type of tissue from each hen. The parameters evaluated were villus height, villus base width, villus surface area, lamina propria thickness, and crypt depth. Morphological parameters were measured using the Image-Pro Plus v 4.5 (Media Cybernetics, Rockville, MD).

The following parameters in H&E-stained sections were measured on one cross-section per bird and intestinal segment:

- Villus height: Ten villi were randomly selected and measured from their base at the level of the crypt's entrance through to their distal tips. Only full finger-shaped and well-oriented villi were used
- Epithelium height: Ten jejunal, duodenal, and ileal epithelial cells of different villi were measured from the basement membrane to the tip of their microvilli
- Crypt depth: Ten crypts were measured from the crypt's base to the closest villus base. The ratio of villus height to crypt depth was calculated by dividing villus height by crypt depth
- Mucosal enlargement factor of the villus: Here the continuous length of the mucosal surface of ten adjacent villi was measured. The length of the corresponding underlying lamina muscularis mucosae was measured.
- Thickness of the tunica muscularis: This parameter was defined as the distance between the lamina muscularis mucosae internally and the tunica serosa externally. Ten measurements were performed per intestinal segment.

Statistical Analysis

All statistical analysis was performed in SAS, version 9.4 (SAS Institute, Inc., Cary, NC). Differences were considered significant when $P \leq 0.05$. Main effects and interaction effects were evaluated for hen strain and diet. The experiment was a completely randomized design, and all data were analyzed with a one-way analysis of variance (ANOVA). Tukey's test was applied to compare the significance of differences between the means. The results were reported as means ± SE.

Results

The overall intestinal length measurements of the small intestine (mm) are illustrated in Table 2. Overall, there were significant differences observed between the two different strains and the diets used. At the strain effect, hens of the 2016 strain were significantly different ($P \leq 0.05$) having longer small intestinal measurements when compared to the hens of the 1940 strain. Similar results were observed in hens fed on the 2016 diet being significantly different ($P \leq 0.05$)

Wall DC, et al., Int J Plant Anim Environ Sci 2023
DOI:10.26502/ijpaes.4490157

when compared to hens fed on the 1940 at the diet effect in which hens fed the 2016 diet had longer small intestinal measurements. There was also a significant (P≤0.5) interaction between strain and diet for the intestinal measurements.

Histological measurements consisting of the villus height, villus tips and bottom width, crypt depths and muscularis of the duodenum, jejunum, and ileum are represented in Table 3, 4, and 5. In the duodenum for the strain effect, hens of the 2016 strain had a significant difference (P≤0.05) with the villus height, villus tip width, and villus bottom width when compared to hens fed on the 1940 strain. However, hens of the 1940 strain had significantly (P≤0.05) deeper crypt depth and thicker muscularis when compared to hens of the 2016 strain. At the diet effect, similar results were observed as the hens fed on the 2016 diet had significant difference (P≤0.05) with higher villus height, and wider villus tip, and bottom width when compared hens fed on the 1940 diet. Yet, hens fed on the 1940 diet were significantly different (P≤0.05) having deeper crypt depth and thicker muscularis when compared to hens fed on the 2016 diet. Significant differences (P≤0.05) of the interaction between strain and diet were observed for each of the parameters measured.

In the jejunum for the strain effect, a slight significance (P≤0.05) was observed between hens of the 1940 strain having a slightly higher villus height when compared to hens of the 2016 hen. There were significant differences (P≤0.05) between hens of the 2016 strain having wider villus tips and bottom width when compared to hens of the 1940 strain. However, hens of the 1940 strain were significantly different (P≤0.05) having a deeper crypt depth and thicker muscularis when compared to hens of the 2016 strain. At the diet effect, similar results were observed between the strains with hens of the 2016 strain having a slight significant difference (P≤0.05) of a higher villus height when compared to hens of the 1940 strain. Hens fed on the 1940 diet had a significant difference (P≤0.05) with having a wider villus tips and bottom width when compared to hens fed on the 2016 diet, however, hens fed on the 2016 diet had deeper crypt depths and thicker muscularis' when compared to hens fed on the 1940 diet. Significant differences (P≤0.05) were observed between strain and diet for all the parameters measured.

Table 2: Overall Intestinal Measurements of Small Intestines (mm).

Length Measurement[1]	
Main Effects	
Strain (S)	
2016	383.28[a]
1940	359.79[b]
SEM	0.02
p-value	0.003
Diet (D)	
2016	391.82[a]
1940	344.76[b]
SEM	0.03
p-value	0.004
Interactions	
S × D	0.027

[1]Values are presented as Means
Note. Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.

Table 3: Histological Measurements of the Duodenum (according to main effects).

	Villus Height (µm)	Villus Tip Width (µm)	Villus Bottom Width (µm)	Crypt Depth (µm)	Muscularis (µm)
Main Effects					
Strain (S)					
2016	1591[a]	186[a]	252[a]	178[b]	161[b]
1940	1345[b]	177[b]	249[b]	193[a]	175[a]
SEM	23.51	4.35	5.49	7.31	5.32
p-value	0.0006	0.011	0.044	0.037	0.013
Diet (D)					
2016	1534[a]	188[a]	258[a]	184[a]	165[b]
1940	1321[b]	175[b]	243[b]	187[a]	171[a]
SEM	23.51	4.35	5.49	3.66	2.65
p-value	≤0.0001	0.054	0.043	0.168	0.015
Interaction					
S x D	≤0.0001	0.028	0.037	0.045	0.054

[1]Values are presented as Means
Note: Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.

Citation: Dannica C Wall, Ramon D. Malheiros, Kenneth (Ken) Anderson, Nick Anthony. Comparative Intestinal Histological Features Observed in 1940 Leghorn vs. 2016 Leghorn-Based Commercial Laying Hens Fed Representative Diets. International Journal of Plant, Animal and Environmental Sciences.13 (2023): 116-125.

Wall DC, et al., Int J Plant Anim Environ Sci 2023
DOI:10.26502/ijpaes.4490157

Table 4: Histological Measurements of the Jejunum (according to main effects).

	Villus Height (µm)	Villus Tip Width (µm)	Villus Bottom Width (µm)	Crypt Depth (µm)	Muscularis (µm)
Main Effects					
Strain (S)					
2016	599b	130a	148.5a	106b	160b
1940	650a	121b	147b	124a	165a
SEM	18.52	6.33	7.74	4.32	6.71
p-value	0.023	0.041	0.042	0.023	0.024
Diet (D)					
2016	633a	138a	163a	119a	173a
1940	617b	114b	133b	112b	153b
SEM	18.53	6.34	7.74	4.33	6.72
p-value	0.053	0.018	0.015	0.052	0.023
Interaction					
S × D	0.047	0.024	0.038	0.039	0.036

[1]Values are presented as Means
Note: Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.

Table 5: Histological Measurements of the Ileum (according to main effects).

	Villus Height (µm)	Villus Tip Width (µm)	Villus Bottom Width (µm)	Crypt Depth (µm)	Muscularis (µm)
Main Effects					
Strain (S)					
2016	599b	130a	148.5a	106b	160b
1940	650a	121b	147b	124a	165a
SEM	18.52	6.33	7.74	4.32	6.71
p-value	0.023	0.041	0.042	0.023	0.024
Diet (D)					
2016	633a	138a	163a	119a	173a
1940	617b	114b	133b	112b	153b
SEM	18.53	6.34	7.74	4.33	6.72
p-value	0.053	0.018	0.015	0.052	0.023
Interaction					
S × D	0.047	0.024	0.038	0.039	0.036

[1]Values are presented as Means
Note: Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.

In the ileum for the strain effect, fluctuation between the strains resulted in significance (P≤0.05). For the villus height, hens of the 1940 strain had the higher height when compared to hens of the 2016 strain. Hens of the 2016 strain had wider villus tips and bottom width when compared to hens of the 1940 strain. The crypt depth was deeper and the muscularis was thicker in hens of the 1940 strain when compared to hens of the 2016 strain. For the diet effect, hens fed on the 2016 diet had significantly different (P≤0.05) higher villus heights, wider villus tips and bottoms, deeper crypt depths and thicker muscularis' when compared to hens fed on the 1940 diet. Significant differences (P≤0.05) were observed between strain and diet for all the parameters measured. Significant (P≤0.05) interactions were observed in the parameters measured.

Organ weights of the proventriculus, gizzard, spleen, liver, pancreas, and small intestines are represented in Table 6. For the strain effect, the weights of the proventriculus, gizzard, spleen, liver, pancreas and small intestines were all significantly different (P≤0.05) in hens of the 2016 strain having heavier weights when compared to hens of the 1940 strain. For the diet effect, the weights of the proventriculus, pancreas, and small intestines were all significantly different

Wall DC, et al., Int J Plant Anim Environ Sci 2023
DOI:10.26502/ijpaes.4490157

Table 6: Allometric organ weights (according to main effects).

	Proventriculus	Gizzard	Spleen	Liver	Pancreas	Small In.
Main Effects						
Strain (S)						
2016	6.62[a]	31.33[a]	1.21[a]	37.57[a]	3.37[a]	90.62[a]
1940	4.5[b]	28.24[b]	1[a]	30.37[a]	2.08[b]	76.53[b]
SEM	0.31	1.08	0.11	1.13	0.38	3.09
p-value	0.023	0.033	0.153	0.042	0.036	0.025
Diet (D)						
2016	5.79[a]	28.33[b]	1.12[a]	31.33[b]	2.66[a]	85.45[a]
1940	3.55[b]	31.24[a]	1.08[b]	36.6[a]	2.79[a]	81.71[b]
SEM	0.31	1.08	0.115	1.13	0.25	3.09
p-value	0.0116	0.024	0.057	0.049	0.057	0.034
Interaction						
S × D	0.0003	0.037	0.055	0.04	0.045	0.038

[1]Values are presented as Means
Note: Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.

(P≤0.05) in hens fed on the 2016 diet having heavier weights when compared to hens fed on the 1940 diet. However, the weights of the gizzard and liver of hens fed on the 1940 diet were significantly different (P≤0.05) having heavier weights when compared to hens fed on the 2016 diet. A slight significant difference (P≤0.05) was observed in the spleen with hens fed on the 2016 having a 3.63% heavier weight when compared to hens fed on the 1940 diet. Interaction significance (P≤0.05) was observed in organs of the proventriculus, gizzard, liver, pancreas and small intestines.

Discussion

The critical roles of the intestinal functional state have gained increased recognition as a contributing factor to overall poultry health and production performance. An essential indicator to assess intestinal health of laying hens that is indicative of absorption capacity and digestion is evaluating intestinal morphology. This study assessed the intestinal histological differences comparing a 1940 leghorn and a 2016 leghorn fed representative diets. The results of this study revealed a positive effect on the parameters that were measured.

Overall Histological Effects

The main effects in this study were strain and diet followed by interaction between strain x diet. Both the strain and diet effects resulted with hens of the 2016 strain and fed on the 2016 diet having longer intestinal length measurements when compared to hens of the 1940 strain and fed on the 1940 diet suggesting that genetic selection had a greater impact on intestinal development which has been known to effect feed intake thus influencing production. The composition of the diet has the ability to significantly affect the feed passage rate according to Marchewka J et al. and the results from this study agree with that claim due to hens fed on the 2016 diet having higher measurements when compared to the hens fed on the 1940 diet. Therefore, since the measurements were shorter in the hens fed on the 1940 diet it can be suggested that nutrient absorption and assimilation were poorer. Similar results were observed in a study conducted by Abelebele et al. [7] when gut histology of broiler and indigenous chickens were evaluated resulting in the broiler chickens displaying better absorption capabilities when compared to the indigenous chickens. The results from the interaction relationships of strain x diet demonstrated that the relationship combined contributed to the histological changes there were observed providing insight into the structure of the intestinal functionality of laying hens from different eras fed representative diets.

Histological Observations

The small intestine is known to be an organ of crucial importance due to its role in maintaining digestive, endocrine, metabolic, and immune functions among domestic animals [8]. Most of the digestive and absorptive processes of ingested feeds take place within the intestine [9]. Nutrients within the diet could induce morphological differences among the intestinal parts and the intestinal absorptive function of each segment [4]. Being able to be to moderately assess intestinal functionality, modifications exhibited within the intestinal morphology measured by villus height and crypt depth are deemed important indicators as well as reflections of the digestive and absorptive capacity [8]. In this study, the length of the small intestines was longer in hens of the 2016 strain and those hens fed on the 2016 diet supporting the claims that longer small intestinal length results in greater digestive and absorptive area.

Wall DC, et al., Int J Plant Anim Environ Sci 2023
DOI:10.26502/ijpaes.4490157

It is reported that an increase in villus height is a direct indication of improved absorptive function [10]. Longer villi are known to provide increased surface area making them capable of greater absorption of available nutrients [11]. According to studies from Lauronen et al. [12], an increase in villus size can be a direct indicator of increased villus length thus providing a greater surface area for the adsorption of available nutrients. In the duodenum, the villus height was the longest in hens of the 2016 strain as well as hens fed on the 1940 diet which may indicate a beneficial effect of the composition of the diet provided but supporting the theory that extensive interactions of host and diet do exist. The duodenum has a major role in nutrient absorption and the decrease in villi size from the duodenum to the ileum is due to the lower absorptive capacity in the last portion of the small intestine which was consistent with findings in a study conducted by Seyyedin et al. [13] that showed the villi height in the duodenum was greater when compared those observed in the jejunum and ileum. The villus height in the jejunum demonstrated a minimal change showing a small significance among hens of the 2016 and 1940 strains as well as hens fed on the 2016 and 1940 diets suggesting that strain nor diet had a major adverse effect on the villus heights. In the ileum, hens of the 1940 strain had longer villus heights when compared to hens of 2016 strain suggesting that the genetic composition of the hens had a greater influence resulting in the differences observed. The results from the heights of the villi decreased from each segment of the small intestines (duodenum, jejunum, and ileum) which were similar to the reports conducted by Rana et al. [14]. Maneevan and Yamauchi [15] explained that the alterations observed within intestinal histology represent the outcome of the availability of nutrients within the intestine of ingested feed differs between the epithelium known to be at the micro-levels and the villi known to be at the macro levels. It was also noticeable that fluctuations were exhibited in the three intestinal segments analyzed from the hens of both strain and fed on both representative diets.

The crypt depth is one of the indicators of the health and functional status of the intestine in chickens, and their size can be a measure of the intensity of intestinal epithelial cell renewal processes. Deeper crypts are known to indicate rapid tissue regeneration to permit the renewal of villi promoting normal sloughing and/or inflammation because of the presence of pathogens or their toxins [16]. This current study showed an overall decrease in crypt depth with each segment with the duodenum having the deepest and the ileum having the shortest indicating an efficient tissue turnover as well as good gut condition. Similar results were observed in research conducted by Sobolewska et al. [17] and in research conducted by Kelly et al. stating that the decrease in the crypt depth could achieve an increase in the enzymatic activity of the small intestine which can affect absorption ability. In the duodenum, hens of the 1940 strain and hens fed on the 1940 diet had deeper crypts suggesting that strain and diet were dependent on each other. Similar results were observed in the jejunum and ileum, however, hens fed on the 2016 diet had deeper crypts suggesting that the composition of the diet had a stronger effect.

The muscularis determines the rate and power of intestinal motility hence the progression of a bolus resulting in the effect of the absorption process that either increases or decreases the contact between the mucosa and intestinal contents. A thick tunica muscularis along with a shorter intestine could lead to a more rapid intestinal passage time with a lower uptake of available nutrients. Results from this study seem to be in correspondence with studies conducted by Mekbungwan et al. [18]; Yamauchi [19,20] stating that morphological differences among the intestinal parts are in fact induced by the nutrients in the diets. In the duodenum, hens of the 1940 strain had a thicker muscularis when compared to hens of the 2016 strain suggesting that those hens had a better improvement rate of the contact between the mucosa and the intestinal content. However, in the jejunum and ileum, at the strain effect, hens of the 1940 strain had thicker muscularis but hens fed on the 2016 diet presented with a thicker muscularis thus suggesting that there was a strong correlation between genetic and nutritional differences. Based on the results, it can be concluded that the differences displayed by both genetic lines are presumably due to genetic differences and are deemed essential as important criteria for ongoing selection for improved performance however, the nutritional differences did relate to the overall growth rate and feed efficiency.

Intestinal morphology is used as an indicator of intestinal health as values are often indicative of digestive and absorptive capacity. The functionality of both anatomical and histological characteristics of the avian gastrointestinal tract are deemed essential to feed conversion efficiency and therefore imperative to focus on the genetic foundation that is linked to feed efficiency such as the potential to promote digestive utilization of feed. In general, both the villus height and crypt depth correlate with intestinal health and proper digestibility. Usually, a combination of high crypt depth, as well as a low villus height: crypt depth ratio is associated with faster mucus turnover, thus causing high energy requirements. In this study, body weight, organ analysis in regard to organ weights, and histological observations of the duodenum, jejunum, and ileum were assessed.

The overall differences between the strains of these histological measurements could be a consequence of the genetic background resulting from selection over the years. It can be suggested that W36 laying hens have been created through intensive selection whereas the W40 laying hens have less intensive development of the intestinal microstructure. Diet presented with significant differences

Wall DC, et al., Int J Plant Anim Environ Sci 2023
DOI:10.26502/ijpaes.4490157

suggesting that the 2016 diet heightened the villus height and width of the small intestines when compared to the 1940 diet due to the concentration of the dietary composition provided. The outcome from this study could be a direct result of the compensatory reactions of the laying hens fed a high fiber yet low nutrient diet. Interactions between strain x diet presented differences as suspected due to several influencing factors. It has been reported that nutrient absorption could have the ability to become enhanced by increasing digestive capacity and it has been shown in this study.

Organ Analysis

Both mammals and birds have demonstrated the digestive ability of the small intestine in response to changes in physiological needs and have been mainly linked to nutrient absorption and organ structure [21]. It has been suggested that internal organ weights undergo modifications, pertaining to size, to accommodate different growth rates [22]. The proventriculus, equivalent to the glandular component of the mammalian stomach, presents as deep gastric glands with lobules and various secretory tubules with the function to secrete gastric acid which is added to the ingesta [23]. The gizzard (ventriculus) is the second compartment of the stomach that demonstrates powerful contractions designed to crush ingested food. It has been determined that the digestive tract adapts rapidly to changes in diet composition, and the gizzard is known to respond particularly rapidly to changes in the diet [24]. In this study, mash feed was given to all birds, and hens of the 2016 strain presented with a heavier organ weight compared to hens of the 1940 strain which could have been a consequence of enhanced gizzard activity due to the retention time being increased based off the composition of the feed. The spleen is known to be the main immune organ of laying hens [25] and these organ weights being strain effect and diet effect from this study were not affected. The liver is an accessory organ of the digestive system and the largest gland of the body playing a major role in the synthesis and metabolism of fat. The fluctuations in liver weights could be indicative of changes in utilization of dietary energy for maintenance thus affecting growth rates. The pancreas, which is responsible for the secretion of insulin synthesis and secretion, displayed differences between the treatment groups. Hens of the 2016 strain presented with had heavier pancreas weights compared to hens of the 1940 strain suggesting that the differences might reflect differences in body growth rate since it has been reported that total body growth does influence the growth of individual organs in chickens [26]. The small intestine, an organ of vital importance, has a vital role in maintaining digestive, endocrine, metabolic, and immune function for domestic animals. The is a process where feed constituents are hydrolyzed into simple molecules in the small intestines in particular; free small peptides, amino acids, free fatty acids, and monosaccharides. These molecules are later absorbed in the duodenum and jejunoileum and then transported via blood circulation to other tissues. Hens of the 2016 strain presented with had heavier weights of the small intestines when compared to hens of the 1940 strain and the differences observed could be a direct result of the strain/diet interactions. It was demonstrated in research conducted by Wang et al. [27] that intestinal weight increases with body weight which displayed similar results in this current study [28-47].

Conclusion

Diet composition is a major factor that has the ability to alter the histological status within the gut. A valuable criterion for estimating the digestive capacity of the small intestine has been the assessment of villus length/ crypt depth ratio. The results from this study suggest that intestinal absorptive function might be activated by available nutrients represented by each diet. The different growth rates are a direct result of a complex interaction of genetic, physiological, and environmental factors. Diet is the major environmental factor assessed in this study. Diet composition, diet form, and feeding strategy were manipulated in the study to delineate differences in outcomes. In this study, the genetic influence appears more relevant than physiology or environment since both lines were maintained under similar conditions. The intestinal histological modifications are largely correlated to the availability and choice of nutrients within the intestine. The differences between the two genetic lines within the small intestines are potentially linked to genetic differences making it an important criterion for ongoing selection for improved performance.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Acknowledgements

The authors would like to extend their gratitude to the farm managers, staff, and personnel from the Prestage Department of Poultry Science, NC State Feed Mill, and the University of Arkansas for participating in this study. We gratefully acknowledge the hard work and dedication it took make this happen.

Author Contributions

Formal analysis: D. C. W and R. D. M; Funding acquisition: K.E. A; Investigation and Metholodology: D. C.W, R. M; Supervision: D. C. W, R. D. M; Writing – original draft: D. C.Wall; Writing – review and editing R. D. M, K. E. A, N. A

Funding

This work was funded by the North Carolina Layer Performance and Management Program.

Wall DC, et al., Int J Plant Anim Environ Sci 2023
DOI:10.26502/ijpaes.4490157

Conflicts of Interest

Declare no conflict of interest regarding this manuscript.

References

1. Alshamy Z, Richardson KC, Hünigen H, et al. Comparison of the gastrointestinal tract of a dual-purpose to a broiler chicken line: A qualitative and quantitative macroscopic and microscopic study Plos one 13 (2018): e0204921.
2. Alshamy Z, Richardson KC, Harash G, et al. Structure and age-dependent growth of the chicken liver together with liver fat quantification: A comparison between a dual-purpose and a broiler chicken line PLoS ONE 14 (2019): e0226903.
3. Svihus B. Function of the digestive system, Journal of Applied Poultry Research 23 (2014): 306-314.
4. Laudadio V, Passantino L, Perillo A, et al. Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poult Sci 91 (2012): 265-270.
5. Kiela PR, Ghishan FK. Physiology of Intestinal Absorption and Secretion. Best practice and research Clinical gastroenterology 30 (2016): 145-159.
6. Karasov WH, Douglas AE. Comparative digestive physiology. Comprehensive Physiology 3 (2013): 741-783.
7. Mabelebele M, Norris D, Brown D, et al. Breed and Sex Differences in the Gross Anatomy, Digesta pH and Histomorphology of the Gastrointestinal Tract of Gallus Domesticus. Brazilian Journal of Poultry Science 19 (2017): 339-346.
8. Gu YF, Chen YP, Jin R, et al. A comparison of intestinal integrity, digestive function, and egg quality in laying hens with different ages. Poultry Science 100 (2021): 100949.
9. Havenstein G, Ferket P, Scheideler S, et al. Carcass composition and yield of 1991 vs. 1957 broilers when fed "typical" 1957 and 1991 broiler diets. Poultry science 73 (1994): 1795-1804.
10. Svihus B. Function of the digestive system. Journal of Applied Poultry Research 23 (2014): 306- 314.
11. Caspary WF. Physiology and pathophysiology of intestinal absorption. Am J Clin Nutr 55 (1992): 299S-308S.
12. Lauronen J, Pakarinen MP, Kuusanmaki P, et al. Intestinal adaptation after massive proximal small bowel resection in the pig. Brit Poult Sci 41 (2000): 416-423.
13. Rana J, Dhote BS, Mrigesh M, et al. Histomorphometry of small Intestine in post-hatch Uttara fowl. Indian J Vet Anat 27 (2015): 1-3.
14. Seyyedin S, Nazem MN. Histomorphometric study of the effect of methionine on small intestine parameters in rat: an applied histologic study. Folia Morphol (Warsz) 76 (2017): 620-629.
15. Maneewan B, Yamauchi K. Intestinal villus recovery in chickens refed semipurified protein-, fat-, or fibre-free pellet diets. Brit Poult Sci 45 (2004): 163-170.
16. Xu ZR, Hu CH, Xia MS, et al. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci 82 (2003): 1030-1036.
17. Sobolewska A, Bogucka J, Dankowiakowska A, et al. The impact of symbiotic administration through in ovo technology on the microstructure of a broiler chicken small intestine tissue on the 1st and 42nd day of rearing. J Animal Sci Biotechnol 61 (2017).
18. Mekbungwan A, Yamauchi K, ThongwittayaN. Histological alterations of intestinal villi in growing pigs fed soybean and pigeon pea seed meals. Can J Anim Sci 83 (2003): 755-760.
19. Yamauchi K-e. Review on chicken intestinal villus histological alterations related with intestinal function. The Journal of Poultry Science 39 (2002): 229-242.
20. Yamauchi K. Buwjoom, T. Koge K. et al. Histological intestinal recovery in chickens refed dietary sugar cane extract. Poultry Sci 85 (2006): 645-651.
21. Pinheiro DF, Cruz VC, Sartori JR, et al. Effect of early feed restriction and enzyme supplementation on digestive enzyme activities in broilers. Poult Sci 83 (2004): 1544-1550.
22. Plavnik I, Hurwitz S. Organ weights and body composition in chickens as related to the energy and amino acid requirements: effects of strain, sex, and age. Poult Sci 62 (1983): 152-163.
23. Scanes CG, Pierzchala-Koziec K. Biology of the Gastrointestinal Tract in Poultry. Avian Biology Research 7 (2014): 193-222.
24. Svihus B. The gizzard: Function, influence of diet structure and effects on nutrient availability. World's Poultry Science Journal 67 (2011): 207-224.
25. Martínez Y, Carrión Y, Rodríguez R, et al. Growth Performance, Organ Weights and Some Blood Parameters of Replacement Laying Pullets Fed with Increasing Levels of Wheat Bran. Brazilian Journal of Poultry Science 17 (2015): 347-354.
26. Nestor KE, Saif YM, Emmerson DA, et al. The influence of genetic changes in body weight, egg production, and body conformation on organ growth of turkeys. Poult Sci 74 (1995): 601-611.

Wall DC, et al., Int J Plant Anim Environ Sci 2023
DOI:10.26502/ijpaes.4490157

27. Wang JX, Peng KM. Developmental morphology of the small intestine of African ostrich chicks. Poult Sci 87 (2008): 2629-2635.

28. Clark CEF, Akter Y, Hungerford A, et al. The intake pattern and feed preference of layer hens selected for high or low feed conversion ratio. PLoS ONE 14 (2019): e0222304.

29. De Verdal H, Mignon-Grasteau S, Jeulin C, et al. Digestive tract measurements and histological adaptation in broiler lines divergently selected for digestive efficiency. Poult Sci 89 (2010): 1955-1961.

30. De Verdal H, Narcy A, Bastianelli D, et al. Improving the efficiency of feed utilization in poultry by selection. 1. Genetic parameters of anatomy of the gastro-intestinal tract and digestive efficiency. BMC Genet 12 (2011).

31. Di Masso RJ, Dottavio AM, Canet ZE, et al. Body weight and egg weight dynamics in layers. Poult Sci 77 (1998): 791-796.

32. Havenstein G, Ferket P, Scheideler S, et al. Growth, livability, and feed conversion of 1957 vs. 1991 broilers when fed "typical" 1957 and 1991 broiler diets. Poultry science 73 (1994): 1785-1794.

33. Yang HM, Wang W, Wang ZY, et al. Comparative study of intestine length, weight and digestibility on different body weight chickens. African Journal of Biotechnology 12 (2013): 5097-5100.

34. Incharoen T, Yamauchi K. Production Performance, Egg Quality and Intestinal Histology in Laying Hens Fed Dietary Dried Fermented Ginger. International Journal of Poultry Science 8 (2009).

35. Isshiki Y. Feed digestibility in different intestinal parts of chickens. Anim Sci J 60 (1989): 1082-1092.

36. Koh-en Yamauchi, Review on Chicken Intestinal Villus Histological Alterations Related with Intestinal Function, The Journal of Poultry Science 39 (2002): 229-242.

37. Pelicano ERL, Souza PA, Souza HBA, et al. Morphometry and ultra-structure of the intestinal mucosa of broilers fed different additives. Brazilian Journal of Poultry Science 9 (2007): 173-180.

38. Popescu RG, Voicu SN, Gradisteanu Pircalabioru G, et al. Effects of Dietary Inclusion of Bilberry and Walnut Leaves Powder on the Digestive Performances and Health of Tetra SL Laying Hens. Animals 10 (2020): 823.

39. Schreiter R, Damme K, Freick M. Relation between Feed Particle Size Distribution and Plumage Condition in Laying Hens on Commercial Farms. Animals 11 (2021): 773.

40. Ruttanavut J, Yamauchi K. Growth performance and intestinal histomorphology in egg-type growing roosters fed recycled food waste containing effective microorganisms. Turkish Journal of Veterinary and Animal Sciences 36 (2012): 509-518.

41. Sharma M, Dinh T, Adhikari P. Production performance, egg quality and small intestine histomorphology of the laying hens supplemented with phytogenic feed additive. Journal of Applied Poultry Research 29 (2020).

42. Svihus B. The Role of Feed Processing on Gastrointestinal Function and Health in Poultry. In: Avian Gut Function in Health and Disease, Perry GC (Eds), Wallingford: CAB International, Cromwell Press (2006): 183-194.

43. Incharoen T, Khambualai O, Yamauchi K. Performance and Histological Changes of the Intestinal Villi in Chickens Fed Dietary Natural Zeolite Including Plant Extract. Asian Journal of Poultry Science 3 (2009): 42-50.

44. Incharoen T, Yamauchi K, Erikawa T, et al. Histology of intestinal villi and epithelial cells in chickens fed low-crude protein or low-crude fat diets, Italian Journal of Animal Science 9 (2010): 4.

45. De Verdal H, Mignon-Grasteau S, Jeulin Grasteau C, et al. Digestive tract measurements and histological adaptation in broiler lines divergently selected for digestive efficiency. Poultry science 89 (2010): 1955-1961.

46. Yokhana JS, Parkinson G, Frankel TL. Effect of insoluble fiber supplementation applied at different ages on digestive organ weight and digestive enzymes of layer-strain poultry. Poultry science 95 (2016): 550-559.

47. Zaefarian F, Abdollahi MR, Cowieson A, et al. Avian Liver: The Forgotten Organ. Animals: An Open Access Journal from MDPI 9 (2019): 63.