ABSTRACT

WALL, DANNICA CHARNAE. Evaluating the Influence of Genetics and Advances in Hen Nutrient Requirements on Performance, Microbial and Intestinal Function, Bone Integrity, and Egg Production Efficacy on 1940 vs. 2016 Commercial Laying Hens. (Under the direction of Dr. Kenneth E. Anderson).

One of the most impactful advances in the last 100 years within the poultry industry has been poultry breeding. The vast majority of these improvements have come from genetic selection for improved feed efficiency, along with establishing the overall production system, and a better understanding of poultry nutrition. To date, there is limited information on the direct comparison of genetic changes and nutritional needs of hens from different eras. Understanding the effect of genetics and nutrition on performance, gut microbial function, bone integrity, and egg production, will help producers to identify steps that can be implemented to reduce stress levels associated with the production environments being selected. Previous research conducted by scientists has centralized their focus upon the modern production strains in modern facilities along with the interaction of the environment on those strains. Comparable research with laying hens to this capacity has not been conducted and unfortunately with the dispersion of the Canadian Random Bred Layer, this type of work has become more challenging to achieve. This current study was developed to provide an understanding of how genetic selection for intensive housing environments and increased egg production affect hens from different eras. This model has the ability to provide the industry with a valuable tool to help interpret and grasp the changes in the hen associated with the genetic response and health, further examining whether the hen's stress indicators are affected by intensive systems. The information generated will enable the evaluation of genetics and nutrition taking into account the changes in laying hens during the last 80+ years, physiological performance, and productivity.

This study has concentrated on understanding the influence of genetic selection and the 1940s perspective of layer nutrition with a 2016 current commercial laying stock in conjunction with its ability to function within the 1940 nutrition and the current nutritional program that is available today. However, distinguishing the interplay of nutrition and selection on performance, microbiome and intestinal function, bone integrity, and egg production is vital in that it may allow the industry to identify a pathway to improve nutrition and or understand the genetics to further enhance overall health in laying hens. The results from this study suggested that the relationship between genetics and nutritional requirements of laying hens do correlate and interact with each other for performance, microbial and intestinal function, bone integrity, and egg production.

Evaluating the Influence of Genetics and Advances in Hen Nutrient Requirements on Performance, Microbial and Intestinal Function, Bone Integrity, and Egg Production Efficacy on 1940 vs. 2016 Commercial Laying Hens

by
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DEDICATION

To my late loving, inspirational, and extraordinary uncle, George Sturdivant. Thank you for the motivation and for always believing in me even when I didn't. I told you I wouldn't let you down. We did it!

BIOGRAPHY

Dannica C. Wall was born on August 5th, 1987, in Raleigh, North Carolina. She is the daughter of Charles and Renee Wall, twin sister to Danielle Wall, and older sister to Marcus Wall. In May 2009, Dannica C. Wall completed her Bachelor of Science degree in Animal Science at North Carolina Agricultural and Technical State University. She then furthered her education and completed her Master of Science degree in Animal Health from North Carolina Agricultural and Technical State University in December 2011. Dannica C. Wall is a candidate for the Doctor of Philosophy in Animal and Poultry Science at North Carolina State University.

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CHAPTER 1: Introduction

In the past, random-bred control strains had been utilized extensively in research investigations providing effectiveness and value within the poultry industry (Therrington et al., 1999; Jones et al., 2001; Anderson et al., 2004). Areas related to management, housing systems, and environments, breeding, and nutrition have been explored establishing new approaches to the evolving industry. Upon those changes, genetic selection has played a vital role economically with enhanced production.

Drastic changes have occurred over the past decades in the poultry industry in the United States. The egg industry and associated research have evolved over the past decades primarily with the expansion of diverse housing systems, however, there are still areas that need to be explored pertaining to the examination of how selection has progressed with preferred production parameters. Farmers and researchers made great strides in the late 1940s to maintain production with the housing that was provided. Production was sustained due to the adaptability of the hen. Subsequently, conventional cage systems made their way into the mainstream adding pressure to the hen's adaptation abilities since they were not accustomed to confinement and ideally genetically or selected to perform in those environments. Feeding became more uniform, health elevated, and egg production heightened. On the contrary, hens' welfare became a concern for consumers and alternative housing systems started to be considered and examined. One of the issues associated with alternative housing systems were hens that were genetically selected to perform in a selective environment. Noticeably, hens that were housed in a conventional cage system performed differently from hens that were housed in non-conventional cage systems. Fluctuations in performance affected the industry economically thus pushing for more research. Over the years, the North Carolina Layer Performance and Management Test has provided

insight into current commercial strains of layers to provide information regarding production in alternative housing systems. While these tests provided viable information, it is unclear whether hens from previous generations are genetically equipped to handle the direction of production for alternative housing systems. In order to maximize genetic potential, it is necessary to compare different strains, those who have been around since the housing system changes to those who have been genetically altered to sustain and produce in alternative housing systems.

The purpose and intent of my research are to provide insight on genetic changes and nutritional needs of hens from different eras in direct comparison. Being able to understand the effect of genetics and nutrition on areas such as immunological function and egg production will help the industry and producers to identify efforts that can be implemented to reduce stress levels associated with production. Decreased hen stress can improve hen health, egg production, egg quality, and food safety, which emphasizes the need for these comparisons.

Havenstein et al. (2003) conducted similar studies on broilers determining the immense influence of both genetic selection and nutrition on body conformation and growth. Reluctantly, comparable research with laying hens of this magnitude has not been conducted and has become challenging to achieve primarily due to the dispersion of the Canadian Random Bred Layer until now. Preliminary studies were conducted using a heritage White Leghorn strain during the 39th North Carolina Layer Performance and Management Test to assess productivity comparing different housing environments.

The egg industry is realigning its production practices to alternative environmental systems in order to enhance the welfare of the hens used to produce shell eggs. The egg industry and research have evolved over the past 100 years to the production systems we are currently using (Anderson, 2012). The shift in production systems was done first to protect the hen, then

enhance their health status and provide a higher quality product to the consumer. In this same time frame, the breeders selected hens to be highly adaptive to these production environments and the egg breeds/strains became more productive.

Thus, the objective of this dissertation is to provide insight on genetic changes and nutritional needs of hens from different eras in direct comparison by comparing the influence of genetics and nutrient requirements on performance, microbial and intestinal function, bone integrity, and egg production efficacy between a 1940's Random Bred strain and 2016's commercial White Leghorns. These objectives will be described and discussed in the upcoming manuscripts within this dissertation.

CHAPTER 2: Literature Review

2.1 Control Strains

Effective controls are imperative for determining an experiment's effects. Control populations of poultry have been extensively utilized in the past having been considered very effective in quantifying changes in poultry genetics. With generational advancements in birds being so immense, it is crucial to have a point of historical reference with which to evaluate such improvements. Control stocks are known to be a maintained point of historical reference illustrating how genetic selection has transformed the hen used by the commercial egg industry with respect to production and physiology. A strain, oftentimes referred to as a bloodline or a line, is considered a family of related chickens. Strains are distinctive by being selectively bred by a single individual or organization over a period of time long enough for all the chickens to be uniquely uniform in some way. Each strain of purebred chickens possesses all the traits that define its breed along with additional unique traits making the strain distinguishable from other strains of the same breed and variety. To date, the most popular layer strains are considered hybrids, meaning they are created by crossing two different breeds. The breeds used in making such a cross are better known as proprietary strains, developed for the unique characteristics each contributes to the hybrid strain. Control strains have been universally used over the years and are considered very useful in quantifying changes in poultry genetics (Collins et al., 2016). According to Gowe et. al. (1959), the ideal control would reflect in its phenotype changes in the environment between generations which would be directly comparable to the changes brought about by the same environment in the phenotype of the selected population. It has been determined that the emphasis of a control strain for a selection study to differentiate between genetic and environmental effects on traits within a population of poultry is vital so that specific

selection traits could be adjusted for the environment's influence (Collins et al., 2016). There has been a considerable number of experimental studies of single control populations, together with analyses of the time trends of their phenotypic and genetic parameters. Comparative populations as these are needed to serve as genetic and environmental controls to 1). To provide benchmarks from which to measure genetic changes in populations under artificial selection; 2). To distinguish between genetic and environmental changes.

The constancy of genetic parameters and absence of genotype-environment interactions between control strains along with years would be indicative that the utilization of random-bred pedigreed control populations has been presented as efficient models for measuring selection change. The history of the populations from which the strains were established, involving a narrow effect on population size, would be expected to reduce the genetic variation for all traits. Genetic changes in a control flock may be of two kinds: (a) random changes in gene frequency due to genetic sampling or "drift"; (b) directional changes due to natural selection. The value of control populations had provided standard material for the evaluation of the level of management in selection programs has become increasingly apparent over the past decades.

Using a White Leghorn control strain, Gowe et al. (1959) showed the importance of a control strain for a selection study to differentiate between genetic and environmental effects on traits. This allows for specific selection traits that could be adjusted for the environment's influence. They concluded that without control, environmental effects can obscure results and conclusions from selection programs. Random mating is the preferred method to maintain a control flock, however, they may be reproduced either as pedigreed, “each member of the flock contributes equally to the next generation”, or random, non-identified matings with “eggs being collected from the flock over a limited period” (Collins et al., 2016).

2.1.1 Random Sample Test

For many years, Random Sample Tests (RST) were conducted at a number of agricultural universities, utilizing results, particularly in both North America and Europe, to compare the performance of commercial strains when grown and tested under the same conditions. The main objective of RSTs was to provide breeders, hatchery personnel, and potential buyers of chicks with impartial information pertaining to poultry stocks that are offered for sale (Becker 1961). Such RSTs were occupying a number of different geographic areas and were utilized comparatively for 30 years in North America. At that time, a committee, pertaining to the Poultry Breeders of America, made recommendations to the various RST managers to assess how testing procedures could potentially be improved. A collaborative summary of all of the North American RSTs that was in operation from 1962 until the 1980s was produced (e.g., USDA-SEA, 1979). With the decline of commercial strains due to the consolidation as well as the disappearance of some strains by breeding companies, interest in RSTs also declined to the point where most were terminated (Carey, 1988).

During their reign, RST programs expanded to accommodate continuous advancements in response to future needs. Advancements regarding purpose, number of tests, the accuracy of comparisons, and completeness of measurements took place to source publications and implementation of test information. The commercial layer industry has been on a steady rise with consistent change over the past decades (Anderson et al., 2013). These changes are a direct result of quantitative genetic selection utilizing a weighted index of economically important traits in order to improve the performance of certain stocks (Toghiani, Sajjad 2012). Hazel, composed the index first to describe how to construct and employ a selection index in addition to industry breeding organizations that generated information on not only genetic traits, but also economic

value by altering those traits, thus resulting in pedigree flock selection (Anderson et al. 2013). Various types of random sample tests used were designed to suit the industry and its needs.

2.1.2 North Carolina Random Sample Test

The North Carolina Random Sample Egg Laying Test was performed more than 63 years ago since the first tests (Kidd et al., 2019). The first test was conducted in 1958 as the North Carolina Random Sample Egg Laying Test until 1980, and the most recent test, the 40th North Carolina Layer Performance and Management Test was conducted and completed in 2019. Not only did the name change, but also the breeds tested, the housing systems utilized, and most importantly the performance of the layer breeds were all included within the enhancement of the tests. The tests conducted over the years included most of the commercial white and brown egg strains, thus providing a broad overview of the general changes that took place in the performance of white egg and brown egg production stocks during the past half-century Performance varied as observed from within these tests have occurred over time, as commercial breeding companies adapted quantitative genetic selection procedures to their breeding flocks. The results from these tests clearly indicate that enhancement with genetic selection has been practiced by egg-type breeders.

2.2 Genetic Diversity

The art of identifying superior animals based on performance or phenotype, particularly for breeding has been practiced for decades. Knowledge gained alongside the needs of the industry has resulted in the changes of selection and breeding programs of the poultry industry (Saxena et al., 2018). The layer breeding industry has significantly evolved during the past decades while coping with new challenges that have resulted in increased egg production, improved feed efficiency and adaptation of egg quality based on consumers' preferences.

Selection itself is done within closed purebred lines that are based on a comprehensive phenotyping scheme (Dekkers, Jack 2007). This is used in pure line birds under highly standardized housing conditions and with crossbred hens under housing conditions that remain closer to the commercial production environment (Dekkers, Jack 2007).

The primary objective for the layers in the egg industry is to obtain the maximum number of saleable eggs per hen housed at a low feed cost per egg or per kg egg mass with the eggs having optimal internal and external qualities (Saxena et al., 2018). The stock should have low mortality and high adaptability to different environments.

The modern chicken known as *Gallus gallus domesticus* was domesticated from the red jungle fowl (RJF), but with some contributions from at least one other closely related species, the grey jungle fowl (Sawai et al., 2010). Domestic chickens have been dissociated into several hundreds of distinct breeds distributed across the world (Qanbari et al., 2019). Extensive gene pools are continuously being evaluated and selected for market demands that must be anticipated at least 5 years in advance within the commercial layer industry (Rudolf et al, 2018). The history of poultry genetics has focused on classical and recently molecular genetics that have been utilized in breeding systems for egg production (M. Tixier-Boichard et al., 2012). During the past four decades, primary breeders have selected traits in the commercial strains of Leghorns that have enhanced the efficiency of egg production (thiruvenkadan et al., 2010). Selection emphasis has changed periodically for numerous traits, some of which included the size of eggs, number of eggs produced, or age at first egg.

Studies conducted throughout the years have isolated some accustomed traits that could be associated with the different control strains and that could be used as a basis for comparison. Previous research conducted by Fairfull and Gowe (1986) described that body weight steadily

declined from the older stocks to the more current stocks reporting that lower body weights of more current stocks have also been reported by other researchers (Fairfull et al., 1986). For many years, early sexual maturity in commercial layers has been an attraction for primary breeders. It has been demonstrated by a research-based laboratory that the age of the first egg has steadily decreased, whereas egg weight has increased in the Canadian control strains formed in 1950, 1959, and 1972 (Jackson et al., 1986). This specific selection emphasis had been in response to consumer demands for larger eggs. Due to this selection pressure, egg size distribution also has changed (Lerner, I. 1951).

The use of control strains in research has (Jones et al., 2001; Anderson et al., 2007) shown that the changes from selection were consistent with the Random Sample Tests conducted over the previous years (Anderson et al., 2013). The problem today is that in many cases these control strains have been dispersed and no longer exist, especially for the leghorn-based white egg strains. The WL40 was evaluated in a modern commercial setting to evaluate its performance potential which showed that egg numbers and size are significantly lower.

2.2.1 Genetic Evolution of the 1940s

Selection and breeding programs within the poultry industry have been evolving to accommodate knowledge that has been gained corresponding to specific needs (Saxena et al., 2018). During the 1940s, individual poultry flocks were evaluated retaining the selected birds and culling surplus birds that were sold as spent fowl (Saxena et al., 2018). Purebred lines were eventually replaced by commercial hybrids considered as terminal crosses while selecting the specialized egg and meat type birds eventually replacing the dual-type birds. However, a negative aspect of this production and reproduction program necessitated the need for specialized male and female lines in both layer and broiler stocks (Saxena et al., 2018). Since 1945, the layer

breeding sector has changed dramatically from hundreds of small breeders per country to three to four companies thus sharing the world market for commercial laying hens. Between 1945 and 1960, hybrid layers were introduced and the production of layers gradually shifted from local breeders with limited capacity to national companies (Leenstra et al., 2016). Annual layer egg production averaged 150 eggs in the 1940s. Today, the utilization of the modern cage system and vaccinations have minimized diseases common in the 1940s. They provided the hens with protection against weather in environmentally controlled housing and predators, while also improving food safety, the environment, in regard to air and water, and lastly animal welfare.

2.2.2 *Genetic Effects on Nutrition*

Nutrition has been defined as the science of investigating interrelationships between the animal's body and its feed comprising of ingestion of foods, digestion, absorption, transport of nutrients, intermediary metabolism, underlying anabolism and catabolism, and excretion of unabsorbed nutrients and metabolites (Sato K. 2016). Nutrition interacts with various gene expressions that are involved with the regulation of copious aspects of animal performances. The interaction established between genotype and nutrition can potentially have adverse effects on various traits that could result in different performance data. Modern layer hens have been selected for high production performance, demanding a correspondingly high food intake to meet requirements for protein, energy, minerals, and accessory nutrients. Conventionally, nutritional recommendations for laying hens consider the breed and housing conditions.

2.2.3 *Genetic Effects on Intestinal Microbial Function*

The genetics of the hen has been recognized as a factor that might influence intestinal microbiota composition. Both the gut microbial community in conjunction with the

health of the gastrointestinal tract (GIT) play essential roles in nutrition absorption, development of immunity, and disease resistance. The microbiome has emerged as a key determinant of several aspects of the organism's biology. The microbiome influences shaping developmental, physiological, and reproductive phenotypes along with being viewed as non-genetic, environmental factors that influence host phenotypes (Henry et al., 2021). Modifications within the GIT microbial community can adversely affect feed efficiency, productivity, and health of the chickens (Shang et al., 2018). Locations within the gastrointestinal tract of chickens are dense and complex microbial communities dominated by bacteria (Shang et al., 2018). The genotype of the host is a component for the preferences associated with the gut microbiota through gut secretions. The job of identifying the pattern of motility, proliferation and lifestyle of the gut microbial community in conjunction with the adjustments of mucosal cell surfaces is the challenge. Interactions between gut microbiota are mediated by host genes; consequently, the change in these gene frequencies in the genotype background will modify the interactions among the gut microbiota (Zhao et al., 2013). This indicated that genetic variation influences the pattern of host–microbe interactions, which drive phenotypic diversity thereby affecting the physiological, immunological, and nutritional status of the hen. The main reason for exploring this interaction is to determine whether genotype influences the heterogeneity of the microbial community organization as in this case selecting different groups of hens for productive performance (Elokil et al., 2020).

2.2.4 *Genetic Effects on Bone Integrity*

Husbandry and nutrition contribute to the effects of skeletal health; however, genetics plays an important role also (Jansen et al., 2021). Skeletal complications observed in layers are oftentimes attributed to selection for increased egg production, suggesting a negative

relationship between laying performance and bone stability (Jansen et al., 2020). Genetic links accompany selection for high laying performance by negative "co-selectional" side effects such as bone quality traits that are supposed to be highly polygenic (Raymond et al., 2018; Habig et al., 2017). One of the main influences attributed to changes in performance potential has been targeted gene selection, which may be associated with undesirable effects on bone stability (Jansen et al., 2020). However, differences may also be due to the phylogenetic origin of these lines, whose distinct breeding history may have influenced the genetic characteristics before selection for increased performance began.

Bone is composed of a mineral matrix, predominantly hydroxyapatite, in an organic matrix dominated by collagen, lipids, proteoglycans, and other bone structural proteins (Feng X, 2009). Bone is continually being remodeled by osteoblasts, which produce bone components, and osteoclasts, breaking them down as needed under coordination with the lay cycle (Rowe et al., 2022). Bone metabolism of female birds is unique due to the production of medullary bone serving as a source of calcium that serves as a reservoir for calcium as needed for the production of the eggshell (Prondvai et al., 2014). There is substantial genetic variation in bone traits in domestic layer chickens which is supported by research, particularly studies involving dietary treatments that evaluate bone strength and its independence from calcium metabolism (Johnsson et al., 2015). The skeletons of layers are composed of two other structural components: cortical (consisting of a dense outer bone surface), and trabecular (consisting of internal woven bone) bone types that form during development.

2.2.5 *Genetic Effects on Egg Production and Performance*

During the past decades, layer flocks have shown significant improvements in both egg production and egg quality. Substantial improvement in egg production for intensity

and persistence has been achieved by the advancements in applications of quantitative genetics. Egg production continues to remain one of the most important traits in layer selection programs. Egg number, egg mass, and egg-laying rate have always been a focus of attention in poultry breeding deeming them as important production traits. Two of the most meaningful traits in a layer breeding program belong to egg number (intensity) and egg-laying rate (persistency), due to considerable selection pressure. Genetic progress directed towards commercial egg-layer breeds by traditional selection for several decades, have reached the point of an egg on almost every day in highly efficient hens (Yuan et al., 2015). Selection for egg production has increased ovulation frequency observed in commercial egg-type hens with current stocks reaching sexual maturity at earlier ages (Lerner, I. 1951). Researchers suggest there has been a loss in genetic variation in the traditional selection traits. Breeders have selected traits in the Leghorn that would enhance the efficiency of egg production (Sitzenstock et al., 2013). Selection emphasis changed periodically for the size of eggs, the number of eggs, or the age of the first egg. Studies conducted throughout the years have isolated some general traits that could be associated with the different control stocks.

2.3 Scientific Findings

Several studies have been conducted comparing various poultry strains under various conditions and housing environments. A major area of concern has been welfare and how hens of different strains perform under similar and different housing environments to improve overall health and production. A lot of these areas of interest heightened with the rapid intensification of the poultry industry during the 1930s and 1940s which resulted in the mechanization and large-scale egg production in laying cages (Singh et al., 2009). Major contributions to the industry were obtained from results of the North Carolina Layer

Management Test as well as previous studies conducted by Havenstein et. al. (1994, 2003, 2004, 2007), who focused on different strains of broilers and turkeys from different eras fed representative diets.

2.4 Dissertation Objectives

The individual objectives that are examined in the research in this dissertation were:

1). Assess differences of nutritional requirements (1940 vs 2016 commercial diets)

2). Evaluating differences between laying hen strain (1940 vs 2016 commercial strain) on rearing and production criteria

3). Contrast between the genetics of the morphological, physical, and chemical properties of eggs and oviduct functionality

4). Contrast between the genetics of the ileal and cecal microbiota profiles

5). Contrast between the genetics of the intestinal histological features of the duodenum, jejunum, and ileum to evaluate nutrient absorbency and efficiency

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CHAPTER 3: Nutrient Maintenance: A Review on Nutrient Requirements for Laying Hens of Different Strains from Different Eras

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3.1 Summary

Feed represents approximately 75% of the expenditures associated with egg production. Nutritional research in laying hens has centered around issues related to identifying barriers to effective digestion and utilization of nutrients, and on approaches for improving feed utilization. Poultry nutritionists have increasingly combined their expertise with that of specialists in other biological sciences, including immunology, microbiology, histology, and molecular biology to further enhance feed utilization. Great advances have been made in defining nutrient requirements for various classes of poultry have been made possible largely by the increasing uniformity of genotypes, housing, and husbandry practices. However, there is limited information on comparative nutrition on strains from different eras in response to representative diet regimes. This review concentrates on the importance and necessity of proper nutrition for laying hens to promote optimal production and performance.

Keywords: White Leghorns, Pullet, Genetics, Nutrition, History

3.2 Introduction

Defining nutrient demands can be difficult due to their influence by several factors as well as being subjected to constant change. Primary factors influencing nutrient requirements are of two major categories: bird-related ones, relating to areas in genetics, sex, and form and stage of production; and external ones, relating to areas in thermal environment, stress, and husbandry

conditions Due to enhanced advances in genetic selection, the production potential has been on a continuous increasing trend in laying hens. The first paper with recommended energy and nutrient requirements labeled as percentage or units per kg of diet amounts required per hen daily of poultry was published in 1944 by the US National Research Council (NRC) (van der Klis et al., 2015). Nutrient requirements vary depending on the purpose for which the bird was developed. To gain clarity and understand nutrient requirements, one must comprehend how they are defined. Both Lesson and Summers (2012), defined a nutrient requirement as "the minimum amount of the nutrient required to produce the best weight gain, feed efficiency, etc., and the lack of any signs of nutritional deficiency" (Leeson et al., 2012).

Accordingly, modifications to nutritional requirements changed extensively as a result of dramatic changes in the genetics of the birds used (Havenstein et al., 2003), and due to improved nutritional knowledge. Laying hens, those intended for producing eggs, utilize a feeding regime that depends on feed consumption and the productivity of the bird. The structure of the diet has also undergone major changes during this period. The use of mash diets, often combined with whole cereals either mixed with the mash or fed separately, was the standard feeding method in the first half of the past century. Nutrient requirements change throughout the bird's life with the different developmental stages of the pullet, starter, grower, developer, and finisher stages activities of the laying hen, breeding, or production environment. Nutrition defined as "enhanced production potential" has had to improve due to increasing genetic selection. However, for laying hens to reach their genetic potential, an adequate understanding of the appropriate nutritional requirements along with good welfare and management practices are essential and need to be implemented. Various dietary factors influence feed formulations including but not limited to energy density, deficiencies, or excesses of nutrients such as carbohydrates, protein,

and minerals. This review presents the different criteria associated with providing laying hens with a sufficient diet to promote and sustain production while exploring the concepts from the past during the 1940s.

This review presents the different criteria associated with providing laying hens a sufficient diet to promote and sustain production while exploring the concepts from the past during the 1940s.

3.3 Historical Perspective on Nutrition

Over the decades, poultry nutrition information has been contradictory and confusing due to the changes in the understanding of nutrients available to the birds themselves, tagging into account their genetic origin, age, and also the system of production. Early research contributed to the focus on dietary components and their effects on the performance and production of laying hens followed by the dietary influence focused on the number of eggs produced, egg mass, and the weight of the eggs. Within cells in poultry the knowledge of nutrient functionality, understanding of how they provide energy, contribute to body structure, and/or regulate chemical processes in the body, has been around since the 1930s. However, it wasn't until the 1940s that the concept of metabolizable energy was introduced, with the energy content of feeds being considered. In 1912, the term 'vitamin' was first proposed to describe the essentiality of thiamine. That term was later extended to cover other essential compounds needed in small quantities. By the 1940s all the remaining 12 vitamins had been identified, and in the 1970s the importance of the vitamin D metabolites was discovered (Elwinger et al., 2016). The importance of calcium and phosphorus for both growing and laying birds was identified in the early stages of poultry keeping and requirements and dietary ratios were established. While, to date, nutrition has been continuously explored to accommodate for genetic potential and continues to this day.

3.4 Pullet Nutrient Requirements

The rearing period is critical for both behavioral and physiological development (Widowski et al., 2018). Nutrition for pullets aims to build a strong base foundation to build upon for future production. Nutrition during this phase has developed to ensure the optimum health status, proper sexual maturity, controlled flock uniformity, and least mortality. Each tissue and organ in the bird develops at a different rate due to the nutritional demands of the various tissues and organs. There is a direct correlation that exists between the pullet's development during rearing and subsequent performance during the lay cycle. The pullet rearing phase is the most rapid growth phase of the hen's whole life having polytropic developmental features that demand requirement parameters to be changed as genetics change.

According to the NRC (1994), there are 4 stages determined by age for the growing phase of pullets; 0 to 6 wk, 6 to 12 wk, 12 to 18 wk, and 18 wk to the age of the first lay at the onset of production. During the first half of the rearing period, the focus is on an optimal supply of digestible amino acids and minerals to ensure the basic growth of the inner organs, muscles, and skeleton. During the second half of the rearing phase, the physiological development of the pullet continues at a slower rate, which offers the chance for the changing feed intake of the pullets, which is critical for the onset of egg production. Diets are provided with adequate high-quality protein to meet the maintenance demands of the pullets making protein the most important factor determining the diet quality of the pullets.

The current NRC (1994) lists the following dietary protein requirements for pullets: 18% for 0 to 6 wk, 16% for 6 to 12 wk, and 15% for 12 to 18 wk of age (Hussein et al., 1996). The Starter diet, a nutrient-dense, recommended to contain about 20% CP and 2770 kcal ME/kg, provided to the pullets from 0 to 6 weeks. At this age, the diet contains all the necessary nutrients

needed for physiological development. The emphasis on protein targets the ability to build muscles, bones, and the formation of the reproductive system. Grower feed, typically composed of 16% CP and high energy of 3000 kcal/kg, is provided to match the bird's increased energy use. Pullets dissipate lots of energy after 6 weeks of age since the growth rate of a layer-type pullet is slower therefore the diets have a medium energy density, and lower protein content as transitions from starter to grower diets are made, reflecting the increasing feed consumption and slowing daily gain. Below is the composition of the diet that was formulated for rearing periods of the research trial expressed previously (**Table 1**) based on the 40th NCLP&MT.

Table 1. Composition of Diet Formulations for Rearing Periods

Ingredient	Starter	Grower	Develop
Corn	1192.0	1172	1193.0
Soybean Meal	592.0	426.0	316.0
Wheat Midds	127.0	316.0	365.0
Limestone, gr.	34.0	37.0	80.0
Coarse Limestone			
Fat	10.0	10.0	10.0
Phosphate Mono/D	20.5	16.4	14.3
Salt	6.0	6.0	6.3
D.L. Methionine	4.1	3.1	2.9
Lysine 78.8%	1.6	2.3	2.1
T-Premix	2.0	2.0	2.0
Sodium Bi-carb	2.0	2.0	1.5
Prop Acid 505	2.0	2.0	2.0
Choline CL 60%	1.4	1.3	1.5
Hy-D 62.5 mg/lb	1.0	0.5	
Trace Min PMX	1.0	1.0	1.0
L-Vitamin PMX	1.0	1.0	1.0
.06% Selenium	1.0	1.0	1.0
Ronozyme HI P (GT)	0.4	0.4	0.4
AMPROL 25 25%	1.0		
Total	2000.0	2000.0	2000.0
Protein %	20.0	17.6	15.5
ME kcal/kg	2926	2860	2805
Calcium %	1.00	1.00	1.80
A. Phos %	0.50	0.48	0.45
Lysine %	1.15	0.98	0.83
TSAA %	0.86	0.74	0.67

[1]Diets were acquired from the North Carolina State University Feed Mill in mash form
[2]Pre-Lay diet fed starting no later than 16 weeks

3.5 Laying Hen Nutrient Requirements

Laying hen nutrition can be precisely divided in various ways according to the demand of phase feeding and the characteristics of birds at different stages of production. The onset of lay is the determining turning point of laying hen nutrition because of the biological characteristics and the production purpose. Various production periods and phase feeding can affect the demand for

laying hen nutrition. Nutrition during the laying phase is to optimize performance, provide a prolonged peak period, and optimum immune function. Typical layer feed contains relatively low energy and high protein with lots of minerals and vitamins, especially phosphorus, calcium, and magnesium. The diet helps to supplement nutrient loss due to laying. The energy content of the feed tends to be low because, at this age, the birds perform fewer activities. Laying hens possess intensive metabolism for egg formulation. Below is the composition of the diet that was formulated for production periods of the research trial expressed previously (**Table 2**).

Table 2. Feed Ingredients and Mash Diet Compositions

Ingredients	2016 Layer Diet	1940 Layer Diet
Corn	940.5	1146.38
Soybean Meal	718.0	232.57
Alfalfa Meal		305.97
Limestone, gr.	145.5	124.2
Coarse limestone	50.0	
Fat	110.0	
Phosphate Mono/D	17.6	
Salt	6.8	5.0
D.L. Methionine	2.9	
T-Premix	1.0	
Sodium Bi-carb	2.0	
Prop Acid 505	1.0	
Choline CL 60%	1.3	4.0
Hy-D 62.5 mg/lb		
Trace Min PMX	1.0	
L-Vitamin PMX	1.0	
.06% Selenium	1.0	
Ronozyme HI P (GT)	0.4	
Total	2000.0	2000.0
	Calculated Analysis	
Protein %	20.8	20.0
ME kcal/kg	2926	1330
Calcium %	4.10	0.90
A. Phos %	0.45	0.42
Lysine %	1.20	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]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt
[4]Vitamin premix provides per kg of diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid. 6 Selenium premix = 1 mg
[5]Selenium premix provides 0.2 mg Se (as Na2SeO3) per kg of diet

3.6 Nutrient Availability

The role of diet composition is discussed in this section and the principal role of the feed ingredients is to provide the nutrients that the bird digests and utilizes for reproductive functions. The onset of lay hens requires nutrients to maintain their current state (maintenance) and to enable body growth (weight gain) and egg production. Historically, recommendations on nutrient requirements have been based on available literature and data from expert groups. Currently, however, because each specific genotype has its own requirements, most commercial feed formulations use minimum requirements recommended by the breeding companies that supply the chicks. Feed itself has six different major nutrients: water, carbohydrates, fats, proteins, vitamins, and minerals.

3.6.1 Water

Water is the most important nutrient playing a vital role in virtually every physiological function of the bird. The intake of water is a sensitive indicator of health, therefore, maintaining and monitoring water consumption is a beneficial tool and guide to assessing health status. Constant supply of water is important to: I) the digestion of feed; II) the absorption of nutrients; III) the excretion of waste products; IV) the regulation of body temperature. Being 90% of the blood's content, water carries nutrients from the digestive tract to cells and aids in the elimination of waste products. Due to the lack of sweat glands, water helps cool the bird through evaporation via respiration. There are several factors that affect the amount of water a bird needs, making it difficult to assess: age, body condition, diet, temperature, quality of water, and humidity. It is assumed that birds consume by weight twice as much water as feed and are directly related. Current high-producing laying hens have higher metabolic demands for water than earlier layer strains. If birds are deprived of water for even a short time, production and

growth are irreversibly affected making it essential for water to be made available at all times. The quality of water is equally important and is often taken for granted, but poor water quality can lead to poor productivity and extensive economic losses. Water is an optimal intermediate for the distribution of contaminants, comparatively both chemicals and minerals, and the proliferation of destructive microorganisms.

3.6.2 Energy

Poultry can derive energy from simple carbohydrates, fat, and protein. Birds cannot digest nor utilize some complex carbohydrates, such as fiber, making feed formulation based on available energy. Metabolizable energy (ME) is the conventional measure of the available energy content of feed ingredients and the requirements of poultry which considers energy losses of both the feces and urine (C. F. M. de Lange et al., 2005). Birds eat primarily to satisfy their energy needs, provided that the diet is adequate in all other essential nutrients, deeming the energy level in the diet a major determinant of the bird's feed intake. When the dietary energy level changes, the feed intake will change, and the specifications for other nutrients must be modified to maintain the required intake. Different classes of poultry need different amounts of energy for metabolic purposes, and a deficiency will affect productive performance. To sustain high productivity, modern poultry strains are typically fed relatively high-energy diets.

3.6.3 Proteins

Proteins are the most important part of a hen's diet as it is a bodybuilding material essential for the formation of flesh, blood, feathers, skin, bone, and eggs. Back in the 1950s, the ideal protein concept was developed as a way to define the essential amino acid requirements of birds for protein accretion and maintenance (Scott et al., 1969). The dietary content of balanced protein has an effect on both egg number and egg size. Laying hens deficient in protein will

display a reduction or cessation in egg production, and or try to meet their protein requirement by feather picking. Poultry does not have a requirement for protein per se, however, an adequate dietary supply of nitrogen from protein is essential to synthesize non-essential amino acids.

Amino acids (AAs) are the building blocks of protein, which is the major dry matter component for growth in chickens and their eggs. Amino acids, calcium, and fatty acids are some of the critical dietary ingredients in a laying hen diet and must be maintained throughout its life. Amino acids promote protein deposition by converting feed utilization into increased growth in preparation for the laying period. Hens will also try to maintain amino acid requirements by increasing their feed intake, resulting in an overall increase in energy consumption. In caged laying hens, increasing lysine and methionine concentrations ameliorate the negative effects of stocking density on cannibalism and mortality. The essential amino acids for poultry are lysine, methionine, threonine, tryptophan, isoleucine, leucine, histidine, valine, phenylalanine, and arginine. Cysteine and tyrosine are considered semi-essential amino acids, due to their ability to be synthesized from methionine and phenylalanine, respectively. Of the ten essential amino acids, lysine, methionine, and threonine are the most limiting in most practical poultry diets. The amino acid requirements of poultry are influenced by several factors, including production level, genotype, sex, physiological status, environment, and health status.

3.6.4 Minerals: Calcium and Phosphorus

Minerals are fundamental for the formation of the skeletal system, for general health, as components of general metabolic activity, and for the maintenance of the body's acid-base balance. Some minerals such as calcium and phosphorus are required in large quantities and are the most abundant mineral elements in the body, being classified as macro-minerals, along with sodium, potassium, chloride, sulfur, and magnesium. Macro-minerals are elements required in

the diet at concentrations of more than 100 mg/kg. For example, laying hens require between 3.5-4% calcium, 0.3-0.4% available phosphorus, and 0.2% sodium in their diets for egg production. Other minerals, such as copper, iron, manganese, zinc, selenium, cobalt, iodine, and molybdenum, are required in milligram quantities but deficiency of these minerals will lead to serious health problems in mild cases and death in severe cases. Trace elements including copper, iodine, iron, manganese, selenium, zinc, and cobalt, function as components of larger molecules and as cofactors of enzymes in various metabolic reactions hence their smaller required amounts in the diet.

Calcium (Ca) and phosphorus (P) are key nutrients in layer diets possessing many essential roles in metabolism, especially in bone development and eggshell formation in the form of hydroxyapatite (99% Ca, 80% P). Defining the calcium and phosphorus requirements of commercial leghorns has been a challenge to both nutritionists and producers due to the demand for these two minerals constantly changing (Roland, David A. 1986). According to the National Research Council (NRC) a hen's calcium requirement has increased by 65% from 1944 to 1984, 2.27 to 375 g/hen/day while the phosphorus requirements have been decreasing (Roland, David A, 1986). Calcium is paramount for the growth of the medullary bone before the first ovulation and eggshell strength. The balance of Ca and P is maintained by the absorption in the small intestine, reabsorption and excretion of the kidney, and deposition and mobilization of bone. In laying hens, Ca and P are involved in the formation of eggshells during the laying period. The chemical composition of the chicken eggshell (by weight) is calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%), and organic matter (4%). Both calcium and phosphorus are more associated with major egg problems, especially shell quality.

In 1940, findings reported by Tyler (1940) suggested that regardless of the level of dietary calcium fed to hens, she would reach a plateau in retention of around 1.5 grams of calcium per day. He also indicated that dietary phosphorus levels that reach way below 0.5% can be fed to hens without being detrimental to performance (Tyler, 1940). In general, 60 to 80 percent of total phosphorus present in plant-derived ingredients is in the form of phytate-phosphorus. Under normal dietary conditions, phytate phosphorus is poorly utilized by poultry owing to the lack of endogenous phytase in their digestive enzymes. It is generally assumed that about one-third of the phosphorus in plant feedstuffs is non-phytate and is biologically available to poultry, so the phosphorus requirement for poultry is expressed as non-phytate phosphorus, rather than total phosphorus. A ratio of 2:1 must be maintained between calcium and non-phytate phosphorus in growing birds' diets, to optimize the absorption of these two minerals. The ratio in laying birds' diets is 13:1 as a result of the very high requirement for calcium for good shell quality (Pelicia et al., 2009).

3.6.5 Vitamins

Vitamins are classified as fat-soluble (vitamins A, D, E, and K) and water-soluble (vitamin B complex and vitamin C). All vitamins, except for vitamin C, must be provided in the diet. Vitamin C is not generally classified as a dietary essential as it can be synthesized by the bird. The metabolic roles of the vitamins are more complex than those of other nutrients. Vitamins are not simple body building units or energy sources but are mediators of or participants in all biochemical pathways in the body.

3.7 Nutritional Approaches that Accommodate Genetic Potential

The science behind poultry nutrition has changed over the past decades to accommodate the changes in production systems and with the availability of various identified nutrients and

their metabolic roles. Genetic selection has affected the amount of nutrients required for maintenance as well as body size. Layers of a light strain will normally consume less feed than a heavier strain, however, both light and heavier strains are very capable of the same production levels. In general, nutritional recommendations for laying hens have considered both breed and housing conditions. As situations change within housing conditions, including the stage of production and season of the year, the amount of each nutrient required will change.

3.8 Conclusion

Over the past decades, the poultry industry has made tremendous strides in health with an emphasis on improvements in nutrition with novel research both in academia and industry. Nutrient recommendations vary depending on strain and management for specific flocks making it critical to understand the interplay of nutritional requirements.

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CHAPTER 4: Influence of genetic selection on rearing parameters and production performance of two Leghorn type pullets, 1940 random-bred control versus W-36 (2016) commercial strain grown under the same regimen

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4.1 Summary

The aim of this study was to expand the period of selection history by evaluating the pullet growth characteristics of two genetic stocks of Leghorns reared under identical conditions. 1940 Leghorn Strain (WL40) from the University of Arkansas and a 2016 Commercial Hy-Line W-36 (WLCC) from Hy-Line were housed in the same environment, comparing rearing production characteristics from the day of hatch until 16 weeks of age. All chicks were hatched at the Prestage Department of Poultry Science at North Carolina State University and raised in the same cage rearing facility, with 14 birds per brooding cage then reduced to 10 birds per cage. Pullets were weighed bi-weekly, and feed weigh backs were concurrent to determine body growth, feed consumption, and utilization. Pullet starter was fed from 0-6 weeks, a grower from 6-12 weeks, and a developer from 12-16 weeks. Diets were provided ad libitum, and mortality was recorded daily. The experiment was a randomized block design using a 2 ×3 factorial design, 2 genetic strains, and 3 dietary phase regimens and all data were statistically analyzed using a one-way ANOVA. Both strains responded similarly, showing an increase in body weights with the 2016 commercial strain exhibiting the heavier weights with no significant differences ($P>0.05$) in all feed phases. Results from this data suggest that layer selection over the years had neutral adverse effects on body weights or feed conversion reared in the same

environment and current commercial diet. This indicated that selection preferences for desirable traits such as body weights and feed conversion have been successfully implemented for optimal performance benefiting the 2016 pullet.

KEYWORDS: Pullet; Nutrition; Genetics; FCR; Body weights

4.2 Introduction

There are few random-bred strains of Leghorns available and there is limited information in strain comparison with reference to rearing practices. Previous research has illustrated that rearing conditions can affect both growth and egg production. The rearing period of pullets is deemed critical and plays an important role in the successful transition to today's diverse housing systems for laying hens. According to de Haas. et al (2014) early life experiences establish long-lasting effects on the brain and body, impacting brain function, behavior, and physical health throughout the lifespan of an animal. Rearing management can also influence bird development. Differences in both physical and social environments experienced between rearing and laying hen facilities can affect bird adaptability and productivity during the layer phase. Rearing pullets in environments that mimic future housing systems during lay is thought to ease the transition between these phases. Despite its key role, research into the rearing housing and management of pullets and its effects over a bird's lifetime has been a relatively neglected field until recently with the focus emphasized on housing systems. Of the heritable traits and characteristics, body weight and feed utilization have remained dominant for the expression of genetic differences. The aim of this study was to expand the period of selection history by evaluating the pullet growth characteristics of two genetic stocks of Leghorns reared under identical conditions. The objective of this study included an assessment of the relationship between body weights and feed conversion within the rearing dietary phases of the different strains.

4.3 Materials and Methods

4.3.1 Strain and Strain Management

A total of 1500 Fertile hatching eggs for the 1940 random-bred control strain were provided by the University of Arkansas, Fayetteville, Arkansas. The 1940 random-bred control strain eggs were set with 720 hatching eggs provided by Hy-Line, NC, from a 2016 white leghorn W36 breeder flock. All chicks were hatched and grown at the Prestage Department of Poultry Science at North Carolina State University in Raleigh, NC. The chicks were hatched, neck-tagged, and subsequently placed with 14 birds per cage in a Petersime battery cage (332 cm^2 per bird). Each cage contained 2 nipple drinkers parallel to each other and a feeder trough. Continuous light was provided at 10ftc. (100 lux) with continuous light for the first 2 days and steadily declined to 10 hr maintained ending at 16 wks of age displayed in **Table 1** (Anderson, 2016). Water and feed were supplied ad libitum. The pullet rearing diets consisted of a starter, grower, and developer. The pullet starter was supplied from 0-6 weeks of age, the pullet grower was supplied from 6-12 weeks of age, and the pullet developer was supplied from 12-16 weeks of age. **Table 2** shows the composition of diet formulations for each dietary phase. **Table 3** illustrates the proximal feed analyses of each dietary phase. Pullets were beak trimmed at 9 d of age using a Lyons Precision beak trimmer with a 7/64-in. guide hole. The trim was a block cut with an approximate blade temperature of 1,100°F (dull red). Beak trimming for all birds was completed in less than 1 d. Pullets were not retrimmed at any point in the rearing period. All birds were vaccinated. All animal use and experimental procedures were approved by the NCSU Institutional Animal Care and Use Committee.

Table 1. Pullet House Light Schedule for 1940/2016 Rearing

Age	Lux	Photoperiod (hr)
Days 1-2	10 ftc. (100 lux)	24
Day 3	1 ftc. (10 lux)	23
Week 1	1 to 0.5 flc. (10 to 5 lux)	22
Week 2	1 to 0.5 flc. (10 to 5 lux)	20
Week 3	1 to 0.5 flc. (10 to 5 lux)	18
Week 4	1 to 0.5 flc. (10 to 5 lux)	16
Week 5	1 to 0.5 flc. (10 to 5 lux)	14
Week 6	1 to 0.5 flc. (10 to 5 lux)	12
Week 7 through	1 to 0.5 flc. (10 to 5 lux)	10
Week 12	1 to 0.5 flc. (10 to 5 lux)	10
Week 13 – 16	1 to 0.5 flc. (10 to 5 lux)	10

[1] Lighting schedule was identical for all birds through 16 weeks of age

4.3.2 Diets Utilized

The assessment of the nutritional contribution to the improvement in pullet growth and efficiency was accomplished by placing both leghorn strains on the same dietary regimens that are currently being utilized in today's industry. The pullet rearing diets were the same as those diets utilized in the 39th NCLP&MT and were delivered in mash form. Descriptions of all the diets involved are provided in **Tables 2** and **3-5**, along with their laboratory analyses for protein, fat, fiber, and ash conducted by the North Carolina Department of Agricultural Consumer Services and Food and Drug Protection Division Laboratory. Both dietary regimens were based on corn and soybean meal.

Table 2. Composition of Diet Formulations for Rearing Periods

Ingredient	Starter	Grower	Develop
Corn	1192.0	1172	1193.0
Soybean Meal	592.0	426.0	316.0
Wheat Midds	127.0	316.0	365.0
Limestone, gr.	34.0	37.0	80.0
Coarse Limestone			
Fat	10.0	10.0	10.0
Phosphate Mono/D	20.5	16.4	14.3
Salt	6.0	6.0	6.3
D.L. Methionine	4.1	3.1	2.9
Lysine 78.8%	1.6	2.3	2.1
T-Premix	2.0	2.0	2.0
Sodium Bi-carb	2.0	2.0	1.5
Prop Acid 505	2.0	2.0	2.0
Choline CL 60%	1.4	1.3	1.5
Hy-D 62.5 mg/lb	1.0	0.5	
Trace Min PMX[3]	1.0	1.0	1.0
L-Vitamin PMX[4]	1.0	1.0	1.0
.06% Selenium[5]	1.0	1.0	1.0
Ronozyme HI P (GT)	0.4	0.4	0.4
AMPROL 25 25%	1.0		
Total	2000.0	2000.0	2000.0
Protein %	20.0	17.6	15.5
ME kcal/kg	2926	2860	2805
Calcium %	1.00	1.00	1.80
A. Phos %	0.50	0.48	0.45
Lysine %	1.15	0.98	0.83
TSAA %	0.86	0.74	0.67

[1]Diets were acquired from the North Carolina State University Feed Mill in mash form
[2]Pre-Lay diet fed starting no later than 16 weeks
[3]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt
[4]Vitamin premix provides per kg of diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid. 6 Selenium premix = 1 mg
[5]Selenium premix provides 0.2 mg Se (as Na2 SeO3) per kg of diet

Table 3. Proximal Feed Analysis of the rearing Diets as fed

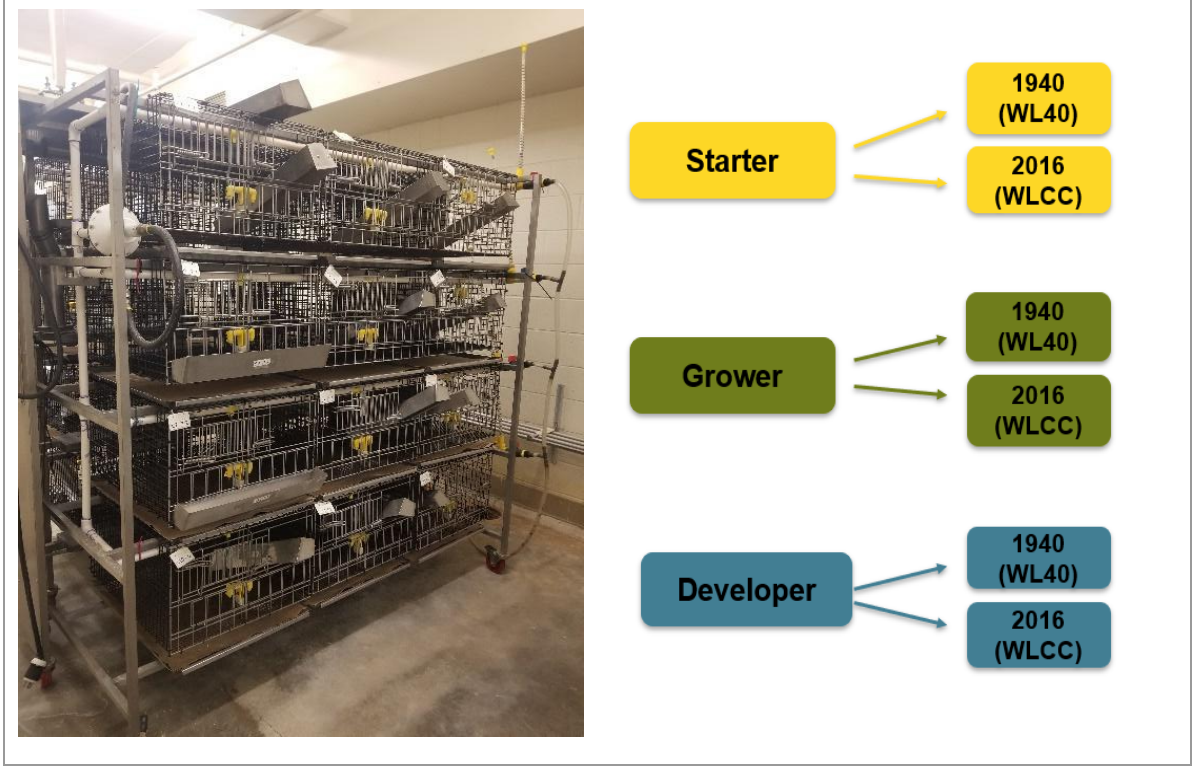
	Unit	Starter	Grower	Developer
Dry Matter	%	90.7	90.29	90.7
Crude Protein	%	20.33	17.88	15.18
Nitrate Ion	%	0	0	0
Neutral Detergent Fiber	%	9.19	8.55	7.49
Acid Detergent Fiber	%	4.06	3.38	3.11
Non-fiber Carbohydrate	%	52.91	55.78	60.66
Fat	%	3.13	2.62	2.74
Calcium	%	1.12	1.08	1.01
Phosphorus	%	0.78	0.64	0.63
Sulfur	%	0.25	0.23	0.21
Magnesium	%	0.13	0.13	0.13
Sodium	%	0.11	0.12	0.09
Potassium	%	0.68	0.66	0.64
Copper	ppm	16	17	13
Iron	ppm	318	285	313
Manganese	ppm	144	143	123
Zinc	ppm	129	150	121
Ash	%	50.13	5.46	4.62
Aflatoxin	ppb	0	0	0

[1]Analyzed by North Carolina Department of Agriculture and Consumer Services Food and Drug Protection Division Laboratory

4.3.3 Experimental Procedures

The birds were placed into 32 Alternative Design Battery Cages in a randomized block design using a 2 ×3 factorial design, 2 genetic strains, and 3 dietary phase regimens. Cages 1-16 housed the 2016 WLC and cages 17-32 housed the 1940 RBC. Mortality and the BW of all mortalities were recorded daily. All birds in each replicate were group weighed according to treatment assignment bi-weekly. Feed consumption was recorded bi-weekly along with feed weigh-back to determine feed conversion ratio (FCR). At 16 weeks of age, all pullets were transported to a grow-out facility.

Figure 1. Experimental Design and Cage Layout



[1]Alternative battery cage system located in Scott Hall at NCSU

4.3.4 Statistical Analysis

All data were statistically analyzed using a one-way ANOVA (SAS 9.4, SAS Institute Inc., Cary NC). The data were analyzed as 0-6 wk (starter), 6-12 (grower), and 12-16 (developer). Feed conversions were analyzed for the entire rearing period. The two main effects were the 1940 WL40 and the 2016 WLCC leghorn strains and 3 dietary phases. All levels of significance were based on a probability value of equal to or less than 0.05.

4.4 Results

The results are presented in two phases: the body weights of pullets from 2 weeks to 16 weeks of age and the feed consumed including feed conversion ratio; and total nutrient intake of

pullets from 0 weeks to 16 weeks of age. The BW, FC, FCR, and total nutrient intake of the pullets are shown in **Tables 4**, **5, 6, and 7.**

4.4.1 Body Weights

During the starter feed phase, illustrated in **Table 4**, both strains showed an increase in body weights over the 6-week period with the 2016 commercial strain exhibiting heavier weights with no significant differences (P≥0.05). During the grower diet phase, both strains showed an increase in body weight with the 2016 strain exhibiting slightly heavier weights. There were no significant differences between both strains during the grower diet phase (P≥0.05). During the developer diet phase, both strains showed an increase in body weight with the 2016 strain exhibiting slightly heavier weights. There were no significant differences (P≥0.05) between the strains during the developer diet phase.

Table 4.	Bi-weekly Body Weights of the Hy-line W-36 and 1940 leghorn: Cage-reared								
Breed	----------(Weeks of Age)----------								
	0	2	4	6	8	10	12	14	16
	----------(kg)----------								
Hy-Line W-36	0.040	0.095	0.231	0.396	0.556	0.739	0.841	1.088	1.185
1940 Leghorn	0.029	0.068	0.186	0.332	0.491	0.658	0.745	0.963	1.036
SEM	0.027	0.031	0.042	0.048	0.053	0.065	0.069	0.071	0.074
p-value	0.059	0.181	0.208	0.364	0.523	0.698	0.793	1.025	1.103

[1]Significant differences (P≤0.05) within both strains are noted by different letters among columns of means

4.4.2 Feed Consumption

Bi-weekly feed consumption per strain, determined in **Table 5**, shows variations but no significant differences. Comparisons of feed efficiency, demonstrated in **Table 6**, were determined by FCR which is feed intake/body weight gain. There were no significant differences between the strains. On the contrary, the 2016 strain exhibited a higher feed conversion in comparison to the 1940 strain during weeks 2 and 4. However, during the remainder of the rearing period, the 1940 strain exhibited a higher feed conversion when compared to the 2016 strain. Total feed nutrient intake, livability, and flock uniformity are outlined in **Table 7.** Similarities were exhibited in both strains for protein, met energy, lysine, and TSAA. Those same similarities were observed for livability and flock uniformity having no significant differences.

Table 5.	Bi-weekly Feed Consumption of the Hy-line W-36 and 1940 leghorn: Cage-reared							
	----------(Weeks of Age)----------							
Breed	0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16
	----------(kg per bird)----------							
Hy-Line W-36	1.43	1.89	1.51	1.56	1.00	1.00	0.53	0.23
1940 Leghorn	1.37	1.79	1.60	1.68	0.95	1.12	0.47	0.32
SEM	0.031	0.061	0.037	0.041	0.037	0.052	0.038	0.026
p-value	0.156	0.184	0.271	0.169	0.313	0.204	0.572	0.273

[1]Significant differences ($P \leq 0.05$) within both strains are noted by different letters among columns of means

Table 6.	Total Nutrient Intake, Livability, and Flock Uniformity of the Hy-line W-36 and 1940 leghorn: Cage-reared					
Breed	Protein	Met. Energy	Lysine	TSAA	Livability (1-112 d)	Flock Uniformity
	----------(per bird to 112 days)----------					(% of pullets within ±10% of *x*)
	(kg)	(kcal)	(g)	(g)	(%)	
Hy-Line W-36	1.051	12,158	43.00	33.00	99.90	96.0
1940 Leghorn	1.013	12,032	42.50	32.00	99.80	90.75
SEM	0.004	50.39	0.018	0.013	0.041	0.037
p-value	0.239	1.724	0.337	0.381	0.519	0.792

[1]Significant differences (P≤0.05) within both strains are noted by different letters among columns of means

4.5 Discussion

Variations in body weight can be attributed to genetic differences which can affect individual performance. Pullets are typically reared in groups according to their body weights in order to achieve improved performance within the flock. Genotype potentially has the ability to affect the body weights of poultry birds according to Korver et al., (2004). A study conducted by Gonzales et al., (1998) supported this claim further explaining that strains have different genetic potentials equipped for growth (Gonzales et al., 1998). Contrary to the thoughts related to growth, there were no significant effects of the rearing environment on growth performance and feed conversion throughout the dietary phases presented in this current study.

One of the major criteria for identifying strains of high performance is through feed conversion. Previous research conducted by Rondelli et al., (2003); Taha et al., (2010) reported significant differences in feed conversion of different chicken strains, however, no differences were observed displaying a reduction in feed conversion in this current study suggesting that the strains used were more genetically alike (Taha et al., 2010; Rondelli et al., 2003). Both strains

consumed around the same amount of feed throughout the rearing phases. It should be noted that the diets of each treatment group were nutritionally equal. As the rearing period progressed, a reduction in FCR in both the 1940 strain and 2016 strain was observed and could be related to potential lower maintenance requirements required by the pullets. These requirements could be due to a rapid growth increase in the proportion of energy used for growth relative to maintenance. Genetic selection can also be a factor determining the effects of the amount of feed required for maintenance as well as body size. This study indicates that selection over the years of layers had little effect on developmental body weights or feed conversion when reared in the same environment using current commercial diets, thus indicating that selection preferences for desirable traits such as body weights and feed conversion have been successfully implemented for optimal performance. The results from this data suggest that the selection of these layers over the 70+ years was focused on uniformity rather than body weights which did impact feed conversion of the pullets reared in the same environment and current commercial diets. This study indicates that selection preferences for desirable traits such as body weights and feed conversion have been successfully implemented for optimal performance subsequently in the layer phase.

4.6 Conclusion and Applications

1. Genetic selection for egg production does not appear to have altered pullet development during any of the rearing phases though the 1940 leghorn was consistently smaller.
2. Feed consumption using modern commercial diets was similar.
3. Understanding the interplay of nutrition and selection on production is important in that it may allow the industry to identify a pathway to improve nutrition and or understand genetics in pullets.

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|CHAPTER 5: Comparing performance, morphological, physical, and chemical properties of eggs produced by 1940 Leghorn or a commercial 2016 Leghorn fed representative diets from 1940 or 2016

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5.1 Summary

Eggshell quality is one of the most significant factors affecting the egg industry as it economically influences the quantity of saleable eggs. Eggshell quality can be improved through optimization of genotype, housing system, and mineral nutrition. The aim of this study was to compare genotype and evaluate the morphological, physical, and chemical properties of eggs (weight, breaking strength, Haugh units, and dry matter) by hens of two different strains fed a 1940 and 2016 representative diet. Egg production was measured daily and there were four periods with ten-week intervals in which eggs were analyzed starting at 30 weeks and ending at 60 weeks of age measuring quality parameters. This study was set as a 2 x 2 factorial. The factors consisted of 2 leghorn based genetic strains that were a 2016 commercial layer (W36) and a 1940 random bred leghorn line, then 2 diets based on 2016 and 1940 dietary standards. 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. Body weights were higher in the 2016 hens when compared to the 1940 hens, however, the feed conversion ratio fluctuated in hens with the 2016 hens on the 1940 diet consuming the most feed throughout the majority of the trial. The 1940s hens came into production later than the 2016 hens; however,

the rate of production was consistent with one another. Oviduct and ovary weights were heavier in the 2016 hens when compared to the 1940 hen. Egg quality, both interior, and exterior were greater in the 2016 hens when compared to the 1940 hens. Parameters measured demonstrated significant differences ($P \leq 0.05$) among treatment groups suggesting that genetics and nutrition both played a role in production rate and egg quality.

Keywords: White Leghorns, nutrition, production, egg quality

5.2 Introduction

The onset of laying age of commercial laying hens has been reduced over the decades with the improvement of laying hens' production performance, depending on many factors, including diet and age of hens (Tumová E et al., 2012). However, increased production has resulted in the decline of egg quality at the end of lay. Negative effects such as small egg size, declined eggshell quality, increased broken rate (percent of cracks), decreased albumen height, and shortened egg storage time has drastically hindered the egg industry. Egg production is a function of genetics, feed consumed, age at point-of-lay, age at peak-of-lay, peak percent lay, percent hen-day egg production, laying period, rearing environment, health care, and overall management of the flock.

Egg quality is a comprehensive concept that encompasses both internal and external quality of quantitative traits that are governed by the acceptability of consumers. External quality consists of eggshell color, egg shape index, eggshell thickness, and eggshell strength. Internal quality focuses on albumen height, egg yolk color, and Haugh unit (Liu, Z et al., 2019). All of these quantitative traits have been affected by genetic selection, especially with the extension of the lay cycle. Nutrition and management of the hen play a vital role in internal egg quality. Interiorly, the hen's egg consists of the yolk and white, or albumen as well as membranes.

Genetically, egg quality characteristics are different between species, breeds, strains, and families within the lines. Differences in egg weight and shell thickness exhibit differences between various lines and have a direct influence based on genotype (Ahn et al., 1997).

5.3 Materials and Methods

5.3.1 Experimental Design, Animal Care and Dietary Treatments

320 White Leghorn layer hens of two different strains were distributed into a 2 × 2 factorial arrangement with the factors being strain consisting of either a 1940 leghorn or a 2016 commercial leghorn strain and diet consisting of a 1940 or a 2016 diet. The factorial 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 had 10 replicates per treatment. Hens were provided with feed and water ad libitum and housed in pairs throughout the duration of the trial. Body and feed weights were recorded on a 28d cycle starting at 20 weeks of age. Egg production was measured daily throughout the trial. Egg quality parameters (physical, internal, and external) were measured at 30, 40, 50, and 60 weeks of age. Egg samples consisting of the previous day's egg production were collected and the shell eggs were analyzed for physical quality (yolk color, albumen height, Haugh unit).

5.3.2 Reproduction Organ Sampling

At the end of the trial, 12 hens were euthanized by cervical dislocation from each treatment group and the reproductive organ was removed. The ovary and oviduct were weighed individually, measured, and photographed.

5.3.3 Egg Production and Egg Quality Traits

Hen-day production per cycle was obtained by calculating the average egg production per bird cycle. For the strain effect, hens of the 2016 strain had a higher hen-day production when

compared to hens of the 1940 strain. Feed consumption was examined by calculating the relationship between the quantity of feed provided and the body weight gained after consumption. Egg numbers were recorded for each laying hen per treatment from sexual maturity until 60 weeks of age. Egg weight was measured with a digital scale in grams with an accuracy of 0.01g. Feed conversion ratio (FCR) was calculated by dividing the total input of the feed by the weight of the number of produced eggs. Candled egg quality of blood and meat spots in the yolk and albumen were detected visually.

5.3.4 Physical Egg Quality

During the collection period, a random sample of 48 eggs/treatment/d was collected on five consecutive days every week (6 eggs per replicate per day for the cage system). Once eggs were broken, eggshells were washed with water and dried to clean the remaining albumen. Shell thickness (with membrane) was measured at the sharp poles, blunt poles, and equatorial parts of each egg using a micrometer to the nearest 0.01 mm. Shell thickness was obtained from the average values of the three parts previously described. The albumen, yolk, and shell percentages were calculated using the individual weight of each egg and the weight of its components. Shell strength was determined utilizing a 3 in. diameter aluminum compression disc (TA-30, Texture Technologies) attached to the unit along with an egg holder with posts with the ability to rotate (TA-650, Texture Technologies). The egg was aligned so that the disc came into contact with the apex of the large end of the egg. A test speed of 2 mm/s and a trigger force of 0.001 kg were used.

5.3.5 Interior Quality

For interior egg quality, 48 eggs per treatment were randomly collected from 30-60 wks of age on a 28-d period. Egg weight (EW) was measured by being weighed to the nearest 10th of

a gram prior to testing. After weighing, the eggs were broken out onto a glass break-out table for albumen height, Haugh unit (HU), yolk weight (YW), and albumen weight (AW) measurements. Eggs used for vitelline membrane and percentage of thin albumen measurements were not weighed. Haugh units were evaluated using the Technical Services and Supplies QDC Egg Quality System. This instrument is connected to a computer equipped with software to automatically record egg weight (in grams) and albumen height (in millimeters) and calculate HU (Haugh, 1937). The second measure was the albumen height in millimeters. Albumen height was measured using an electronic height gauge that is part of the Technical Services and Supplies QDC Egg Quality System (TSS, York, UK) was used to measure albumen height after being broken out on a flat surface. Yolk color score was measured.

Component percentages were determined on a sample of 6 eggs from each replicate. The eggs' each replicate from the experimental treatments were individually weighed and identified. The yolks were separated from adhering albumen by being rolled, then weighed. The shells with membranes were then washed with distilled water and dried. After the shell was dried, they were weighed, and the albumen weight was calculated by subtraction of the shell with membrane weight and the yolk weight from the whole egg weight. The component percentages were then calculated for analysis.

Vitelline membrane strength was determined using a Texture Technologies TA-XT2i Texture analyzer which determines vitelline membrane strength using static compression (Texture Technologies, Scarsdale, NY). A set of 6 eggs, with cracked or broken eggs excluded from the analysis, were used. Each egg was individually broken into a shallow dish and the yolk was positioned under a 1-mm, rounded end, stainless steel probe. Due to reports outlined by Lyon et al. (1972) indicating that the strongest section of the vitelline membrane is near the

chalazae, precise precautions were considered to ensure that measurements were not obtained from this area. Direct pressure was applied to the yolk until the vitelline membrane ruptured and the probe penetrated the yolk. Compression measurements were made using a 0.5kg load cell, 0.1 g of trigger force, and 3.2 mm/s test speed. Vitelline membrane breaking strength was recorded as grams of force/mm^2 required to rupture the membrane. The force required to break the vitelline membrane corresponds to its strength and elasticity is the flexure of the vitelline membrane in mm prior to the penetration of the membrane.

Table 1. Feed Ingredients and Mash Diet[1] Compositions

Ingredients	2016 Layer Diet [2]	1940 Layer Diet [2]
Corn	940.5	1146.38
Soybean Meal	718.0	232.57
Alfalfa Meal		305.97
Limestone, gr.	145.5	124.2
Coarse limestone	50.0	
Fat	110.0	
Phosphate Mono/D	17.6	
Salt	6.8	5.0
D.L. Methionine	2.9	
T-Premix	1.0	
Sodium Bi-carb	2.0	
Prop Acid 505	1.0	
Choline CL 60%	1.3	4.0
Hy-D 62.5 mg/lb		
Trace Min PMX[3]	1.0	
L-Vitamin PMX[4]	1.0	
.06% Selenium[5]	1.0	
Ronozyme HI P (GT)	0.4	
Total	2000.0	2000.0
	Calculated Analysis	
Protein %	20.8	20.0
ME kcal/kg	2926	1330
Calcium %	4.10	0.90
A. Phos %	0.45	0.42
Lysine %	1.20	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]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt
[4]Vitamin premix provides per kg of diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid. 6 Selenium premix = 1 mg
[5]Selenium premix provides 0.2 mg Se (as Na2SeO3) per kg of diet

5.3.6 Statistical Analysis

All statistical analysis was performed in SAS, version 9.4 (SAS Institute, Inc., Cary, NC). Differences were considered significant when $P \leq 0.05$. Tukey's test was used to detect differences among treatments. Data are reported as the mean ± SD. Main effects and interaction effects were evaluated for hen strain and diet.

5.4 Results

Overall performance parameters measuring eggs per dozen, hen-day production per cycle (%), feed consumed (g/bird/day), mortality (%), feed conversion ratio (grams of feed/grams of egg) and average egg weight by strain, diet, and interactions are displayed in **Table 2.** Overall, hens of the 2016 strain consumed more feed when compared to hens of the 1940 strain. Mortality was higher in hens of the 1940 strain when compared to hens of the 2016 strain. Hens of the 2016 strain had a higher FCR when compared to the hens of the 1940 strain. The average egg weight was heavier in hens of the 2016 strain when compared to the hens of the 1940 strain. For the diet effect, hens fed the 2016 diet had a higher hen-day production when compared to hens fed the 1940 diet. Hens fed the 1940 diet consumed more feed when compared to hens fed the 2016 diet. Mortality was higher in hens fed the 2016 diet when compared to hens fed the 1940 diet. Hens fed the 1940 diet had a higher FCR when compared to hens who were fed the 2016 diet. Hens fed the 2016 diet had a higher average egg weight when compared to hens fed the 1940 diet. Three interactions were distinguished between strain x diet, strain x period, and period and diet. With the parameters measured, there was no significant interactions.

Table 2. Effect of strain and diet on overall performance of the laying hens

	Hen Day Production (%)	Feed Consumed[1] (g/bird/day)	Mortality (%)	Feed Conversion Ratio (feed grams/egg grams)	Average Egg Weight (g)
Main Effects					
Strain (S)					
2016	92.03[a]	173.50[a]	0.82[b]	1.67[a]	60.41[a]
1940	68.75[b]	123.00[b]	4.95[a]	1.42[b]	41.15[b]
SEM	1.256	0.876	3.341	0.030	2.576
p-value	0.003	0.001	0.002	0.001	0.033
Diet (D)					
2016	90.01[a]	146.23[b]	3.95[a]	1.26[b]	51.83[a]
1940	80.45[b]	151.09[a]	1.82[b]	1.38[a]	49.73[b]
SEM	0.422	0.654	1.103	0.073	0.134
p-value	0.006	0.003	0.001	0.011	0.002
Interactions					
S x D	0.379	0.183	0.241	0.344	0.352
S x Period	0.321	0.167	0.133	0.321	0.204
D x Period	0.311	0.165	0.131	0.317	0.211

[1]Feed consumed includes the feed which was wasted by the hens which would equate to feed disappearance

Note. Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.

Body weights per period are represented in **Table 3**. At the strain effect during the beginning of the trial, hens of the 2016 strain were heavier in weight when compared to hens of the 1940 strain resulting in significance (P≥0.05). Similar trends were observed during the end of the trial when hens of the 2016 strain had heavier body weights when compared to hens of the 1940 strain. At the diet effect, hens fed on the 2016 diet presented with significantly (P≥0.05) heavier body weights when compared to hens fed on the 1940 diet during the beginning of the trial. At the end of the trial, it was observed that hens fed on the 2016 diet were significantly (P≥0.05) heavier in body weight when compared to hens fed on the 1940 diet. An interaction between strain and diet did exist in both the beginning and the end of the trial.

Table 3. Effect of strain and diet on overall body weight

	Beginning Body Weight (kg)	Ending Body Weight (kg)
Main Effects		
Strain (S)		
2016	1.54[a]	1.59[a]
1940	1.40[b]	1.50[b]
SEM	0.037	0.041
p-value	0.011	0.013
Diet (D)		
2016	1.51[a]	1.68[a]
1940	1.44[b]	1.49[b]
SEM	0.042	0.047
p-value	0.012	0.014
Interaction		
S x D	0.036	0.041

[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.

The results of ovary weights and oviduct lengths at strain and diet effects are displayed in **Table 4** which were measured at the end of the trial. Significant differences (P≤0.05) were observed in both the ovary weights and the oviduct length. Hens of the 2016 strain had heavier weights when compared of hens of the 1940 strain. Similar results were observed for the length of the oviduct demonstrating that hens of the 2016 strain had longer oviducts when compared to hens of the 1940 strain. For hens fed on the 2016 diet, ovary weights were significantly (P≤0.05) heavier when compared to hens fed on the 1940 diet. However, there were differences observed in either ovary weights or oviduct length of hens fed on either the 2016 or 1940 diet. Interaction between strain x diet were significant (P≤0.05) in observed ovary weights and oviduct length.

Table 4. Ovary Weight and Oviduct Length at 69 weeks of age (g, inches)

	Ovary Weights	**Oviduct Length**
Main Effects		
Strain (S)		
2016	7.33[a]	26.36[a]
1940	5.62[b]	20.01[b]
SEM	0.695	0.445
p-value	0.077	0.119
Diet (D*)*		
2016	7.08[a]	22.82[a]
1940	5.88[b]	23.61[a]
SEM	0.695	0.445
p-value	0.132	0.236
Interaction		
S x D	0.049	0.047

[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.

The results of physical egg quality parameters measured from both strain and diet at 30, 40, 50, and 60 wks of age are displayed in **Tables 5**, **6**, **7**, and **8**. At 30 wks of age with the strain effect, hens of the 2016 strain were significantly different (P≤0.05) when compared to hens of the 1940 strain for egg weight, albumen height and Haugh unit. On the contrary, no significant differences (P≤0.05) were observed in shell color and yolk color for both strains. With the diet effect, there were no significant differences (P≤0.05) observed for shell color, egg weight, albumen height and Haugh unit for hens fed on both the 2016 and 1940 diets. However, there was a significant difference (P≤0.05) in yolk color with hens fed on the 1940 diet having a higher color when compared to hens fed on the 2016 diet. Interaction between strain x diet were significant (P≤0.05) in observed in the parameters measured at 30 wks of age with the exception of shell color.

Table 5. Physical egg quality components as measured at 30 wks of Age

	Shell Color %	Egg Weight (g)	Albumen Height	Haugh Unit (HU)	Yolk Color
Main Effects					
Strain (S)					
2016	78.70[a]	57.41[a]	9.52[a]	97.31[a]	4.76[a]
1940	76.52[a]	38.34[b]	6.18[b]	85.49[b]	4.45[a]
SEM	0.473	0.435	0.165	0.713	0.120
p-value	0.058	0.031	0.028	0.033	0.057
Diet (D)					
2016	77.48[a]	48.62[a]	7.72[a]	90.41[a]	3.83[b]
1940	77.73[a]	47.28[a]	7.98[a]	92.39[a]	5.38[a]
SEM	0.485	0.430	0.210	0.715	0.115
p-value	0.067	0.056	0.057	0.059	0.038
Interaction					
S x D	0.272	0.050	0.038	0.048	0.038

[1]Shell color was measured based on the light reflectance from the surface of the egg with pure white having a light reflectance of 83.3%
[2]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.

At 40 wks of age, significant differences (P≤0.05) were observed for the strain effect, hens of the 2016 strain had a higher shell color, egg weight, albumen height, Haugh unit and yolk color when compared to hens of the 1940 strain. For the diet effect, there no were significant differences observed for shell color, egg weight, albumen height, and Haugh unit. However, a slight difference was observed for yolk color where hens fed on the 1940 diet had a higher yolk color when compared to hens fed on the 2016 diet. Interaction between strain x diet were significant (P≤0.05) in observed in the parameters measured at 40 wks of age with the exception of shell color.

Table 6. Physical egg quality components as measured at 40 wks of age

	Shell Color %	Egg Weight (g)	Albumen Height	Haugh Unit (HU)	Yolk Color (DSM fan)
Main Effects					
Strain (S)					
2016	84.16[a]	60.75[a]	8.47[a]	91.35[a]	4.54[a]
1940	81.42[a]	41.39[b]	5.59[b]	80.43[b]	3.76[b]
SEM	0.345	0.500	0.135	0.780	0.038
p-value	0.057	0.037	0.039	0.027	0.047
Diet (D)					
2016	82.97[a]	52.06[a]	7.03[a]	85.68[a]	3.87[b]
1940	82.61[a]	50.07[a]	7.02[a]	86.11[a]	4.43[a]
SEM	0.345	0.435	0.135	0.775	0.039
p-value	0.057	0.051	0.159	0.059	0.045
Interaction					
S x D	0.239	0.049	0.053	0.052	0.040

[1]Shell color was measured based on the light reflectance from the surface of the egg with pure white having a light reflectance of 83.3%
[2]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.

At 50 wks of age, for the strain main effect, hens of the 2016 strain were significantly different (P≤0.05) by having a higher shell color, egg weight, albumen height, and Haugh unit when compared to hens of the 1940 strain. However, no significance was observed for yolk color between hens of the 2016 strain and hens of the 1940 strain. For the diet effect, no significance was observed for shell color, egg weight, or albumen height for hens fed on the 2016 diet and for hens fed on the 1940 diet. However, significance (P≤0.05) was present for Haugh unit and yolk color where hens fed on the 1940 diet had a higher unit when compared to hens fed on the 2016 diet. Interaction between strain x diet were significant (P≤0.05) in observed in the parameters measured at 50 wks of age.

Table 7. Physical egg quality components as measured at 50 wks of age

	Shell Color %	Egg Weight (g)	Albumen Height	Haugh Unit (HU)	Yolk Color (DSM fan)
Main Effects					
Strain (S)					
2016	82.57[a]	60.54[a]	8.54[a]	91.62[a]	3.06[a]
1940	79.94[b]	41.77[b]	5.99[b]	82.90[b]	3.03[a]
SEM	0.365	0.540	0.560	0.815	0.075
p-value	0.034	0.040	0.0397	0.034	0.121
Diet (D)					
2016	81.49[a]	52.54[a]	7.08[a]	86.01[b]	2.97[b]
1940	81.34[a]	50.26[a]	7.45[a]	88.52[a]	3.12[a]
SEM	0.370	0.540	0.140	0.815	0.080
p-value	0.060	0.051	0.054	0.031	0.033
Interaction					
S x D	0.173	0.049	0.056	0.047	0.039

[1]Shell color was measured based on the light reflectance from the surface of the egg with pure white having a light reflectance of 83.3%
[2]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.

At 60 wks of age, for the strain main effect, significance (P≤0.05) was observed for hens of the 2016 strain having the highest shell color, egg weight, albumen height, and Haugh unit when compared to hens of the 1940 strain. However, the yolk color was significantly (P≤0.05) higher in hens of the 1940 strain when compared to hens of the 2016 strain. For the diet main effect, hens fed on the 2016 diet were significantly (P≤0.05) higher in egg weight and albumen height, but hens fed on the 1940 diet were significantly (P≤0.05) higher in shell color, Haugh unit, and yolk color. No significance was present for the albumen height for hens fed on the 2016 diet or hens fed on the 1940 diet. Interaction between strain x diet were significant (P≤0.05) in observed in the parameters measured at 60 wks of age with the exception of shell color. Interaction between strain x diet were significant (P≤0.05) in observed in the parameters measured at 60 wks of age.

Table 8. Internal egg quality components measured at 60 wks of age

	Shell Color %	Egg Weight (g)	Albumen Height (mm)	Haugh Unit (HU)	Yolk Color (DSM fan)
Main Effects					
Strain (S)					
2016	79.25[a]	62.36[a]	8.06[a]	88.72[a]	4.69[b]
1940	76.61[b]	43.12[b]	6.29[b]	84.09[b]	5.15[a]
SEM	0.515	0.535	0.145	0.871	0.185
p-value	0.037	0.038	0.036	0.032	0.026
Diet (D)					
2016	77.82[b]	54.13[a]	7.08[a]	85.55[b]	4.33[b]
1940	78.03[a]	51.35[b]	7.27[a]	87.26[a]	5.51[a]
SEM	0.515	0.540	0.150	0.870	0.100
p-value	0.033	0.045	0.161	0.038	0.027
Interaction					
S x D	0.030	0.037	0.050	0.041	0.033

[1]Shell color was measured based on the light reflectance from the surface of the egg with pure white having a light reflectance of 83.3%
[2]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.

The results of external egg quality from both strain and diet at 30, 40, 50, and 60 wks of age are displayed in **Tables 9**, **10**, **11**, and **12**. At 30 wks of age, for the strain effect, hens of the 2016 strain were significantly different (P≤0.05) from hens of the 1940 strain having heavier whole egg weights, shell weights, yolk weights and thicker shell thickness. For the diet effect, hens fed on the 2016 diet were significantly different (P≤0.05) from hens fed on the 1940 diet by having heavier whole egg weights and thicker shell thickness. However, there were no significance in from hens fed on the 2016 diet and hens fed on the 1940 diet in shell weight or yolk weight. Interaction between strain x diet were significant (P≤0.05) in observed in the parameters measured at 30 wks of age.

Table 9. External egg quality at 30 wks of age

	Whole Egg Wt.[1] (g)	Shell Wt.[1] (g)	Yolk Wt.[1] (g)	Shell Thickness[1] (mm)
Main Effects				
Strain (S)				
2016	56.52[a]	5.4[a]	13.55[a]	0.33[a]
1940	37.83[b]	3.66[b]	11.04[b]	0.29[b]
SEM	0.560	0.435	0.150	0.003
p-value	0.048	0.035	0.043	0.025
Diet (D)				
2016	47.59[a]	4.73[a]	12.41[a]	0.33[a]
1940	46.76[b]	4.33[a]	12.18[a]	0.30[b]
SEM	0.675	0.075	0.230	0.004
p-value	0.031	0.120	0.303	0.027
Interaction				
S x D	0.032	0.046	0.057	0.030

[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.

At 40 wks of age, for the strain main effect, hens of the 2016 strain were significantly (P≤0.05) heavier in whole egg weight, shell weight, yolk weight and shell thickness when compared to hens of the 1940 strain. For the diet main effect, hens fed on the 2016 diet were significantly (P≤0.05) heavier for whole egg weight and yolk weight when compared to hens fed on the 1940 diet. However, shell thickness was significantly (P≤0.05) thicker in hens fed on the 1940 diet when compared to hens fed on the 2016 diet. There was no significance in shell weight for hens fed on the 2016 diet and the hens fed on the 1940 diet. Interaction between strain x diet were significant (P≤0.05) in observed in the parameters measured at 40 wks of age with the exception of shell color.

Table 10. External egg quality components measured at 40 wks of age

	Whole Egg Wt.[1] (g)	Shell Wt.[1] (g)	Yolk Wt.[1] (g)	Shell Thickness[1] (mm)
Main Effects				
Strain (S)				
2016	60.87[a]	5.82[a]	15.26[a]	0.32[a]
1940	41.2[b]	4.01[b]	12.5[b]	0.30[b]
SEM	0.535	0.065	0.140	0.003
p-value	0.037	0.021	0.030	0.022
Diet (D)				
2016	51.86[a]	4.93[a]	14.29[a]	0.31[b]
1940	50.21[b]	4.94[a]	13.47[b]	0.32[a]
SEM	0.535	0.065	0.140	0.003
p-value	0.046	0.100	0.030	0.032
Interaction				
S x D	0.042	0.050	0.038	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.

At 50 wks of age, for the strain main effect, hens of the 2016 strain were significantly different (P≤0.05) by having heavier whole egg weights, shell weights, yolk weights, and thicker shell thickness when compared to hens of the 1940 strain. For the diet main effect, hens fed on the 2016 diet were significantly different (P≤0.05) by having heavier whole egg weights, slightly heavier shell weights, yolk weights and thicker shell thickness when compared to hens fed on the 1940 diet. Interaction between strain x diet were significant (P≤0.05) in observed in the parameters measured at 50 wks of age.

Table 11. External egg quality components measured at 50 wks of age

	Whole Egg Wt.[1] (g)	Shell Wt.[1] (g)	Yolk Wt.[1] (g)	Shell Thickness[1] (mm)
Main Effects				
Strain (S)				
2016	61.64[a]	5.45[a]	15.49[a]	0.32[a]
1940	44.03[b]	4.11[b]	13.31[b]	0.29[b]
SEM	0.695	0.075	0.165	0.003
p-value	0.037	0.020	0.027	0.022
Diet (D)				
2016	53.35[a]	4.89[a]	14.85[a]	0.31[a]
1940	52.33[b]	4.67[b]	13.94[b]	0.30[b]
SEM	0.695	0.080	0.165	0.003
p-value	0.040	0.042	0.031	0.032
Interaction				
S x D	0.043	0.040	0.039	0.028

[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.

At 60 wks of age, for the strain main effect, hens of the 2016 strain were significantly different (P≤0.05) by having heavier whole egg weights, shell weights, yolk weight and thicker shell thickness when compared to hens of the 1940 strain. For the diet main effect, hens fed on the 2016 diet were significantly (P≤0.05) heavier in whole egg weights and yolk weights when compared to hens fed on the 1940 diet. There was no significance of the hens fed on the 2016 diet and hens fed on the 1940 diets for shell weight or shell thickness. Interaction between strain x diet were significant (P≤0.05) in observed in the parameters measured at 60 wks of age.

Table 12. External egg quality component measured at 60 wks of age

	Whole Egg Wt.[1] (g)	Shell Wt.[1] (g)	Yolk Wt.[1] (g)	Shell Thickness[1] (mm)
Main Effects				
Strain (S)				
2016	62.33[a]	5.58[a]	15.97[a]	0.32[a]
1940	43.02[b]	4.07[b]	13.36[b]	0.30[b]
SEM	0.560	0.075	0.170	0.003
p-value	0.046	0.013	0.022	0.038
Diet (D)				
2016	53.91[a]	4.92[a]	15.15[a]	0.31[a]
1940	51.44[b]	4.73[a]	14.18[b]	0.31[a]
SEM	0.560	0.075	0.170	0.003
p-value	0.030	0.104	0.027	0.130
Interaction				
S x D	0.038	0.045	0.030	0.039

[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.

The results of the vitelline membrane, membrane elasticity, shell strength and strength elasticity at both strain and diet effects are displayed in **Tables 13**, **14**, **15**, and **16** measured at 30, 40, 50, and 60 wks of age. At 30 wks of age, for the strain main effect, hens of the 1940 strain had significantly (P≤0.05) stronger vitelline membranes when compared to hens of the 2016 strain. However, for the diet main effect, no significance was observed for hens fed on the 2016 diet and hens fed on the 1940 diet. At 40 wks of age, for the strain main effect, hens of the 2016 strain had significantly (P≤0.05) stronger vitelline membranes when compared to hens of the 1940 strain. However, for the diet main effect, no significance was observed for hens fed on the 2016 diet and hens fed on the 1940 diet. At 50 wks of age, for the strain main effect, hens of the 2016 strain had significantly (P≤0.05) stronger vitelline membranes when compared to hens of the 1940 strain. Similar results were observed at the diet main effect where hens fed on the 2016 diet had significantly (P≤0.05) stronger vitelline membranes when compared to hens fed on

the 1940 diet. At 60 wks of age, for the strain main effect, hens of the 1940 strain had significantly (P≤0.05) stronger vitelline membranes when compared to hens of 2016 strain. However, hens fed on the 1940 diet had significantly (P≤0.05) stronger vitelline membranes when compared to hens fed on the 2016 diet. Interaction between strain x diet were significant (P≤0.05) in observed in the vitelline membrane at 30, 40, 50 and 60 wks of age.

Table 13. Vitelline Membrane[1] (g/mm²)

	30 Wks	40 Wks	50 Wks	60 Wks
Main Effects				
Strain (S)				
2016	1.938[b]	1.764[a]	1.608[a]	1.642[b]
1940	2.007[a]	1.516[b]	1.593[b]	1.855[a]
SEM	0.070	0.055	0.060	0.050
p-value	0.032	0.029	0.027	0.031
Diet (D)				
2016	1.985[a]	1.683[a]	1.648[a]	1.559[b]
1940	1.960[a]	1.642[a]	1.553[b]	1.937[a]
SEM	0.070	0.060	0.060	0.050
p-value	0.003	0.114	0.027	0.035
Interaction				
S x D	0.036	0.041	0.030	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.

At 30 wks of age, for the strain main effect, hens of the 2016 strain were significantly different (P≤0.05) by having stronger vitelline membrane elasticity when compared to hens of the 1940 strain. However, there were no significance observed at the diet main effect for hens fed on the 2016 diet and hens fed on the 1940 diet. At 40 wks of age, for the strain main effect, hens of the 2016 strain were significantly different (P≤0.05) by having stronger vitelline membrane elasticity when compared to hens of the 1940 strain. However, no significance was observed for

the diet main effect between hens fed on the 2016 diet and hens fed on the 1940 diet. Similar results were observed at 50 wks of age for the strain main effect where hens of the 2016 strain had significantly (P P≤0.05) stronger vitelline membrane elasticity when compare to the hens of the 1940 diet. Again, no significance was observed for the diet main effect between hens fed on the 2016 diet and hens fed on the 1940 diet. At 60 wks of age, for the strain main effect, hens of the 2016 strain had significantly (P≤0.05) stronger vitelline membrane elasticity when compared to hens of the 1940 strain. On the contrary, for the diet main effect, hens fed on the 1940 diet had significantly (P≤0.05) stronger vitelline membrane elasticity when compared to hens of the 2016 diet. Interaction between strain x diet were significant (P≤0.05) in observed in the vitelline membrane elasticity measured at 30, 40, 50, and 60 wks of age.

Table 14. Vitelline Membrane[1] Elasticity (mm)

	30 Wks	40 Wks	50 Wks	60 Wks
Main Effects				
Strain (S)				
2016	3.638[a]	3.677[a]	2.811[a]	3.297[a]
1940	3.010[b]	2.691[b]	2.286[b]	3.079[b]
SEM	0.130	0.120	0.013	0.140
p-value	0.037	0.031	0.028	0.038
Diet (D)				
2016	3.384[a]	3.198[a]	2.562[a]	2.754[b]
1940	3.355[a]	3.171[a]	2.534[a]	3.623[a]
SEM	0.160	0.010	0.150	0.120
p-value	0.253	0.212	0.155	0.037
Interaction				
S x D	0.049	0.043	0.038	0.029

[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.

At 30 wks of age, for the strain main effect, hens of the 2016 strain had significantly ($P \leq 0.05$) greater shell strength when compared to hens of the 1940 strain. Similar results were observed for the diet main effect where hens fed on the 2016 diet had significantly ($P \leq 0.05$) stronger shell strength when compared to hens fed on the 1940 diet. At 40 wks of age, for the strain main effect, hens of the 2016 strain had significantly ($P \leq 0.05$) stronger shell strength when compared to hens of the 1940 strain. However, for the diet main effect, hens fed on the 1940 diet had significantly ($P \leq 0.05$) stronger shell strength when compared to hens fed on the 2016 diet. At 50 wks of age, for both the strain and diet main effects, hens of the 2016 strain and hens fed on the 2016 diet had significantly ($P \leq 0.05$) stronger shell strength when compared to hens of the 1940 strain and hens fed on the 1940 diet. At 60 wks of age, for the strain main effect, hens of the 2016 strain had significantly ($P \leq 0.05$) stronger shell strength when compared to hens of the 1940 strain. However, at the diet main effect, hens fed on the 1940 diet had significantly ($P \leq 0.05$) stronger shell strength when compared to hens fed on the 2016 diet. Interaction between strain x diet were significant ($P \leq 0.05$) in observed in the shell strength measured at 30, 40. 50 and 60 wks of age.

Table 15. Shell Strength[1] (g)

	30 Wks	40 Wks	50 Wks	60 Wks
Main Effects				
Strain (S)				
2016	4083.79[a]	4221.49[a]	4354.64[a]	3846.29[a]
1940	3427.74[b]	3518.14[b]	3688.92[b]	3182.77[b]
SEM	97.53	100.29	111.27	91.92
p-value	0.021	0.024	0.025	0.024
Diet (D)				
2016	3913.58[a]	3784.82[b]	4197.39[a]	3418.19[b]
1940	3597.95[b]	3954.80[a]	3846.17[b]	3611.36[a]
SEM	97.52	100.29	111.52	91.90
p-value	0.027	0.022	0.028	0.025
Interaction				
S x D	0.0268	0.0276	0.023	0.0217

[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.

At 30 wks of age, for the strain main effect, hens of the 2016 strain had significantly (P≤0.05) stronger shell strength elasticity when compared to hens of the 1940 strain. However, there were no significance observed at the diet main effect for hens fed on the 2016 diet and hens fed on the 1940 diet. At 40 wks of age, for the strain effect, hens of the 2016 strain had significantly (P≤0.05) stronger shell strength elasticity when compared the hens fed on the 1940 diet. However, there were no significance observed at the diet main effect for hens fed on the 2016 diet and hens fed on the 1940 diet. At 50 wks of age, for the strain effect, hens of the 2016 strain had significantly (P≤0.05) stronger shell strength elasticity when compared the hens of the 1940 diet. A slight significance difference (P≤0.05) was observed for the diet main effect between hens fed on the 2016 diet and the hens fed on the 1940 diet. Hens fed on the 2016 diet had a slight stronger shell strength elasticity when compared to the hens fed on the 1940 diet. At 60 wks of age, for the main effect, hens of the 2016 strain had significantly (P≤0.05) stronger

shell strength elasticity when compared the hens of the 1940 strain. However, there were no significance observed at the diet main effect for hens fed on the 2016 diet and hens fed on the 1940 diet. Interaction between strain x diet were significant (P≤0.05) in observed in the shell strength elasticity at 30, 40, 50 and 60 wks of age.

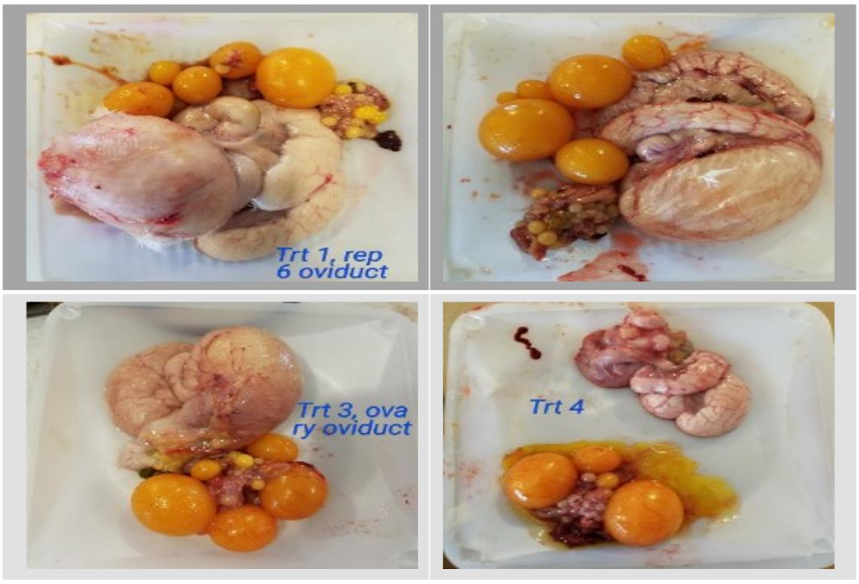
Table 16. Shell Strength[1] Elasticity (mm)

	30 Wks	40 Wks	50 Wks	60 Wks
Main Effects				
Strain (S)				
2016	0.524[a]	0.547[a]	0.241[a]	0.216[a]
1940	0.478[b]	0.482[b]	0.228[b]	0.202[b]
SEM	0.009	0.008	0.001	0.005
p-value	0.031	0.031	0.040	0.038
Diet (D)				
2016	0.500[a]	0.515[a]	0.239[a]	0.209[a]
1940	0.502[a]	0.513[a]	0.231[b]	0.208[a]
SEM	0.008	0.008	0.001	0.055
p-value	0.113	0.135	0.03	0.104
Interaction				
S x D	0.040	0.039	0.040	0.047

[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.

Figure 1. Visual Comparison representation of Ovaries and Oviducts from the treatment groups



[1]Treatment groups were as follows: 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

5.5 Discussion

Selection for particular characteristics such as production or egg weight can affect other characteristics of the hen. As a result of laying hen genetic selection, different strains of laying hen vary significantly in eggshell quality, egg size, and production and there are clear differences between modern commercial birds and those of traditional breeds. In this study, production performance including body weight and feed conversion, and egg quality parameters were evaluated and compared.

5.5.1 Overall Performance Effects

In this study, the interactions between strain x diet did exist suggesting that strain was dependent on diet for parameters that were measured. However, strain independently of diet

resulted in observed differences. With strain being one of the main effects, hens of the 2016 strain outperformed hens of the 1940 strain in hen-day production, feed consumed, feed conversion ratio, and average egg weight suggesting that the differences that were observed were attributable to genetic selection. With diet being the other main effect, hens of the 1940 strain outperformed hens of the 2016 strain for feed consumption suggesting that the increase could be linked to the variation in maintenance energy of the diet provided. On the contrary, hens fed on the 2016 diet outperformed hens fed on the 1940 diet in hen-day production, mortality, and average egg weight yet again suggesting that the differences that were observed were attributable to genetic selection. In a study conducted by Grady, G. A (1973), it was observed that hen-day production was increased in the modern commercial strain producing more eggs which was similar to the findings in this current study. The strain effect on feed consumption was greater in hens of the 2016 strain suggesting that it could be correspondent to the higher productivity rate of this strain, however, the diet effect on feed consumption was greater in hens fed on the 1940 strain suggesting that the hens were consuming more feed to meet their nutritional requirements but due to the lack of the diet's completeness to meet those requirements. In a study conducted by Pottgüter (2013), it was concluded that modern laying hens can consume adequate amounts of feed due to their genotypes when provided and similar observations were seen in this current study. The results of this study could provide clarity in explaining why higher nutrient diets sometimes lead to improved performance and has been reported in a study conducted by Harms et al., (2000) who concluded that Hy-Line W-36 hens consumed significantly more feed that was low in energy and consumed less feed that was high in energy. Mortality with the strain effect was higher in hens of the 1940 strain which could be connected to the level of egg production. There was also a higher level of prolapse observed with this strain which was associated with

increased egg production. Similar results of mortality and prolapse were seen in a study conducted by Jones, DR et al., 2001. Average egg weight was higher in hens of the 2016 strain when compared to the hen of the 1940 strain for both the strain and diet effects which supports the claim that egg weight is positively correlated to body weight and the 2016 strains were heavier than the 1940 strains throughout the production cycle. Interactions weren't explored further due to the primary focus of this research was to investigate and compare relationships between strains, and diets of parameters measured.

5.5.2 Egg Production Performance

Research conducted by Renema et al., (2001) found that modern layer strains started to lay 9.1 days earlier than an antique strain when photostimulated at the same age (Arafa, A et al., 1982). Research has also shown that laying hen egg production is adversely affected by inadequate dietary energy, protein, or calcium and requires a nutritionally balanced diet for optimal egg production. In this current study, hens of the 2016 strain produced more eggs on average as well as came into production earlier when compared to hens of the 1940 strain suggesting that genetics played a role in maturity rate further affecting the production rate. On the contrary, hens that were fed the 2016 commercial diet on average produced more eggs compared to the hen fed the 1940 diet which further supported evidence collected from Dao, Thi Hiep, et al., (2018) who stated that nutritional factors can in fact significantly impact egg production. However, egg production was not affected by body weight, which was similar to findings in a study conducted by Akbas et al., (2005) who found that body weight was positively correlated to egg production. The genetic differences associated with both strains might have been a more important determinant for the onset of lay.

Hen body weight (BW) is an important contributing factor to egg weight and feed intake. BW was influenced by the difference in feed consumption, whereas the heavier strain, the 2016 strain consumed more feed when compared to that of the than medium-type strain, the 1940 strain which was in agreement with Anderson et al. (2004) who stated that the differences in body size and weight will affect feed consumption. Typically, laying hens gain weight during the egg production periods as well as after the early growth of reproductive tissues with the gain being the main result of increased fat deposition (Kumar, D. et al., 2018). Feed consumption is known to be dependent on factors including environmental temperature, body maintenance requirements, and feed metabolizable energy (Sterling K, et al., 2003). In this current study, hens fed on the 2016 diet had heavier BW when compared to hens fed on the 1940 diet resulting in more feed consumption overall indicating that hens consumed feed to satisfy their nutritional requirements for energy maintenance and production. The results from this study also showed hens that consumed more feed were in accordance with Harms et al., (2000) who reported that Hy-Line W-36 hens consumed significantly more feed that was low in energy as opposed to those that consumed less feed that was high in energy.

5.5.3 Reproductive System Characteristics

The avian oviduct is a complex biological organ that undergoes a series of hormonal, neural, biochemical, and cellular changes during the formation of an egg. The oviduct is defined as having five distinct segments: the infundibulum, magnum, isthmus, uterus, and vagina. The ovary and oviduct undergo a steady increase in measurements, weight, and development until the age of sexual maturity and its heath is critical to production performance. The size and weight of the hen's ovary and oviduct are influenced by age as well as the reproductive phase. In this study, hens of the 2016 strain had a higher ovary and oviduct weight when compared to hens of the

1940 strain suggesting that generational selection contributed to maximal reproductive efficiency, and similar results were observed for those hens fed on the 2016 diet and those fed on the 1940 diet. Interestingly enough, hens that presented with heavier oviduct weights produced heavier eggs resulting in heavier egg necessity for higher oviduct function (Muir WI et al., 2022). The potential reason attributing to the weight differences observed among the hens could be primarily affected by the layer strain given that the environmental conditions were identical. Diet did appear to have an effect however; the strain effect was greater. It should be noted that the reproductive system characteristics were measured at only a one-time point being at the end of the trial with no assessment of the follicles therefore, different time points in measuring the ovaries and oviduct in conjunction with follicle count should be taken into consideration.

5.5.4 Egg Quality: Physical Characteristics

Egg quality, both external and internal, is deemed an important economic trait possessing a genetic basis. Intensive genetic selection of layer hens for economically important production traits results in considerable differences in the mechanisms of growth and development (Buzała M, et al., 2015). Egg quality can be influenced by several factors that include housing environment, hen strain, and nutritional values. Differences in egg quality parameters have been known to be displayed between different strains (Heflin et al., 2018). Egg quality parameters can also be affected by laying periods. Overall, the hens of the 2016 strain had better egg quality when compared to hens of the 1940 strain indicating that genetic selection played a positive role in enhancing egg quality and supporting those phenotypic correlations. In this study, differences observed in egg quality parameters measured suggest that strain had more of an adverse effect than diet with hens of the 2016 strain displaying better quality when compared to hens of the 1940 throughout the trial.

5.5.5 Egg Weight

The weight of the hen egg is an important criterion due to its influence for both preference and quality in the layer industry (Anene DO et al., 2020). Genetically, egg weight is related to all three of the major components belonging to shell, albumen, and yolk. There is a positive correlation between egg weight and body size, therefore, hens of smaller size typically produce smaller eggs when compared to larger hens that produce larger size eggs. Egg size can also be influenced by diet due to the energy amount, crude protein, amino acids, linoleic acid, and total fat a well as the intake of these nutrients by the hen (Leeson S, et al., 2009). In this current study, as expected, egg weight from hens of the 2016 strain presented a higher weight when compared to hens of the 1940 strain which were in agreement with reports concluded by Akbar et al. (1983) and Jackson et al. (1986) who found that eggs from a 1950 and 1950 control strain were indeed smaller than that of the current commercial strain utilized in their trials. Research reported from Tumová E and Gous RM (2009) stated that egg weight does increase with hen age which was also shown in this current study. Collection time could also affect egg weight but collection time was identical all hens, however, oviposition time (which was not measured) can contribute to egg weight as eggs laid in the afternoon produce better quality characteristics. Results from Silversides and Budgell (2004) stated that genetic improvement in egg weight permitted the attainment of an egg with higher albumen weight, however, not altering the yolk weight. The difference in egg weight in this current study may have been attributed to the hen's genotype and nutrition, with the purebred having the smallest egg weight.

5.5.6 Yolk Color and Weight

The yolk of an egg contains about 50% solids mainly composed of proteins and lipids. As the hen ages, the percentage of yolk increases, and albumen decreases. Eggshell quality, color,

and strength can be affected by many factors such as strain, age, and nutrition (Küçükyılmaz, K et al., 2012). Yolk color depends on the consumption of pigmented substances (i.e. carotenoids) found within the feed and is deemed an important parameter for consumers. During the measured time points for yolk color assessment, hens of the 2016 strain had an overall greater yolk color value compared to hens of the 1940 strain which could have been a direct reflection of the genetic variability of the two strains. With diets provided, the hens fed on the 1940 diet has a high portion of the alfalfa meal which could have been a direct result of the increased yolk color value in comparison to hens fed on the 2016 diet. Yolk weight has been shown to be a function of the age of the bird which may differ between strains of laying hens (B. De Ketelaere et al., 2002). Previous studies have concluded that the lay intensity of hens has a direct influence on the viscosity of the yolk presumably affecting the weight which was observed in this study (Williams, K. 1992). According to Johnston SA et al., (2007) the proportion of yolk is negatively related to egg size meaning that at any given hens' age, larger eggs typically have greater absolute amounts of components, in this case, yolk, when compared to smaller eggs. In this current study, it was demonstrated that differences did exist between hens of the 2016 strain that displayed a heavier yolk weight when compared to hens of the 1940 strain suggesting that genetics played a greater role in the differences observed among the strains for yolk weights. It has been established that long-term genetic selection in White leghorns has indeed increased egg weight however, the percentage of yolk has decreased.

5.5.7 Haugh Unit and Albumen Height

The standard for interior egg quality is determined by the Haugh Unit (HU) within the egg industry. The albumen, having a major influence on overall interior egg quality, is primarily composed of water with albumen solids, and albumen proteins ranging from approximately 11%

to 13% (Kumar, D et al., 2018). Albumen height and Haugh unit score are important indicators of egg quality by measuring the viscosity of the thick albumen. Haugh Unit is a logarithmic function of the height of thick albumen adjusted for egg weight. Determining the HU score is an accepted way to assess the egg's internal quality (Williams, K. 1992). The Haugh unit in this current study was influenced by the hens' strain and subsequently their diet as well due to the HU being greater in hens of the 2016 strain when compared to the hens of the 1940 strain. Research from previous studies reported that the Haugh unit does in fact decrease with increasing age which was demonstrated in this study from hens throughout the lay cycle measurements. In this current study, it was demonstrated that albumen height did decrease overall with the hen's age which was shown throughout the measurement periods suggesting that age was a factor that affects albumen height. The results from this study were in agreeance with research conducted by Carvalho et al., (2003) and Ramos et al., (2008) who reported that albumen height was negatively affected as the age of the hen increased. It can be suggested that the decline and fluctuation in the Haugh unit and albumen height could be due to the physiological changes that occurred as egg production progressed. A decline in albumen height has been known to be a result of increasing hen age in conjunction with Haugh unit variability.

5.5.8 Vitelline Membrane Strength and Elasticity

The vitelline membrane (VM), being a multilayered structure by providing protection and gives shape to the egg yolk is important for egg breaking because it separates the yolk from albumen to reduce yolk contamination of the albumen (Mann, K 2008) (Marzec et al., 2019). In addition to the chalaza, the VM also has the responsibility of keeping the egg yolk in the central part of the egg, thus preventing its integration with the shell membranes (Damaziak K et al., 2020). VM strength was evaluated on account of its breaking force along with elasticity while

elasticity was measured by the distance from the point of the membrane deformation to its breaking. Reports from Damaziak et al., (2020) demonstrated that differences were found in VM, not only between species but within the structures and fibers that form the membranes as well. Other studies that have been conducted have demonstrated that the main reason for differences observed in the strength of VM is a result of yolk viscosity due to different chemical compositions maintained within the yolk (Marzec et al., 2016). At the strain effect, in the beginning, hens of the 1940 strain presented with better VM strength which could have been due to better membrane structure, however, as time progressed, hens of the 2016 strain presented with better VM strength. At the diet effect, hens fed on the 2016 diet presented with better VM strength suggesting that diet played a role in the outcome. Differences in overall VM strength and elasticity observed in this study indicated that age had an adverse influence due to weakness as time progressed in conjunction with genetic differences and nutritional compositions provided by the diets. These results were similar to the results found in research conducted by Jones DR et al., (2018) who found an interaction between hen strain and hen age for vitelline membrane strength. A steady trend was observed over the time points measured where hens of the 2016 strain had overall stronger vitelline strength and elasticity when compared to hens of the 1940 strain suggesting genetics had a greater impact.

5.5.9 Egg Shell Color, Strength, Elasticity, and Thickness

Several factors can affect eggshell quality, color, and strength such as strain, age, and nutrition. Eggshell color may be monitored by visual comparison with a series of graded standards or it may be measured by shell reflectivity, which is the detection of the proportion of incident light that is reflected from the surface of the egg, under controlled conditions (Mishra, B et al., 2019). Shell reflectivity, known to be a traditional scientific method that is expressed as a

percentage, is an indication of shell color lightness meaning that the higher the value, the lighter the color of the eggshell and vice versa (Samiullah, S et al., 2015). Results from this current study show that hens of the 2016 strain had similar overall eggshell color when compared to hens of the 1940 strain as well as those hens on their representative diets which can be indicative of the strain itself, but also a reflection of age and environment. It is also important to note that the meter used to measure reflectivity is not as accurate as other methods for measuring shell color due to the meter only taking one reading by targeting a small circular surface area, approximately 1 cm, of the eggshell (Samiullah, S et al., 2015). According to Dearborn et al., (2012), it was indicated diet, identity, and time are deemed important factors that affect the eggshell color of laying hens, however, results from this study regarding time and identity did not support these claims due to the lack of differences observed.

The most important quality traits of the eggshell are its strength and thickness. According to Hammerle (1961), eggshell strength can be regarded as a reflection of an egg's mechanical and physical properties. Genetic differences in eggshell quality characteristics exist between species, and between breeds, strains, and families within the lines (Buss, E.G., 1982). Breaking strength provides an indicator of static shell resistance that is correlated with shell thickness. In this study, genetic characteristics played a role in both strain and diet effects. The results of this study indicated that eggshell strength was highly correlated with other eggshell quality traits with hens of the 2016 strain having an overall stronger shell strength when compared to hens of the 1940 strain. The higher eggshell strength observed in the hens of the 2016 strain could be a result of better utilization in conjunction with an effective metabolism of both calcium and phosphorus. However, it should be noted that due to the destructiveness of the measurement making it impossible to perform repeated measurements on the same egg, discrepancies could arise in the

case of eggshell strength. The amount and thickness of the eggshell have been found to be linked to eggshell strength (Mishra, B et al., 2019) making it a trait of high genetic inclusive variability. The results from this study have shown to be a direct correlation between shell thickness and shell strength as mentioned in the previous claims with the hens of the 2016 strain possessing a greater thickness compared to the hens of the 1940 strain as well as hens fed the representative diets. It is imperative for hens to have good shell thickness because those hens with lower shell thickness could be predisposed to greater breakage thus leading to higher economic losses.

5.6 Conclusion

Egg production and quality traits are critical in layer programs due to their effect on profitability within the egg production industry. In conclusion, the results of this study have demonstrated differences that were observed between the laying hen strains comparing morphological, physical, and chemical properties of eggs. Strain selection is important for the productivity of laying hens and over the 70+ years of selection that this study highlights, the productivity, and quality of the eggs produced have improved the industry's performance. Strain difference and nutrition had effects on performance and egg quality characteristics including egg, shell, albumen, and yolk weights.

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CHAPTER 6: Evaluating the ileal and cecal microbiota composition of a 1940 heritage genetic line and a 2016 commercial line of white leghorns fed representative diets from 1940 and 2016

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6.1 Summary

This study was conducted to identify and evaluate the differences between the microbiome composition of the ileum and ceca of 1940 and 2016 genetic strains of white leghorns fed representative contemporary diets from those times. Ileal and cecal samples were aseptically collected from both genetic lines at 69 weeks of age. The genomic DNA of the ileal and cecal contents were extracted and the V4 region of the 16S rDNA was sequenced on an Illumina Miseq. Microbiota data were filtered and aligned using the QIIME2 2020.2 pipeline. Alpha and beta diversity metrics were generated and the Analysis of Composition of Microbiomes (ANCOM) was utilized to determine significantly different taxa. Data were considered significant at $P \leq 0.05$ for main effects and $Q \leq 0.05$ for pairwise differences. Alpha diversity of the ileum and ceca were significantly different ($P = 0.001$; $Q = 0.001$; however, no differences between genetic lineage were observed ($P > 0.05$; $Q > 0.05$). The beta diversity between the ileum and ceca, as well as between the genetic lines (1940 vs. 2016) were significantly different from one another ($P = 0.001$; $Q = 0.001$). Using ANCOM, *Proteobacteria* and *Actinobacteriota* were significantly different than other phyla ($P < 0.05$) with a higher relative abundance of *Proteobacteria* being observed among treatment groups 2 and 3, while

Actinobacteriota had higher relative abundance in treatment groups 1 and 4. Among the significantly different genera in the ileum, *Pseudomonas*, *Rhizobiaceae*, *Leuconostoc*, and *Aeriscardovia* were different ($P < 0.05$) with treatment groups 1 and 4 having a higher relative abundance of *Aeriscardovia*, while treatment groups 2 and 3 had higher relative abundance in both *Pseudomonas* and *Leuconostoc*. In the ceca, *Proteobacteria*, *Firmicutes*, *Actinobacteriota*, and *Euryarchaeota* were significantly different phyla ($P < 0.05$) with *Firmicutes* having the highest relative abundance across all treatment groups. Among the significantly different genera (*Pseudomonas*, *Leuconostoc*, *Alloprevotella*, and *Aeriscardovia*), *Alloprevotella* had the highest relative abundance across all treatment groups 1 and 2, while *Leuconostoc* and *Pseudomonas* had the highest relative abundance in treatment group 4. Results from this study suggest that genetic makeup in conjunction with the nutritional composition of laying hens influences the cecal and ileal microbiota of corresponding hens.

Keywords: Leghorn; Nutrition; Genetics; Ileum; Ceca; Microbiome

6.2 Introduction

Diet directly impacts and shapes the composition and subsequent functions encoded by the microbiota within the gastrointestinal tract of poultry (Hubert, S. et al, 2019). The integrity of the gastrointestinal tract (GIT) and the gut microbial community play vital roles in nutrient absorption, development of immunity, and disease resistance (Shang, Y et al., 2018). As such, the microbiota is in constant interaction with the host and has been shown to influence several major functions such as the immunological, physiological, and nutritional status of birds. In addition, poultry is an ideal model for host-microbiota interactions as the microbiota establishment of commercial poultry is relatively independent of that of the parent stock (Zhao L et al., 2013; Rychlik, 2020). Research conducted by Kers et al. (2018) demonstrated differences

in the intestinal microbiota composition. Within the same study having controlled conditions they showed that genetics plays a factor that could influence microbiota composition.

The microbiota of the small intestine has been demonstrated to have relatively low diversity. It is estimated that 50% of the total ileal microbiota can be formed by one to five genera only (Rychlik, 2020). Although the ileum may have a relatively smaller abundance of microbiota, the microbiota present within directly impacts ileal digestibility (Ptak, A et al., 2015). Unlike the ileum, the ceca possess the highest microbial richness without poultry and are mainly colonized by anaerobic and microaerophilic microorganisms. Therefore, fermentation of nutrients indigestible to the host occurs with the ceca due to the longer retention time of feed within the ceca (Clench, M. et al., 1995). Compared to the ileum, the cecum microbiota is more diverse and more stable.

A large and diverse microbiota exists within the ceca of laying hens, prolonging the retention time of digesta to approximately 12–20 h., a process known as microbial-based metabolism (Huang, C. B., et al., 2019). It has been estimated that over 10 Log10 CFU per gram of digesta and approximately 100 different taxa exist within the ceca of poultry (Oakley BB, et al. 2014; Kogut, Michael, 2018). These taxa belong to the two major phyla; Gram-positive *Firmicutes* and Gram-negative *Bacteroidetes*, followed by two major phyla: *Actinobacteria* (Gram-positive) and *Proteobacteria* (Gram-negative) (Oakley BB, et al. 2014; Kogut, Michael, 2018). *Firmicutes* and *Bacteroidetes* are generally equally distributed in the cecal microbiota of healthy adult hens comprising 45% of the total microbiota (Schreuder, J., et al., 2020); whereas the abundance of *Actinobacteria* and *Proteobacteria* is only 2–3% of total microbiota (Rychlik, 2020).

Due to the rapid development of DNA sequencing technologies, the composition of the gut microbiota within poultry is rapidly being described. However, there is little understanding of how the microbiota has been altered with the genetic selection pressures utilized in the poultry industry to enhance poultry health and performance. Additionally, there is limited knowledge on how improved diet formulation has altered the microbiota of poultry. Therefore, the aim of the current study was to describe and identify potential differences of the ileal and cecal microbiota composition of two genetic strains of white leghorns; a heritage line from 1940 and a commercial line from 2016 fed diets representative of 1940 and 2016.

6.3 Materials and Methods

6.3.1 Bird Management and Diet

At 16 weeks of age, a total of 320 white leghorn laying hens (WL40 and WL36) were transported and housed in conventional cages at a laying facility at the North Carolina Chicken Education Unit in Raleigh, NC. The rearing of these birds was carried out in accordance with the Institutional Animal and Use Committee at North Carolina State University (NCSU IACUC) [30]. All birds were randomly divided into 4 treatment groups with 80 hens per treatment N = 320; n = 80; k = 4). Treatment groups were as follows: 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. There were 2 hens per cage consisting of 8 replicate cages. 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. All animal management and sampling procedures were in accordance with the NCSU IACUC [30].

Table 1. Feed Ingredients and Mash[1] Diet Compositions

Ingredients	2016 Layer Diet[2]	1940 Layer Diet[2]
Corn	940.5	1146.38
Soybean Meal	718.0	232.57
Alfalfa Meal		305.97
Limestone, gr.	145.5	124.2
Coarse limestone	50.0	
Fat	110.0	
Phosphate Mono/D	17.6	
Salt	6.8	5.0
D.L. Methionine	2.9	
T-Premix	1.0	
Sodium Bi-carb	2.0	
Prop Acid 505	1.0	
Choline CL 60%	1.3	4.0
Hy-D 62.5 mg/lb		
Trace Min PMX[3]	1.0	
L-Vitamin PMX[4]	1.0	
.06% Selenium[5]	1.0	
Ronozyme HI P (GT)	0.4	
Total	2000.0	2000.0
	Calculated Analysis	
Protein %	20.8	20.0
ME kcal/kg	2926	1330
Calcium %	4.10	0.90
A. Phos %	0.45	0.42
Lysine %	1.20	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]Mineral premix provides per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt
[4]Vitamin premix provides per kg of diet: 13,200 IU vitamin A, 4000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 2 mg menadione (K3), 2 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 4 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid. 6 Selenium premix = 1 mg
[5]Selenium premix provides 0.2 mg Se (as Na2SeO3) per kg of diet

6.3.2 Ileum and Cecal Sample Collection

On the last day of the trial, at 69 weeks of age, 12 hens from each treatment group were randomly selected and euthanized by cervical dislocation. Both the ileum and cecal digesta contents were aseptically collected and stored at -80°C, until further analysis.

Microbiome Analysis: 16S rDNA Sequencing

Ileal and cecal digesta samples were shipped at ambient temperature to the University of Arkansas (< 72 hrs) for further analysis. The genomic DNA from both ileum and ceca (200mg) were extracted according to the manufacturer's recommendations using a Qiagen Stoll Mini Kit (Qiagen, Hilden, Germany). Genomic DNA was eluted and quantitated using a NanoDrop 1000 (Thermo Scientific; Waltham, MA, USA). Genomic DNA was diluted to 10ng/µL in Buffer AE (Qiagen, Hilden, Germany). Using custom primers described by Kozich et al., [31] Using a high-fidelity polymerase, Accuprime *pfx* (Invitrogen, Waltham, MA, USA), the V34, V4, and V45 region of 16S rDNA was amplified in a Mastercycler® X50 thermal cycler according to the manufacturer recommendations (Eppendorf, Hamburg, Germany). Using gel electrophoresis, amplification was confirmed, and individual samples were normalized using the SequalPrep™ normalization kit (Applied Biosystems™, Waltham, MA, USA). After samples were in equal molar concentrations, the library was pooled using 5 µL of each sample. The final library size was confirmed using a Qubit fluorometer (Invitrogen, Waltham, MA, USA) and a KAPA qPCR library quantification kit (Roche Sequencing, Pleasanton, CA, USA). Confirmation of the amplified region size (bp) was confirmed using an Agilent 2200 TapeStation (Aglient, Santa Clara, CA, USA).

The library and PhiX control v3 (Illumina, Carlsbad, CA, USA) were diluted to 20nM in HT1 Buffer and denatured in 0.2 N NaOH to generate a final concentration of 12 pM. The

diluted library was combined with the subsequent PhiX control v3 (20%, v/v) and loaded onto a MiSeq v2 (500 cycles) reagent cartridge (Illumina, Carlsbad, CA, USA). The resulting sequences were uploaded to BaseSpace (Illumina, San Diego, CA, USA), NCBI Sequence Read Archive (Project Accession), and Github (Lab repository).

6.3.3 Statistical and Bioinformatics Analysis

The QIIME2 pipeline (version 2020.11) was utilized for sequencing data processing (Bolyen, E. et al., 2019). Demultiplexed reads were downloaded from the Illumina BaseSpace website and were uploaded into Qiime2-2020.11 using Casava 1.8 paired-end demultiplexed format (via Qiime tools import). Demultiplexed reads were denoised and filtered in DADA2 via q2-dada2 (Callahan, B. J., et al, 2016). Using mafft, the observational taxonomic units (OUT's) were aligned and a rooted phylogenetic tree was generated with fastree2 via q2-phylogeny (Price, M. N et al., 2010). Punitive OTUs were identified against SILVA (Silva 138 99% OTUs full-length sequences) (Quast, C., et al., 2013; Yilmaz, P., et al., 2014; Glöckner, F. O., et al., 2017) using the sk-learn Bayesian algorithm (via q2-feature-classifier) (Bokulich, N. A., et al., 2018).

Alpha and Beta diversity were analyzed using core metrics results in QIIME2. Alpha diversity was determined using the Shannon diversity index. Observed OTUs, Pielou's Evenness, and Faith's phylogenetic diversity, which included the Kruskal-Wallis tests for pairwise differences (Kruskal, W., et al., 1952). Beta diversity metrics were analyzed with qualitative and quantitative indices, Bray Curtis, Jaccard, and Unweighted and Weighted UniFrac Distance Matrix (Lozupone, C., et al., 2005; Lozupone, C. A., et al., 2007). PERMANOVA, a multivariate form of ANOVA with permutations to reduce bias, was used to determine if the distribution and abundances among beta diversity metrics were different. Interactions between the organ site and

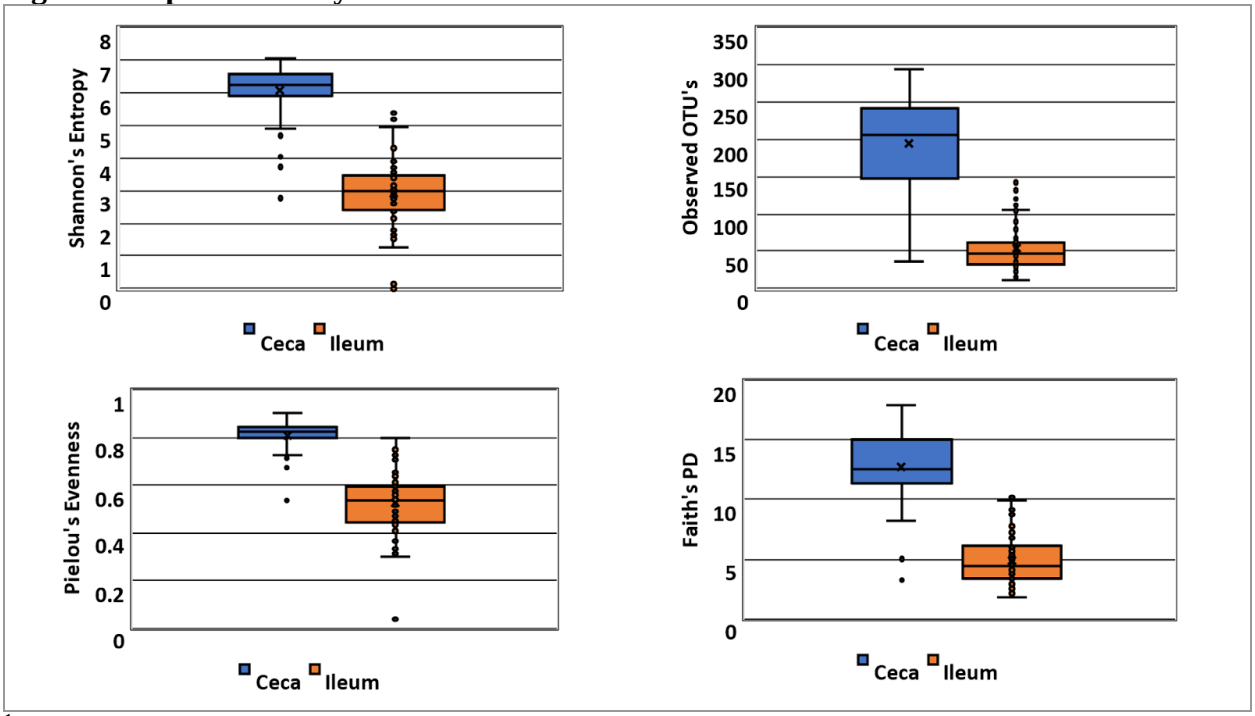
treatment were identified using ANOVA (via q2-longitudinal (Bokulich, N.A., et al., 2018) and ADONIS (Anderson, M.J. 2001) for Alpha and Beta diversity metrics. Pairwise differences for all diversity metrics (Kruskal Wallis and PERMANOVA) were considered significant when $P \leq 0.05$ and $Q \leq 0.05$. The Q values were used as they include the False Discovery Rate, which accounts for the occurrence of type I errors, rejecting a true null hypothesis (false positive) when conducting multiple comparisons. Lastly, ANCOM (analysis of the composition of microbiomes) was conducted according to Mandal et al. (2015) to determine differentially abundant taxa.

6.4 Results

6.4.1 Alpha Diversity

There were 4 metrics used to evaluate alpha diversity. The Species diversity or Shannon index determined how diverse or how different the species were within an ecosystem. Species richness or OTU count determines how many different and unique OTUs can be detected in a microbial ecosystem within a sample. Pielou's evenness determined how evenly the species were distributed within an ecosystem. Faith's Phylogenetic Diversity was used to determine diversity within the ecosystem using the sum of all the phylogenetic branch lengths of the OTUs in the ecosystem. Among all the diversity indices, significant differences were observed between the microbiota of both the ceca and ileum ($P = 0.001$; $Q = 0.001$; **Figure 1**) however, no differences were observed between the four treatment groups ($P > 0.05$; $Q > 0.05$; **Supplemental Table 1**).

Figure 1. Alpha Diversity of Ceca and Ileum



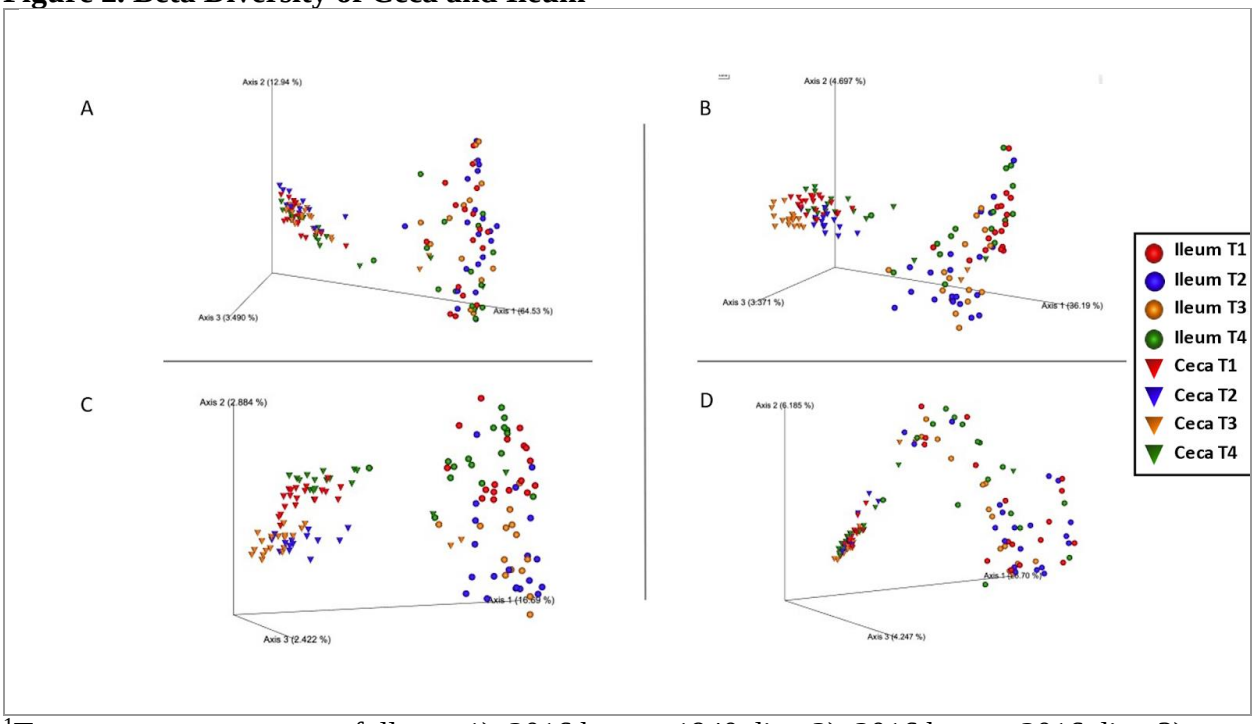
[1]Alpha diversity indices of ileum and ceca

6.4.2 Beta Diversity

There were 4 metrics used to evaluate beta diversity. Bray-Curtis dissimilarity compared the microbial ecosystems to one another based on the differences in microbial abundances between two samples on a scale from 0 to 1 with 1 meaning completely different species abundances. Jaccard distance compared the microbial ecosystems to one another based on the presence or absence of species on a 0 to 1 scale with 1 meaning no species in common. Weighted UniFrac compared microbial ecosystems to one another based on the sequence distance in the phylogenetic tree and is based on the branch lengths of the phylogenetic tree that are weighted by relative abundances. Unweighted UniFrac compared microbial ecosystems to one another based on the sequence distance in the phylogenetic tree and is based on the sequence distance alone. Differences were observed between the Bray Curtis Dissimilarity between the ceca and ileum (P

= 0.001; Q = 0.001; **Figure 2A**). Additionally, the Bray Curtis Dissimilarity was different between all treatment groups (P = 0.001; Q < 0.05; **Figure 2A**). Similar to Bray Curtis Dissimilarity, the Jaccard Diversity index was significantly different between that of the ceca and ileum (P = 0.001; Q = 0.001); **Figure 2B**). As well, the Jaccard Diversity index of the treatment groups was significantly different from one another (P = 0.001; Q < 0.05; **Figure 2B**.

Figure 2. Beta Diversity of Ceca and Ileum



[1]Treatment groups were as follows: 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
*Beta Diversity of ceca and ileum from all treatment groups. A). Bray Curtis B). Jaccard distance C). Unweighted UniFrac D). Weighted UniFrac

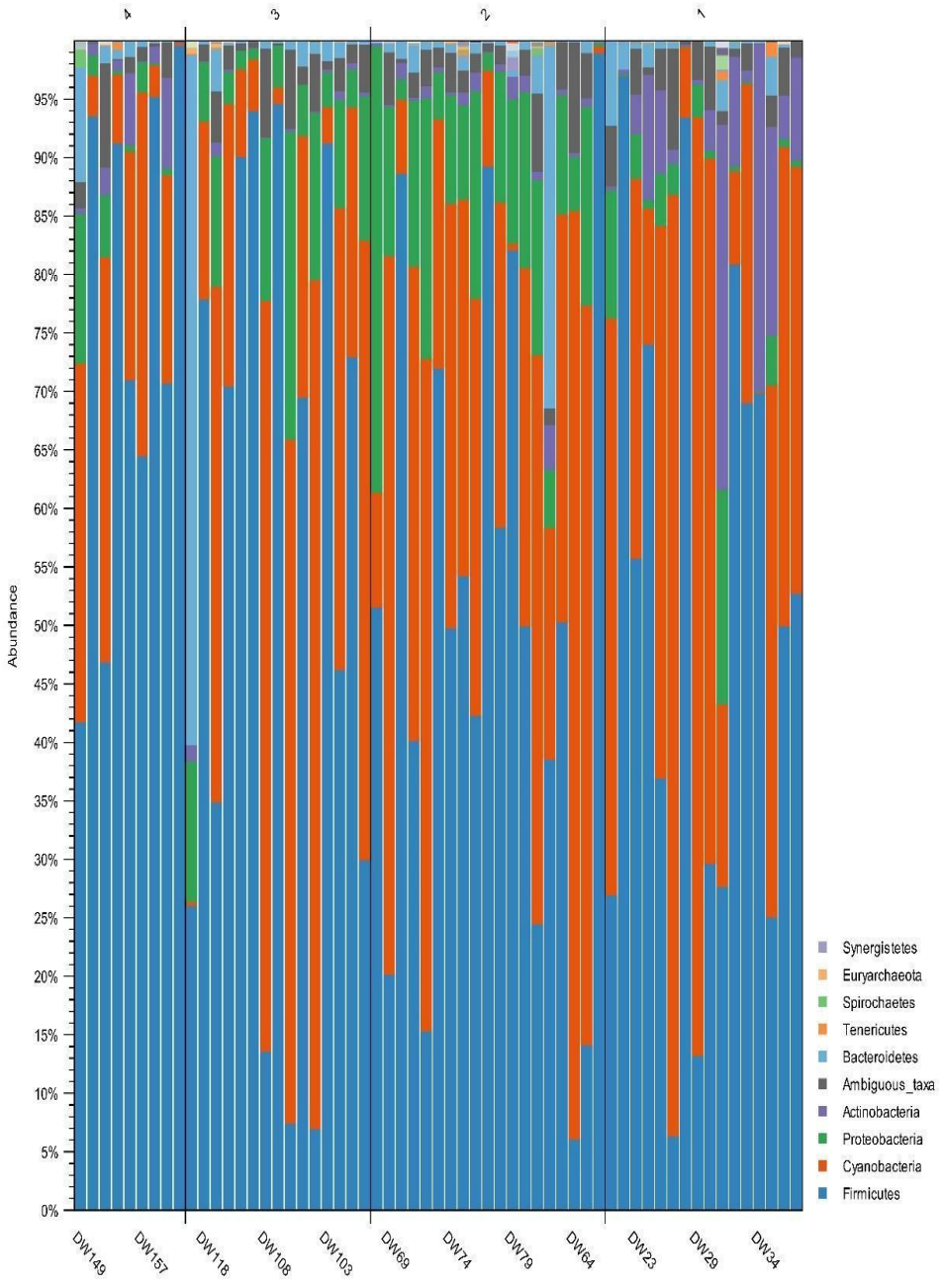
The Operational Taxonomic Unit (OTU) distribution among hens from all treatment groups of the ileum and ceca is illustrated in **Figures 3** and **4**. OTU distribution at the phylum level among treatment groups of the ileum outlined the abundance of microbial communities which had no significant differences but illustrated Firmicutes as having the highest abundance

in all treatment groups (**Figure 3A**). OTU distribution at the phylum level among treatment groups of the ceca outlined the abundance of microbial communities which had no significant differences but illustrated Bacteroidetes as having the highest abundance in all treatment groups (**Figure 3B**). OTU distribution at the genus level among treatment groups of the ileum outlined the abundance of microbial communities which had no significant differences, the top ten are illustrated which showed the highest abundance (**Figure 4A**). OTU distribution at the genus level among treatment groups of the ceca outlined the abundance of microbial communities which had no significant differences, the top ten are illustrated which showed the highest abundance (**Figure 4B**).

Figure 3. OTU Distribution at Phylum Level of Ileum and Ceca

[1]Treatment groups were as follows: 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 [2]Diagram A represents OTU distribution of phylum within the ileum [3]Diagram B represents OTU distribution phylum within the ceca

A)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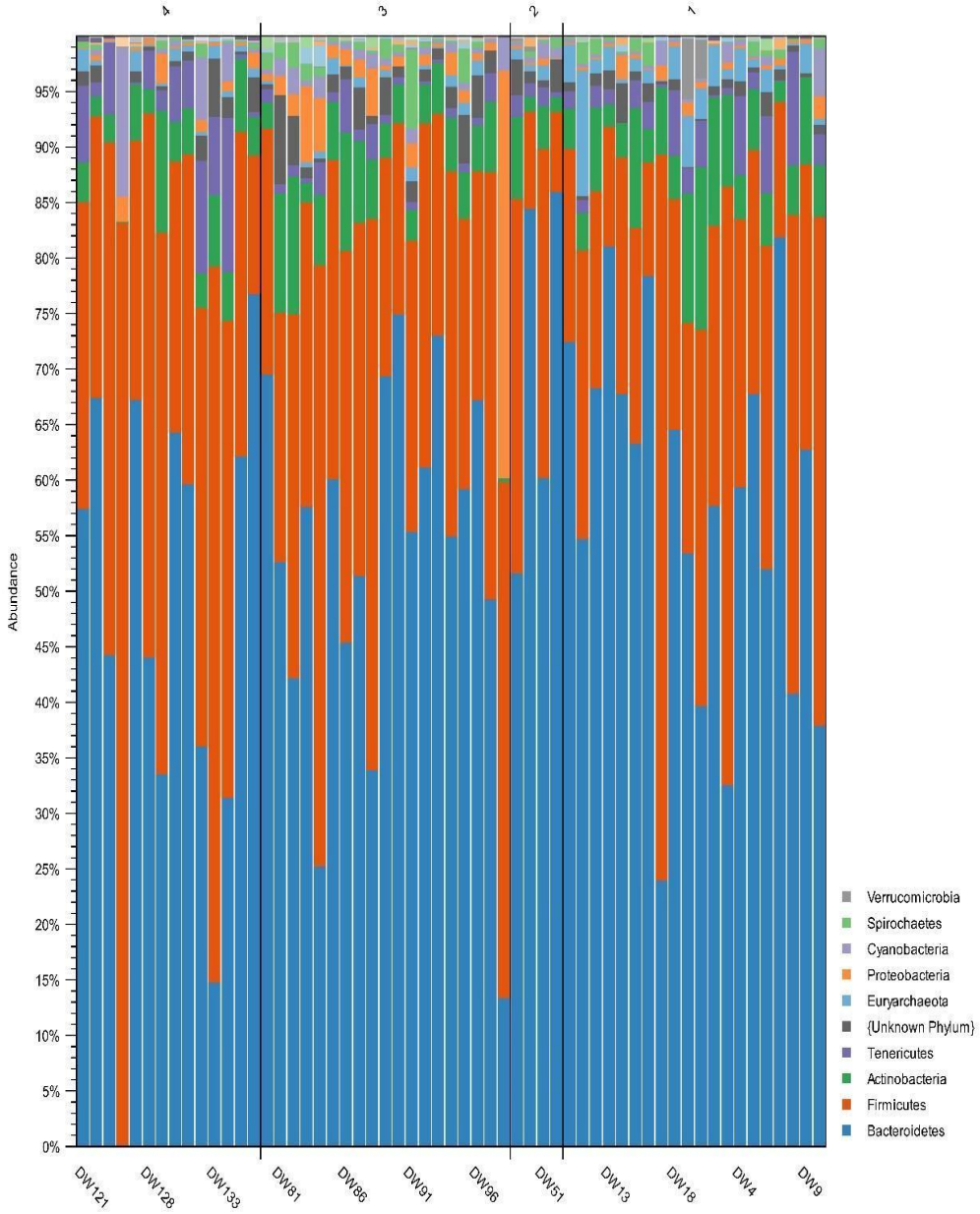
DW69
DW74
DW79
DW64
DW23
DW29
DW34
Synergistetes
Euryarchaeota
Spirochaetes
Tenericutes
Bacteroidetes
Ambiguous_taxa
Actinobacteria
Proteobacteria
Cyanobacteria
Firmicutes

B)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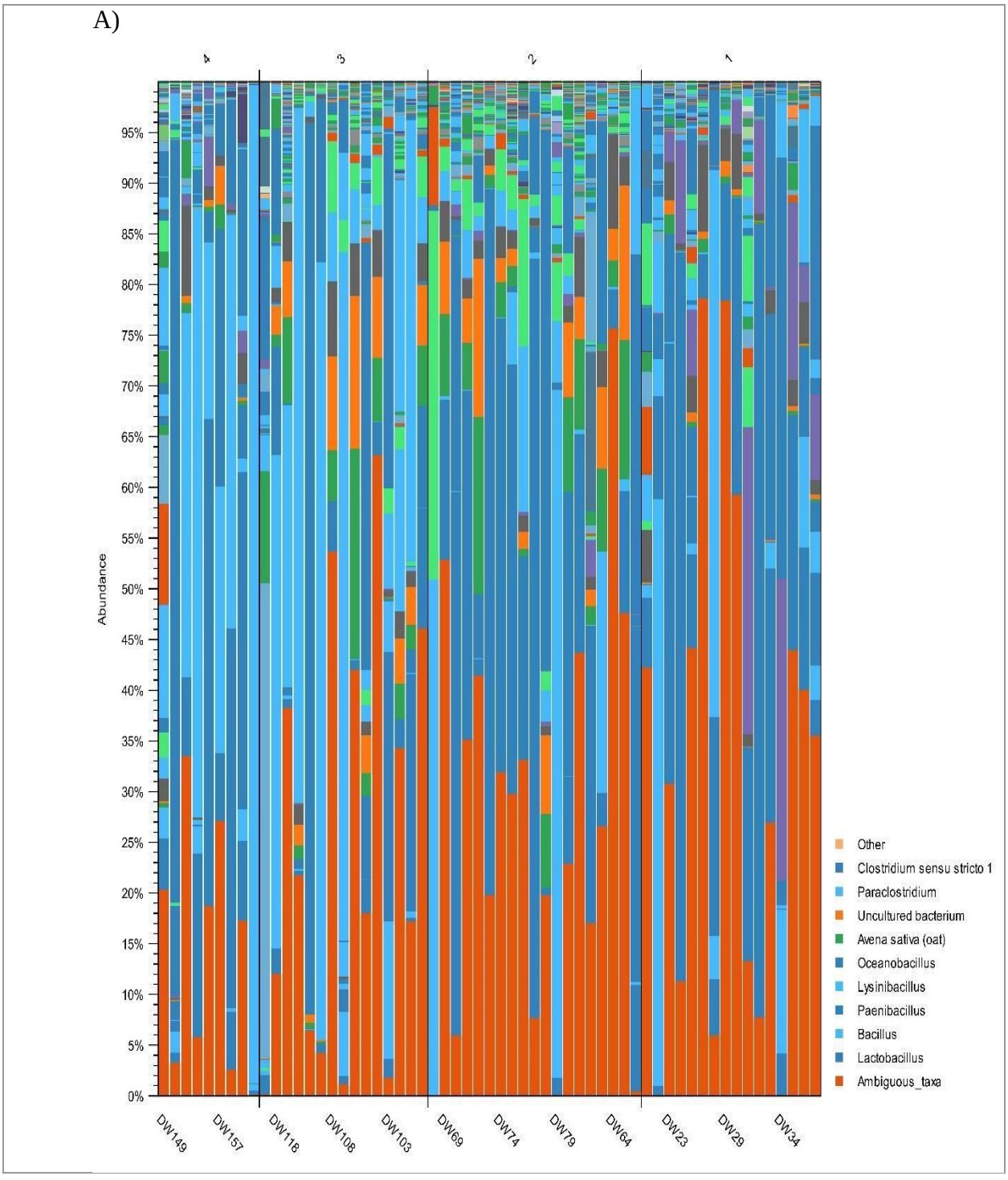
DW51
DW13
DW18
DW4
DW9
Verrucomicrobia
Spirochaetes
Cyanobacteria
Proteobacteria
Euryarchaeota
{Unknown Phylum}
Tenericutes
Actinobacteria
Firmicutes
Bacteroidetes

Figure 4. OTU Distribution at Genus Level of Ileum and Ceca

[1]Treatment groups were as follows: 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 [2]Diagram A represents OTU distribution of genus within the ileum [3]Diagram B represents OTU distribution genus within the ceca

A)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DW149
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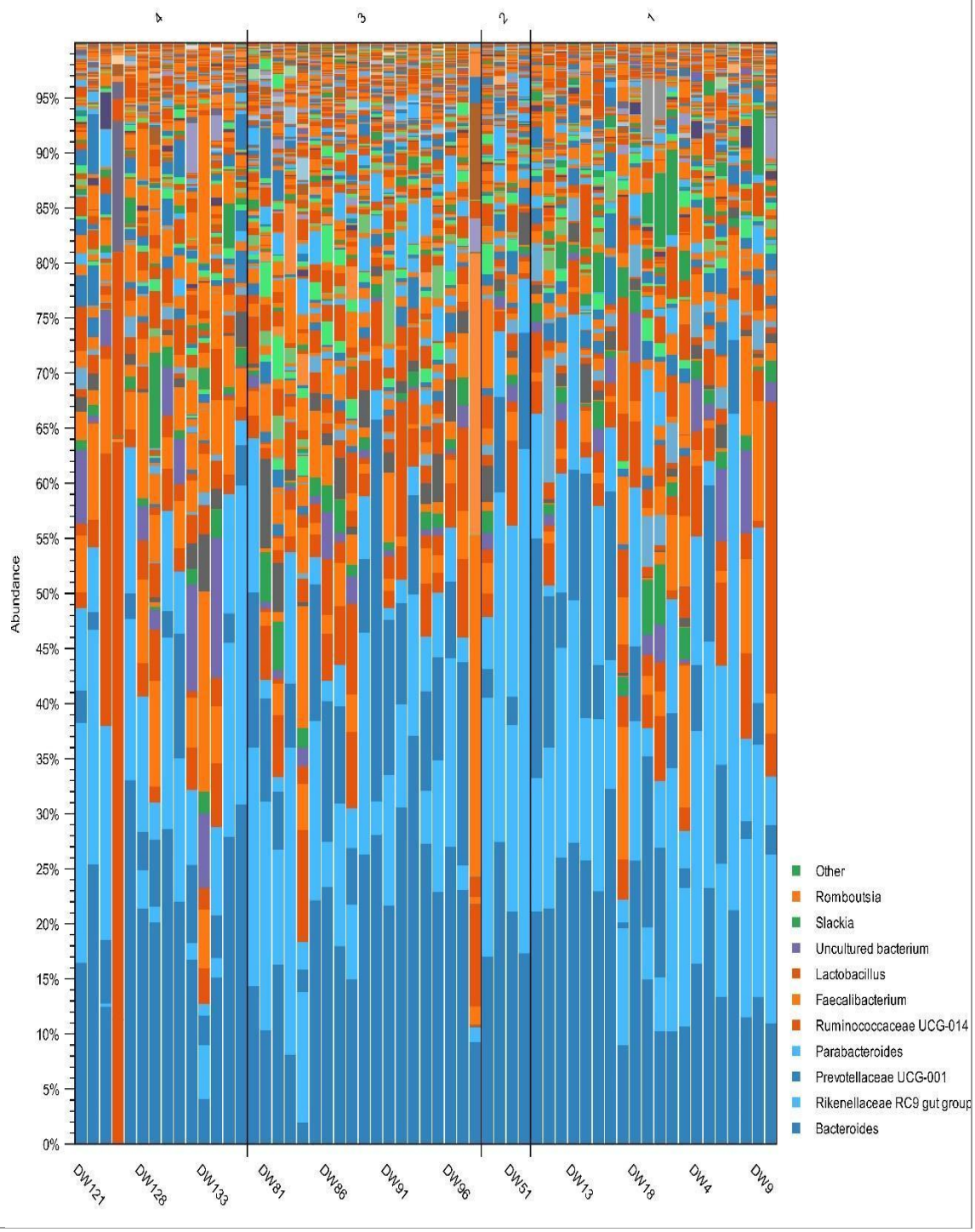
DW118
DW108
DW103
DW69
DW74
DW79
DW64
DW23
DW29
DW34
Other
Clostridium sensu stricto 1
Paraclostridium
Uncultured bacterium
Avena sativa (oat)
Oceanobacillus
Lysinibacillus
Paenibacillus
Bacillus
Lactobacillus
Ambiguous_taxa

B)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Abundance
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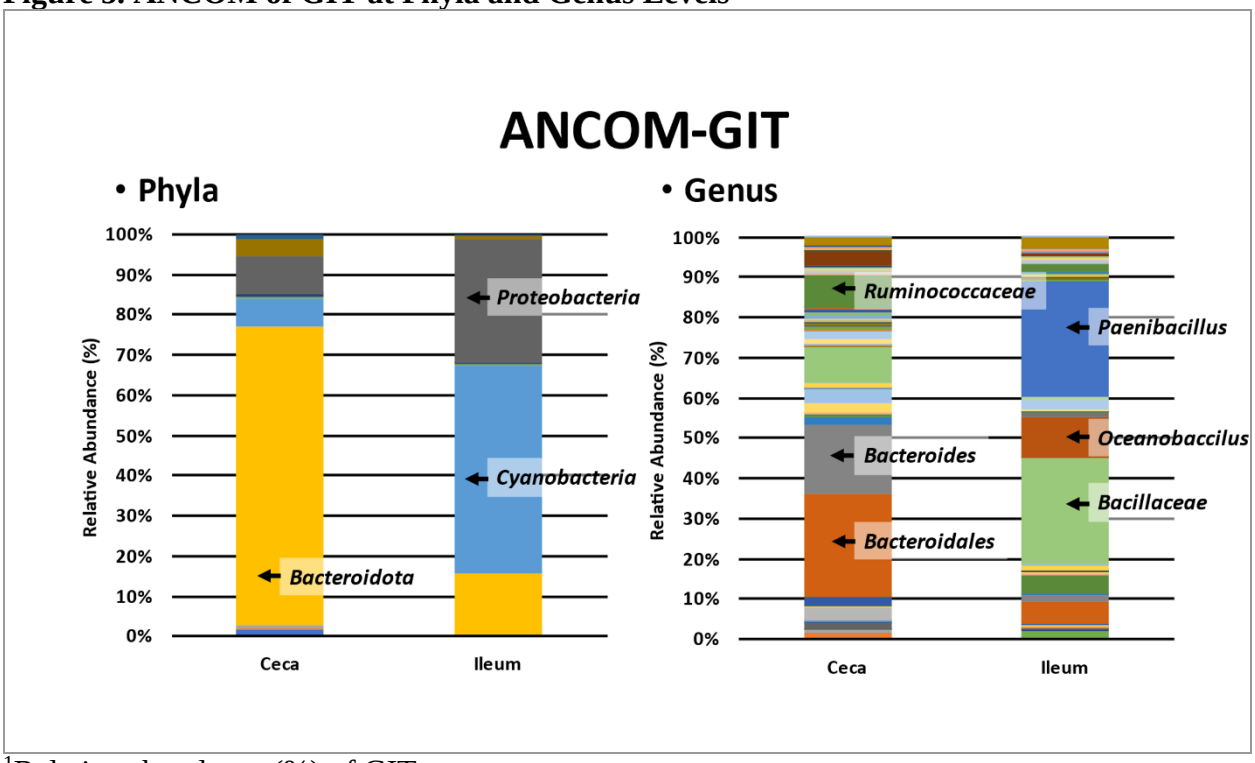
DW128
DW133
DW81
DW86
DW91
DW96
DW51
DW13
DW18
DW4
DW9
Other
Romboutsia
Slackia
Uncultured bacterium
Lactobacillus
Faecalibacterium
Ruminococcaceae UCG-014
Parabacteroides
Prevotellaceae UCG-001
Rikenellaceae RC9 gut group
Bacteroides

6.4.3 ANCOM

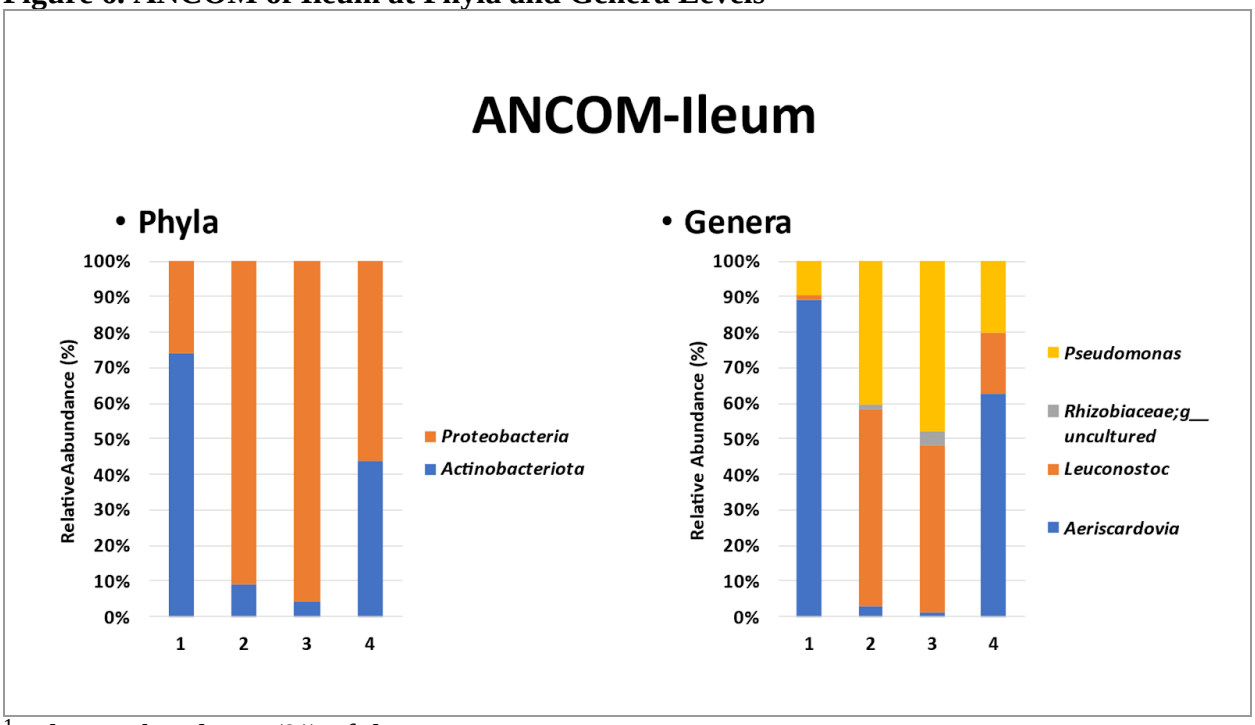
ANCOM, known as analysis of the composition of microbiomes, detected the differences in the microbial mean taxa abundance to provide exact differences when compared to beta diversity. In the ileum for ANCOM metrics, *Proteobacteria* had higher relative abundance in hens from treatment groups 2 and 3, while *Actinobateriota* had higher relative abundance in hens from treatment groups 1 and 4 at the Phyla level (**Figure 5A**). At the Genera level, hens from treatment groups 1 and 4 had higher relative abundance in *Aeriscardovia*, while hens from treatment groups 2 and 3 had higher relative abundance in both *Pseudomonas* and *Leuconostoc* (**Figure 5B**). In the ceca for ANCOM metrics, *Firmicutes* had the highest relative abundance across all treatment groups at the Phyla level (**Figure 6A**). At the Genera level, *Alloprevotella* had the highest relative abundance in hens from treatment groups 1 and 2, while *Leuconostoc* had the highest relative abundance in hens from treatment group 4, and *Pseudomonas* in hens from treatment group 4 (**Figure 6B**).

Figure 5. ANCOM of GIT at Phyla and Genus Levels



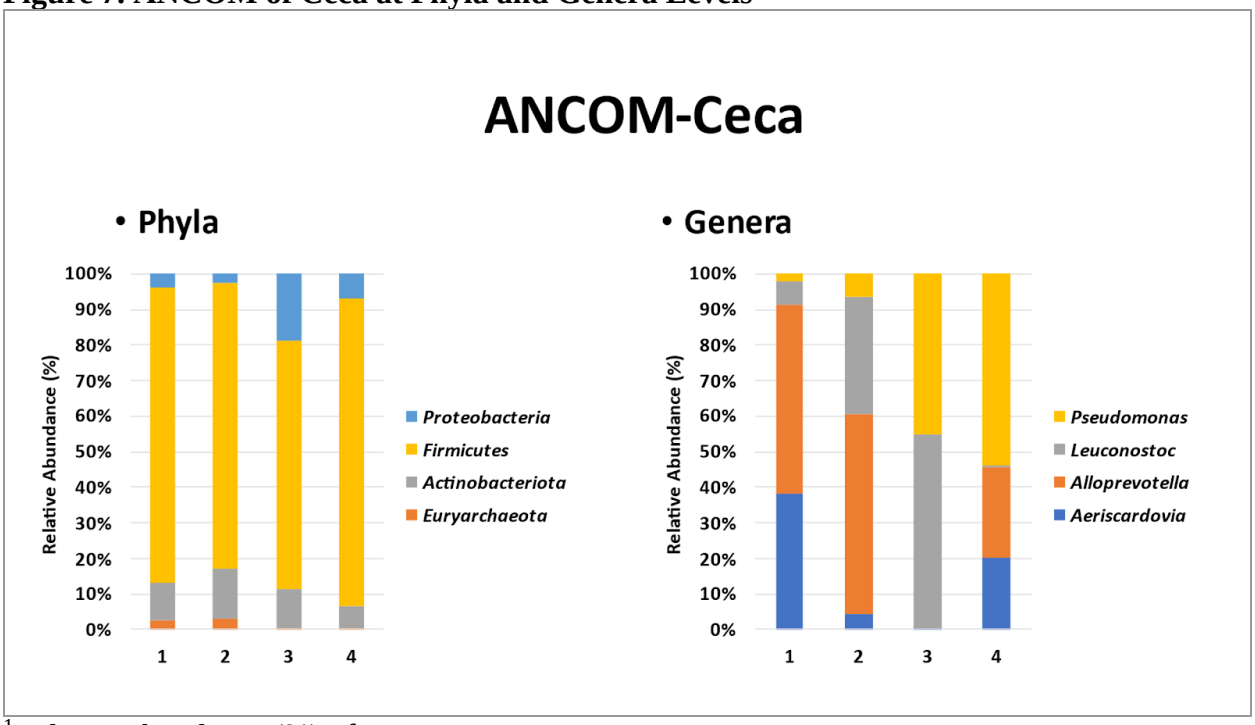
[1]Relative abundance (%) of GIT

Figure 6. ANCOM of Ileum at Phyla and Genera Levels



[1]Relative abundance (%) of ileum
[2]ANCOM at the phyla level of the ileum in **6A**
[3]ANCOM at the genera level of ileum in **6B**

Figure 7. ANCOM of Ceca at Phyla and Genera Levels



[1]Relative abundance (%) of ceca
[2]ANCOM at the phyla level of the ileum in **7A**
[3]ANCOM at the genera level of ileum in **7B**

6.5 Discussion

A healthy gut has been deemed as an indicator of a healthy host, which in turn digests nutrients more efficiently for optimal production. The balance of microflora is the most important characteristic of a healthy gut. According to Lu et al., (2003) low bacterial diversity may be associated with reduced immunological and gut protective functions, as a rich and diverse bacterial population usually correlates with positive health status (Lu J, et al., 2003). The intestinal microbiota of chickens is affected by various factors and has been widely studied in conjunction with breed and host genotypes both having the ability to influence gut microbiota communities (Kers, J. G., et al., 2018). According to Mi et al., (2017), the host genotype has the greatest effect on the diversity of microbiota communities as well as the genotype of the host

(Elokil AA, et al., 2020). Another contributing factor responsible for shaping the composition and function encoded by the microbiota in the gastrointestinal tract is diet. The efficiency of feed utilization is generally considered stable; however, it varies considerably among individuals fed identical diets and reared under the same conditions. The compounds within diets are important, providing growth substrates or microbes. In this study, ileum, and ceca microbiota composition was evaluated among two dissimilar leghorn strains that were fed on two different representative diets. Changes in the community composition, diversity, and richness of microbial communities in the ileum and ceca within the Alpha. Beta and ANCOM were identified. Factors such as diet and feeding regime could affect the population structure of chicken microbiota within host species (Huang, C. B et al., 2019).

6.5.1 Alpha Diversity

The compartments of the digestive tract differ from one another, both morphologically and functionally, and have previously shown that their microbial composition is similarly distinct. Alpha diversity indices are used to estimate the diversity of microbial communities and it is believed that the richer the alpha diversity and species composition in microbiota, the better stability within the gut micro-ecosystems. Alpha diversity in this study measured the variation in the structure of the microbial community within individual samples of the ileum and ceca. Alpha diversity has been strongly correlated with variations in a specific gene function suggesting that gut microbiota with high diversity could potentially be more stable or labeled healthier than those with low diversity. The non-significance in alpha diversity among treatment groups measured within the ileum and ceca illustrated that the microbial communities were more evenly distributed. The low richness and evenness of some suggest that there were low numbers of species in both ileum and ceca consisting of a few dominating taxa. The microbiota of the small

intestine, the section of the ileum as described in this study, could positively contribute to nutrient digestion and absorption processes, more specifically water and mineral absorption. Microbial compositions of both the environment and gastrointestinal tract vary considerably in poultry production and can be affected by factors such as age and production batch, therefore, we tried to limit many factors such as age, breed, gender, and house to minimize the fluctuation that exists within the gut microbiome. Results indicate that alpha diversity measured in this study can contribute to functional inference involving the underlying microbiota mechanism. The lack of differences can be attributed to the hens being exposed to the same environmental sources of microorganisms that are known to have an effect on overall diversity as well as the richness of the microbiome in poultry.

6.5.2 Beta Diversity

Different nutrient requirements may also affect the composition and abundance of microbiota in different intestinal segments. Beta diversity was used to compare differences in species diversity between multiple samples. Beta diversity in this study measured the similarity or dissimilarity of microbial communities between samples of the ileum and ceca; thus, making it a useful technique to observe changes within the community composition. Although beta diversity consisting of Bray-Curtis, Jaccard, and Unweighted UniFrac produced significant variations in the ileum and ceca, there was no significant effect among hens within treatment groups with Weighted UniFrac indicating that distinct phylogenetic groups are possible, but the prevalent abundance of taxonomic groups persist. Lower UniFrac distance indicates a more similar bacterial composition between the samples. The two most dominant bacterial phyla in chicken belong to Firmicutes and Bacteroidetes (Magne, F. et al., 2020). Firmicutes species are associated with the decomposition of polysaccharides and the production of butyrate (Flint, H. J

et al., 2012). Bacteroidetes species degrade complex carbohydrates and synthesize propionate via the succinate pathway.

6.5.3 ANCOM

ANCOM accounts for the underlying structure in the data and can be used for comparing the composition of microbiomes in two or more populations (Siddhartha Mandal et al., 2015). The microbiome compositional diversity did not group the samples at family and species levels according to the diet. The function of the ileum has a major role in water and mineral absorption with evidence of contributing to starch and fat digestion and is generally known to harbor a diverse and numerically more significant population of bacteria when compared to the other two intestine segments. The ileum microbiota at times can intermix with the microbiota of the ceca. Within the ileum, at the phyla level, Proteobacteria (gram-negative) had higher relative abundance followed by Actinobacteriota (gram-positive), both non-spore-forming bacteria. Hens belonging to treatment group 2 (2016 hen on 2016 diet) and treatment group 3 (1940 hen on 1940 diet) had an increased abundance of Proteobacteria suggesting that genetics had a greater influence on observed bacteria. In that same respect, similar results were seen in hens belonging to treatment group 1 (2016 hen on 1940 diet) and treatment group 4 (1940 hen on 2016 diet) having an increased abundance in Actinobacteria suggesting that the influence of diet may have been attributed to the observed presence of bacteria. Actinobacteria have a number of important functions, including the degradation/decomposition of all sorts of organic substances such as cellulose, polysaccharides, protein fats, organic acids, etc. The abundance of Actinobacteria in hens belonging to treatment groups 1 and 4 may be a direct result of the composition of the diets provided to these hens. The findings in this study were similar to the results from research conducted by Ngunjiri et al., (2019) and Wang et al., (2019) where the majority of phyla

belonged to both Proteobacteria and Actinobacteria of the ileum (Wiersema, M. L et at., 2021). At the genera level, hens belonging to treatment groups 1 (2016 hen on 1940 diet) and 4 (1940 hen on 2016 diet) had a dominant abundance of Aeriscordovia. Hens belonging to treatment groups 2 (2016 hen on 2016 diet) and 3 (1940 hen on 1940 diet) had a dominant abundance of Leuconostoc.

A complex bacterial community is found in the ceca primarily due to the longer digestive transit times making it an important site for the recycling of urea, water regulation, and carbohydrate fermentations while contributing to intestinal health and nutrition. The abundance of Firmicutes, the major bacterial phyla observed, was dominated in all treatment groups within the ceca which were similar to research conducted by Hamid et al., (2019). Proteobacteria and Actinobacteria were the second and third abundant components observed at the phyla level in the ceca which typically accounts for 2-3% of total microbiota. Despite variations observed, representatives of the phyla mentioned above are found in the ceca of nearly all adult chickens. Hens belonging to treatment groups 1 and 2 of the 2016 strain were more abundant in Alloprevotella (a fiber degradation bacteria) at the genera level and hens belonging to treatment groups 3 and 4 of the 1940 strain were more abundant in Pseudomonas suggesting that genetics had a greater influence on bacteria observed rather than diet, however, diet may have attributed to the differences of bacteria amounts found. The variations validated by the ANCOM results suggest that there were some bacteria that persevered genetically throughout the treatment groups despite the differences in the nutritional composition of the diets provided. Direct comparison of the microbiota composition established between laying hen strains has the ability to provide valuable insight into the association between microbiota, genetic and nutritional composition (James J. Kozich et al., 2013).

6.6 Conclusion

The chicken gut microbiota is colonized with complex microbial communities, known to play an important role in overall health and performance. Distinct microbiota between the ileum and ceca were observed for both alpha and beta diversity and ANCOM. Results from this study suggest that genetic makeup in conjunction with the nutritional composition of laying hens has the ability to influence both ileal and cecal microbiota. The comparison of microbiota composition between laying hen breeds can provide valuable insight into the linkage between microbiota distribution and genetic architecture. This information can collectively be beneficial in explaining the behavior of the intestinal ecosystem in order to proactively modulate necessary changes needed to enhance overall health status. Furthermore, it remains to be determined whether the interplay of diet and genetics singlehandedly fosters the changes observed within the microbiome of the gastrointestinal tract, however, the results from this study provide a vital step in identifying the roles exhibited by the various bacteria mentioned above.

6.7 References

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CHAPTER 7: Influence of nutrition and genetics on bone parameters of 1940 Leghorn and 2016 commercial White Leghorns

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7.1 Summary

Bones play an essential role in poultry production and are responsible for the support of the body mass, protection of internal organs, and providing musculature attachment sites on the bird. In laying hens, bones serve as a reservoir for eggshell mineralization during the production phase. Targeted genetic selection constitutes the main contribution to changes in body morphometry and performance potential and could be inadvertently associated with undesirable associated effects on bone stability. In this study, bone parameters were compared between a contemporary and a heritage line for the effect of strain and dietary regimen. A total of 320 White Leghorn laying hens (69 weeks of age) 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, creating 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. Keel bones were assessed for deviations and or fractures. Significant differences ($P \geq 0.05$) were observed for both deviations and/or fractures with the 2016 strain having more when compared to the 1940 strain. Humerus and tibia bones were analyzed for bone mineral density, breaking strength, and bone ash. Humerus weights which included both pre (with meat attached) and post weights (without meat attached) had significant differences ($P \leq 0.05$) in the pre-weight

in the 2016 hens, however no significant differences in the post weights. The same results were exhibited in the tibia weights. Results suggest that genetics played a role in the differences observed with the bone parameters measured and nutrition had few adverse effects.

Keywords: White Leghorns, Genetics, Bone Quality, Keel, Humerus, Tibia

7.2 Introduction

Poultry nutrition programs are often designed with production parameters in mind. Nutrient levels are set to maximize the production of eggs and meat, and those requirements are set and reviewed by the National Research Council guidelines (Fleming RH. 2008)2. Nutrition has been identified as a critical factor impacting skeletal growth and bone strength in poultry (Muszyński S, et al., 2018). Nutritional criteria for hens have usually been based on optimizing responses in egg production and shell quality, with less focus on bone quality. Within the layer industry, bone weakness and associated fractures represent considerable welfare and economic problems (Raymond, B. et al, 2018). Unlike mammals, laying hens have a unique bone turnover synchronized with a day laying cycle, indicating that rapid remodeling occurs in laying hen bones (Kim, Woo Kyun, et al., 2012). The most important nutritional factor influencing bone quality is the supply of Ca in adequate amounts and from through the diet.

There is substantial genetic variation in bone traits in domestic-layer chickens. Bones are dynamic tissues, and their quality is influenced by nutritional, hormonal, and physiological factors including mechanical stress and the extent of physical activity. Bone structure can adapt its mass, shape, and internal architecture according to the mechanical loading experienced within an environment (Hart, N. H., et al., 2017). Bone metabolism in female birds is special in that they produce a medullary bone, which serves as a reservoir for calcium used in the production of the eggshell. Maintaining optimum bone strength is a particular problem for modern layers

because of the intense selection that the strains have been subjected to and the demands that an extremely high egg production rate places on calcium and calcium metabolism (Silversides FG, et al., 2012). The structural skeleton of the laying hen becomes fully developed during the rearing period (Whitehead CC. 2004). A dramatic adjustment occurs within the bone biology of the hen during the onset of sexual maturity, with the function of osteoblasts changing from lamellar cortical bone to the production of medullary bone (Whitehead CC. 2004). Bone-breaking strength and bone mineral density (BMD) are two important parameters to assess bone quality in layers. Factors influencing these traits include nutrition, sex, age, exercise, genetics, and certain diseases (Guo, J., et al 2017).

7.2.1 Keel Bones

The keel bone is prone to damage in terms of fractures and deviations due to the anatomical position, especially in modern layers with small breast muscles (Riber, A. B. et al., 2018). Keel bone fractures are characterized by sharp bends, shearing, and/or fragmented sections of the keel bone. Fractures can extend from the ventral to the dorsal surface in the sagittal plane however, can also be cranial to caudal or a combination of the two (Casey-Trott T, et al., 2015). One of the major risk factors for keel bone fractures in layers is a result of a collision with housing structures combined with weakened bone strength (Riber, A. B. et al., 2018). A less often mentioned type of keel bone damage is deviation. A normal keel bone follows a straight line, but deformation may occur, leading to deviations from this line. These can be vertical or horizontal, showing an s-shaped appearance, bumps, or notches. Deviations are known to be disruptions located in the periosteal surface of the keel making them not the direct result of a fracture or potential impact injury (Riber, A. B. et al., 2018). Contrary to fractures, which typically occur amid an isolated event, the development of deviations likely takes place

over a period of time due to bone remodeling in response to regular loading pressures (Riber, A. B. et al., 2018). Results from studies conducted have reported keel bone deviations in 6–59% of laying hens aged 60–62 weeks depending on the type of production and the housing system (Riber, A. B. et al., 2018).

7.2.2 Humerus and Tibia Bones

The humerus is a pneumatic bone with a hollow inner cavity contained with air instead of marrow (Whitehead CC. 2004). When the bird is static, the humerus lies adjacent to the thoracic cavity and connects with the glenoid cavity of the pectoral girdle. The top end of the humerus is rounded and fits into a cup-shaped depression in the scapula, or shoulder bone, forming a ball-and-socket joint. Ball-and-socket joints allow for circular movement. The humerus is part of the upper or thoracic portion of the body (non-weight bearing). The humerus has been mainly studied in laying hens due to its different mineralization properties when compared with the tibia (Van Wyhe RC, et al., 2012).

The tibia bone is longer in length when compared to that of the fibula bone while being much thicker at the proximal end than it is at the distal end. Historically, tibia ash has been the main method by which bone mineralization has been determined. The amount of available calcium and phosphorus in conjunction with other ingredients within the diet causes sensitivity to tibia ash (Hall LE, et al., 2003). Direct interaction has been exhibited between increased bone ash, a greater supply of available calcium and phytate phosphorus in the diet, as well as increased BW gain (Hall LE, et al., 2003). Fractures of the tibia, humerus, and keel tend to be the most common potentially due to the thinning of cortical bone and loss of trabecular integrity resulting in bones becoming weaker, making them more susceptible to fracture as the result of trauma

(Whitehead CC. 2004). The aim of this study was to evaluate the relationship of bone integrity regarding the comparison of nutrition and genetic effects of laying hens fed representative diets.

7.3 Materials and Methods

7.3.1 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 identical and carried out in accordance with the NCSU IACUC. All birds were randomly divided into 2 hens per cage consisting of 10 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 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 starting at 17 wks of age. All animal management and sampling procedures were in accordance with the NCSU IACUC.

Table 1. Feed Ingredients and Mash Diet[1] Compositions

Ingredients	2016 Layer Diet[2]	1940 Layer Diet[2]
Corn	940.5	1146.38
Soybean Meal	718.0	232.57
Alfalfa Meal		305.97
Limestone, gr.	145.5	124.2
Coarse limestone	50.0	
Fat	110.0	
Phosphate Mono/D	17.6	
Salt	6.8	5.0
D.L. Methionine	2.9	
T-Premix	1.0	
Sodium Bi-carb	2.0	
Prop Acid 505	1.0	
Choline CL 60%	1.3	4.0
Hy-D 62.5 mg/lb		
Trace Min PMX[3]	1.0	
L-Vitamin PMX[4]	1.0	
.06% Selenium[5]	1.0	
Ronozyme HI P (GT)	0.4	
Total	2000.0	2000.0
	Calculated Analysis	
Protein %	20.8	20.0
ME kcal/kg	2926	1330
Calcium %	4.10	0.90
A. Phos %	0.45	0.42
Lysine %	1.20	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.

7.3.2 Sampling

At the end of the trial, 12 hens were randomly selected, and euthanized by cervical dislocation. The right humerus and tibia were excised and cleaned of connecting tissue and then placed in storage at -20 C until analyzed. Both humerus and tibia were thawed out overnight at room temperature before further analyses were conducted. Both humerus and tibia bones were measured for length and weight using a digital caliper and precision scales (0.01 g), respectively.

7.3.3 Keel Analysis

The keel bones were dissected, with adherent muscle tissue being removed, and stored frozen at −20 ◦C for further analysis. The dissected keel bones were scored by palpation and visual analysis for keel bone deviation (KBD) and keel bone fractures (KBF). Palpation was performed by running two fingers down the edge of the keel bone to detect alterations like S-shaped deviations, bumps, depressions, or proliferations. The following scoring system was used: 4 = intact keel bone, 3 = slight deviation, 2 = moderate deviation counted as fracture, 1 = severe deviation counted as fracture. For fractured assessment, scores 3 and 4 were combined as "no fractures" vs. scores 1 and 2 as "fractured." If fractures occurred, the type of fracture (transversal or longitudinal) as well as the number of fractures (1 to 3 or ≥4), and their location in the cranial, intermediate, or caudal third of the carina sterna were recorded (Käppeli S et al., 2011).

7.3.4 Bone Mineral Density

Bone mineral density (BMD) is the mass of material per volume of bone, which includes both organic, representing collagen, and inorganic, representing mineral hydroxyapatite, components. The BMD was determined in excised bones of both the humerus and tibia and the

correlation was determined using the Schick AccuDEXA BMD Portable Bone Densitometer – tabletop DEXA scanner.

7.3.5 Bone Breaking Strength

Bone breaking strength measures resistance to fracture from a force applied perpendicular to the bone at mid-shaft (Kittelsen KE et al., 2021). Bones were cradled on 2 support points measuring 3 cm apart. Using a 50-kg load cell and a crosshead speed of 100 mm/min, the force of an attached shear plate measuring 8 cm in length and 1 mm wide was applied to the midpoint of the same anteroposterior plane of each bone using the TA. HD Plus texture analyzer machine (Stable Micro Systems, Hamilton, MA). Breaking strength was recorded.

7.3.6 Bone Ash

Ashing of the bone yields, by burning off all organic material, the total mineral content of a given known dry weight of bone. Ash weight is a fundamental measure of bone mineral content, expressed most often as a percentage of fat-free dry weight. Both humerus and tibia bones were cleaned of surrounding muscles and soft tissues. The tibia was separated from the fibula, and both humerus and tibia were cut into pieces to fit into a Soxhlet for ether extraction for 48h. Ether extracted bone pieces were dried and weighed and placed in crucibles, and were ashed in a Thermolyne furnace (30400; Barnstead International) at 600°C for 10 h and weighed to determine ash percentage. Bone ash concentration was calculated as bone ash weight per unit of volume. Percentage bone ash was calculated by dividing bone ash by bone weight and multiplying by 100.

7.3.7 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.

7.4 Results

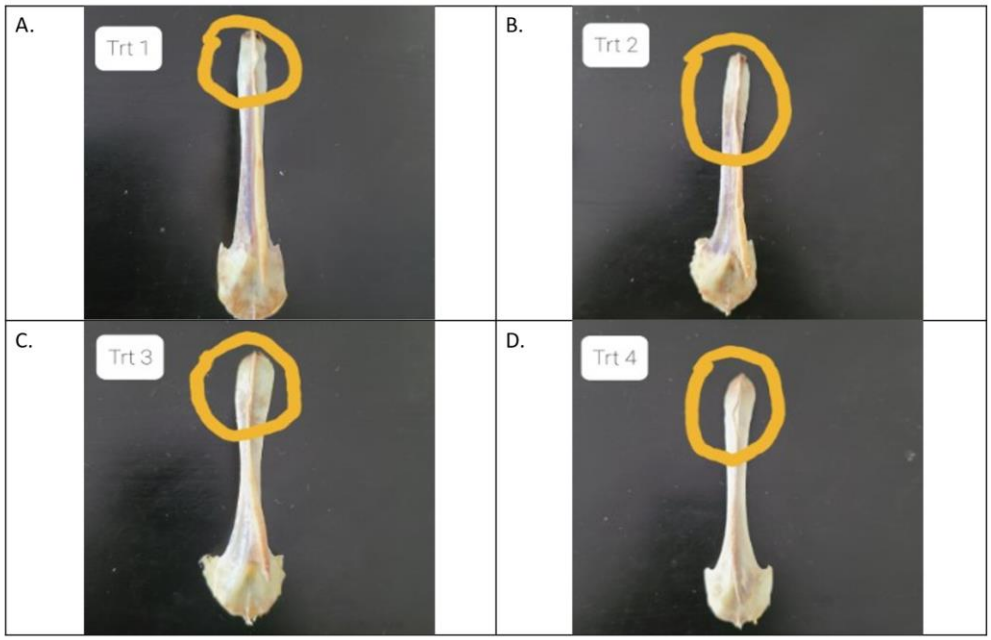
Keel bone characteristics for length, deviations, and fractures are shown in **Table 2**. There were significant differences ($P \leq 0.05$) observed in keel bone lengths among treatment groups. Hens of the 2016 strain had longer keel bones by approximately 0.57 mm when compared to the hens of the 1940 strain. At the strain effect for deviations, hens of the 2016 strain had significantly ($P \leq 0.05$) more deviations when compared to hens of the 1940 strain. Similar results were observed for fractures at the strain effect where hens of the 2016 strain had significantly ($P \leq 0.05$) more fractures when compared to hens to the 1940 strain. At the diet effect for deviations, no significance was observed, however, fractures at the diet effect showed a significantly ($P \leq 0.05$) higher incidence of fractures when compared to hens of the 1940 strain. Significance at the interaction level between strain x diet did occur for keel bone length, deviations and fractures.

Table 2. Keel Bone Characteristics

	Length (cm)	Deviations	Fractures
Main Effects			
Strain (S)			
2016	10.34[a]	1.2[a]	0.95[a]
1940	9.76[b]	0.7[b]	0.42[b]
SEM	0.115	0.429	0.395
p-value	0.003	0.051	0.022
Diet (D)			
2016	10.11[a]	0.95[a]	0.67[b]
1940	9.98[b]	0.95[a]	0.70[a]
SEM	0.116	0.214	0.403
p-value	0.004	0.457	0.038
Interaction			
S x D	0.016	0.014	0.012

[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.

Figure 1. Keel Bone Deviations



[1]Treatment groups were as follows: 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

Humerus bone weight, mineral density, breaking strength and length and diameter measurements are displayed in **Table 3**. There were no significant differences ($P \leq 0.05$) in humerus bone weights for either the strain or diet main effects. Similar results were seen for the bone mineral density as well displaying no significance for the strain or diet effect, however hens of the 2016 strain and hens fed on the 2016 diet had a higher bone mineral density when compared to hens of the 1940 strain and hens fed on the 1940 diet. Breaking strength, which is separated by bending moment expressed as kg/mm, peak force expressed as kg, and diameter expressed as mm was identical for both strain and for hens fed on both diets resulting in no significance. Humerus length showed significance ($P \leq 0.05$) at the strain effect with hens of the 2016 strain having longer humerus lengths when compared to hens of the 1940 strain. However, different results were observed at the diet effect with hens fed on the 1940 diet had longer humerus lengths when compared to hens fed on the 2016 diet. The diameter showed no significance ($P \leq 0.05$) between hens of either strain and for hens fed on either diet. The only interaction between strain x diet was observed for length of the humerus bone.

Table 3. Humerus Bone Mineral Density, breaking strength and measurements

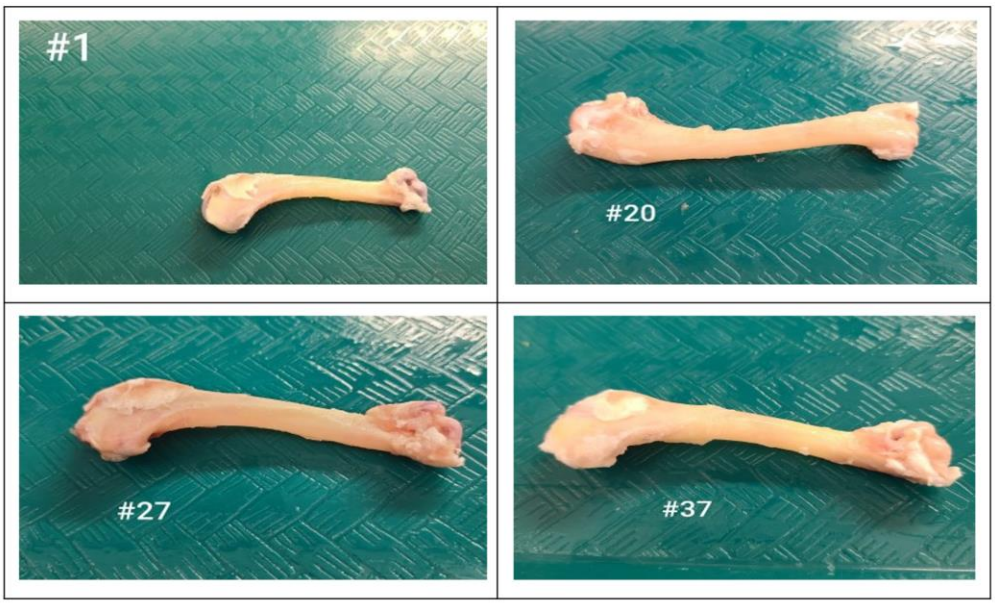
	Weight (g)	BMD Test Results (g/cm²)	Breaking strength (g/mm²)	Length (mm)	Diameter (mm)
Main Effects					
Strain (S)					
2016	3.20[a]	0.29[a]	0.05[a]	71.13[a]	40.92[a]
1940	3.17[a]	0.27[a]	0.05[a]	69.09[b]	40.96[a]
SEM	0.663	0.21	0.012	0.179	0.590
P value	0.323	0.649	0.291	0.322	0.303
Diet (D)					
2016	3.24[a]	0.29[a]	0.05[a]	81.08[b]	40.98[a]
1940	3.20[a]	0.28[a]	0.05[a]	85.85[a]	40.89[a]
SEM	0.473	0.2	0.011	0.017	0.582
P value	0.308	0.648	0.297	0.0314	0.319
Interaction					
S x D	0.341	0.233	0.591	0.043	0.323

[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.

*Bone mineral density test results were provided by the accuDEXA Bone Densitometry Report

Figure 2. Humerus bones of both strains



[1]Treatment groups were as follows: 1). 2016 hen on 1940 diet (1); 2). 2016 hen on 2016 diet (27); 3). 1940 hen on 1940 diet (37); and 4). 1940 hen on 2016 diet (20)

Tibial bone weight, mineral density, breaking strength and length and diameter measurements are displayed in **Table 4**. Tibia weights expressed in grams showed significance ($P \leq 0.05$) with the strain effect with hens of the 2016 strain having heavier weights when compared to hens of the 1940 strain. However, there were no significant differences ($P \leq 0.05$) with the diet effect for tibia weights, but hens fed on the 2016 diet had slightly heavier tibia bones when compared to hens fed on the 1940 diet. No significant differences ($P \leq 0.05$) were exhibited among the main effects of strain or diet for tibia bone mineral density. However, hens fed on the 2016 diet had a higher bone mineral density when compared to hens fed on the 1940 diet. Tibia breaking strength which is separated by bending moment expressed as kg/mm, peak force expressed as kg, and diameter expressed as mm showed that hens of the 2016 strain had a stronger breaking strength when compared to hens of the 1940 strain, however, with the diet effect, hens fed on the 1940 diet had stronger breaking strength when compared to hens fed on the 2016 diet. The length of the tibia at the strain effect was longer in hens of the 2016 strain resulting in significance ($P \leq 0.05$) but, there were no significance observed between hens fed on the 2016 diet or 1940 diet at the diet effect. Similar results were observed for the diameter measurements at the strain effect of the tibia in which hens of the 2016 strain had a significantly ($P \leq 0.05$) bigger diameter when compared to hens of the 1940 strain, however, no significance was observed between hens fed on the 2016 diet and those hens fed on the 1940 diet. Interactions between strain x diet were observed for both breaking strength and tibia bone length.

Table 4. Tibial Bone Mineral Density, breaking strength and measurements

	Weight (g)	BMD Test Results (g/cm^2)	Breaking strength (g/mm^2)	Length (mm)	Diameter (mm)
Main Effects					
Strain (S)					
2016	6.21[a]	0.71[a]	0.20[a]	117.34[a]	32.02[a]
1940	5.79[b]	0.71[a]	0.11[b]	112.76[b]	30.91[b]
SEM	0.185	0.021	0.218	0.422	0.733
p-value	0.053	0.7	0.041	0.045	0.049
Diet (D)					
2016	6.01[a]	0.72[a]	0.12[b]	114.19[a]	31.29[a]
1940	5.95[a]	0.69[a]	0.19[a]	113.61[a]	31.63[a]
SEM	0.185	0.01	0.161	0.422	0.734
p-value	0.348	0.631	0.053	0.351	0.218
Interaction					
S x D	0.243	0.378	0.045	0.056	0.121

[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.

*Bone mineral density test results were provided by the accuDEXA Bone Densitometry Report

Percent bone ash of the humerus and tibia are displayed in **Table 5.** There was no significant difference (P≤0.05) among the strain main effect for humerus percent bone ash, however, a significance (P≤0.05) was observed among the diet effect where hens fed on the 2016 diet had a high percent bone ash when compared to hens fed on the 1940 diet. On the contrary, there was no significant difference (P≤0.05) among the strain main effect or for the diet main effect for tibia percent bone ash. For the strain effect, the tibia of the 1940 strain presented with a higher percent bone ash when compared to the 2016 strain, but for the diet effect, hens fed on the 2016 diet presented with a higher percent bone ash when compared to hens fed on the 1940 strain. The interaction between strain and diet showed no significance for the humerus or tibia.

Table 5. Percent Bone Ash of Humerus and Tibia

	Humerus (%)	Tibia (%)
Main Effects		
Strain (S)		
2016	8.82[a]	46.03[a]
1940	8.59[a]	46.92[a]
SEM	0.685	0.700
p-value	0.280	0.289
Diet (D)		
2016	9.05[a]	46.67[a]
1940	8.36[b]	46.29[a]
SEM	0.685	0.705
p-value	0.053	0.244
Interactions		
S x D	0.395	0.283

[1]Values are presented as Means ± SEM

Note. Mean values down the column having the same alphabets are not significantly different at P≤0.05 according to Tukey's tests.

7.5 Discussion

The aim of this study was to evaluate the genetic variation of bone properties of the keel, humerus, and tibia bones of hens representing different eras and diet regimens. Concerns acquired from egg producers, veterinarians, nutritionists, and geneticists have been placed on bone health along with bone metabolism of laying hens. Bone health crucially impacts skeletal support along with eggshell quality deeming it vital in meeting these industry targets. The most important factors influencing these features are locally acting stresses and strains created by intrinsic muscle forces as well as the external loads. Body mass or physical activity which can lead to an increase in bone load can stimulate bone formation as well as increases bone mass. Decreased bone load or hypoactivity can induce bone loss or reduced mass in chickens because of modeling and remodeling that is mediated by the activity of osteoblasts and osteoclasts. Bone strength was not deemed a top productive trait in past selection programs making it possible that over several generations of genetic selection for production traits pertaining to high egg

production has resulted in a negative impact with hens having genetically weaker bones (Raymond, B., et al., 2018). The occurrence of bone breakage and bone quality is understood to vary among different laying hen strains.

7.5.1 Overall Effects

Several factors have been identified that can affect the strength and integrity of bones in laying hens. The focus in this study was related to genetics and nutrition on the bone parameters that were measured. The main effects in this study were strain and diet followed by interaction of strain x diet. Genetics plays a role in the functionality of the skeletal system of laying hens. A study conducted by Bishop et al., (2000) reported that approximately 40% of bone strength variation can be accounted for due to genetic differences among laying hens. Another study conducted by Stratmann et al., (2016) reported that genetic lines that were selected for high bone strength had few keel bone fractures and higher bone mineral density. In the past selection programs, bone quality was not accounted for unlike other traits such as egg production. Therefore, it has been assumed that modern laying hens has been negatively affected by genetic selection resulting in weaker bones. Surprisingly, the results in this current contradicted the studies previously mentioned related to bone mineral density of the humerus and tibia showing no significant difference in both genetic lines suggesting that both lines are more alike than different genetically and diet was not a major contributing factor. Similar results were presented in breaking strength of both the humerus and tibia of both lines as well as the keel bone analysis of deviations and fractures. Dietary effects weren't apparent in comparison to genetic effects. With the results from this study, it can be determined that genetic selection can provide a more permanent solution for improving bone quality of laying hens being of both a welfare and economic concern in comparison to nutritional interventions however, the issue can also be

addressed with proper nutrition. The interactions of the bones analyzed in this study are mainly additive and can contribute to overall bone quality either independently or jointly. It should be noted that sampling took place at one time point, at the end of the trial, so the lack of variation could be attributed to the absence of measurements throughout the hens' lifetime during the single lay cycle.

7.5.2 Keel Bone Analysis

Several production parameters are potentially connected to keel bone fractures (KBF); particularly being an early onset of lay (Kittelsen KE, et al., 2021). In a study conducted by Schütz et al., (2001), it was reported that the White Leghorn hybrid came into production relatively earlier (19.9 weeks) when compared to the Red Jungle fowl (24.9), thus theorizing that the ossification of the caudal part of the keel was not completed until around 30-40 weeks of age further suggesting that the modern hens started laying before the keel was mature (Schütz K, et al., 2002). Results from this study were in agreement with this theory due to hens of the 2016 strain coming into production earlier than the hens of the 1940 strain. Due to the higher impact force when experiencing a collision incident, heavier hens are more likely to suffer from keel bone damage (KBD) (Kolakshyapati M, et al., 2019). The prevalence of keel bone fractures depends not only on the housing condition but also on the rearing system that was used during the pullet development, alongside age and strain. Keel bone deviations and fractures are assumed to have a different etiology, with deviations believed to be a result extended perching and a long-term pressure exhibited on the keel bone (Kittelsen KE, et al., 2021). In this study, the prevalence of KBD and KBF were both observed in both strains and in both representative diets. It was demonstrated that KBD occurred more in the lighter strain hens (1940 strain) when compared to the heavier strain hens (1940 strain) suggesting that the weight of those hens had

impact of their ability to move more freely when compared to the heavier hens thus resulting in keel damage. Due to the lack of significance with diet, it can be suggested that the nutritional composition had little effect on preventing or reducing the damage of the keel bone. KBF was also observed in both strains, but hens of the 2016 strain had a higher occurrence suggesting that the positive correlation was due to body weight due to the 2016 strain being heavier than the 1940 strain. It can also be concluded that diet did impact the severity of KBF with hens fed on the 1940 diet being presented with more fractures than hens fed on the 2016 diet due to being unable to meet the high calcium demands for egg production. According to Bain et al., (2016), this is a classical explanation for justifications in KBFs as this phenomenon potentially induces resorption or breakdowns the bone matrix, thus releasing contained material leaving the bones weak and brittle.

7.5.3 Bone Mineral Density

Dual-energy X-ray absorptiometry (DEXA) consists of a moving X-ray generator that produces photons at 2 energy levels. A collimated scintillation detector moves simultaneously on the opposite side of the bone measuring flux. As the beam passes through the limb or bone, photon output is filtered to produce 2 distinct peaks that distinguish soft tissue from bone, generating bone density values. It should also be noted that the positioning of the bone being measured is critical in determining BMD when using the DEXA due to its 2-dimensional display according to Markel et al., (1994). In chickens, there has been positive correlation with bone breaking force ($r = 0.58$ to 0.68; $P < 0.001$) and bone ash weight ($r = 0.73$ to 0.99; $P < 0.001$) with bone mineralization as determined through utilization of the DEXA (Schreiweis, M, et al., 2005). The humerus representing the wing, has one of the greatest fracture rates of all bones

(Rayan, Gamal et al., 2020) and had a lower total bone mineral density measurement when compared to the humerus in this current study. According to Jendral MJ, et al., (2008), the lower total BMD likely reflects excessive bone mineral loss by birds whose movements were highly restricted due to heavier size, however, in this current study, the results contradicted those claims due to insignificance of humerus BMD of both strains in identical cage systems. According to research conducted by Schreiweis MA et al., (2003) adult White Leghorn hens consuming diets that have decreased calcium levels approximately around 5.4, 3.6, and 1.8% Ca, have shown a linear decrease in both tibia and humeral bone mineral density (BMD) together with bone mineral content. The tibia had higher BMD when compared to the humerus. The tibia possesses a greater medullary component when compared to the humerus which most likely contributed to the results of higher BMD. The similarities of the interaction between strain and diet could have been a result of bone mineralization not being affected over the course of the single lay cycle. The BMD of both humerus and tibia was not influenced significantly by the main effects of hen strain or diet.

7.5.4 Breaking Strength

According to Leyendecker et al., (2001) genetics can influence bone stability and bone breaking strength in addition to exercise and nutrition, however, Bishop et al., (2000) showed that bone stability could potentially be enhanced via selection occurring within a few generations (Leyendecker M, et al., 2005). Bone breaking strength using material testing equipment is a common means of determining functional characteristics of bone as a material. The humerus, known to normally be a pneumatized bone, devoid of mineral within its trabecular space with various degrees of humeral pneumatization, have been reported by Flemming et al., (1996, 1998) to have the presence of medullary bone which in turn appears to increase humeral density and

bone strength (Robison CI, et al., 2019). The results from this study demonstrated that humeral density was potentially increased to promote greater bone strength without negative implications from either strain or diet due to the lack of significance. The tibia is often used by researchers to represent the leg of a chicken. The tibiae of heavy hens were found to have greater breaking strength, diameter, length, bone weight, and total bone volume compared to the tibiae of lighter hens in research conducted by Kolakshyapati et al., (2019) which agreed with results from this current study. Comparable differences can be attributed to the overall body size with the knowledge that bone geometry responds to the changes in body weight as well as additional body mass thus increasing the loading strain that is applied to the skeletal system, and therefore its composition and strength (Kolakshyapati et. al., 2019). The results in this study demonstrated that the tibia breaking strength differed significantly among the genetic groups and diets but did not for humerus breaking strength suggesting that layer lines do differ with respect to body size. The results could also be triggered by both environment and nutrition in conjunction with underlying genetic factors.

7.5.5 Bone Ash

Utilizing the ash content of a bone typically provides estimates of the total mineral content (Robison CI, et al., 2019). Bone ash occurs when bones calcinate due to being heated at high temperatures. Ash is known as the inorganic residue resulting after water along with other materials are vaporized and organic substances are burned in the presence of oxygen (Robison CI, et al., 2019). Although the percentage of tibia ash is commonly and routinely used as a measure of bone mineralization, the actual weight of the tibia ash may be a more sensitive indicator of bone mineralization (Hall LE, et al., 2003). In conventional cages, all of these activities are constrained by both the small surface area of the cages and the absence of a suitable

amenity, and movement is likely insufficient to prevent loss of mineralized bone (Jendral MJ, et al., 2008). The results from this study suggest diet had a stronger influence on humerus ash compared to strain, but pressure of strain or diet did not impact bone ash of the tibia.

7.6 Conclusion

Quantity and quality are both important for overall bone health. Although bone strength was not likely part of the genetic selection programs during the greater part of the last 70+ years, the genetic determination of bone strength is high. Since skeletal problems in laying hens are important economic, welfare, and health issues for the poultry industry, a better understanding of bone metabolism in laying hens is important to enhance productivity and improve animal welfare.

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Chapter 8: Comparative intestinal histological features observed in 1940 Leghorn vs. 2016 Leghorn-based commercial laying hens fed representative diets

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8.1 Summary

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

8.2 Introduction

The avian gastrointestinal tract comprises functional anatomical and histological characteristics that are critical for feed conversion efficiency (Alshamy, Z., et al., 2018). 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 (Svihus, B. 2014). The chicken's gastrointestinal tract consists of 3 components belonging to the glandular stomach (proventriculus), gizzard (ventriculus), and intestine (small and large) (Alshamy, Z., et al., 2018). To promote maximal absorption of dietary components the intestinal mucosa is highly convoluted and specialized (Laudadio V, et al., 2012). The feeding process occurs when the feed material is ingested, moisturized, ground into small particles, acidified, and attacked by endogenous enzymes (Birger Svihus, 2014). 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.

8.2.1 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 (Birger Svihus, 2014). 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 (Birger Svihus, 2014). 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 (Kiela, P. R., et al., 2016). 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 (Kiela, P. R., et al., 2016). 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 (Alshamy, Z., et al., 2018). This increases the small intestinal surface area for absorption by about 600-fold resulting in a higher capacity for nutrient absorption (Karasov, W. H. et al., 2013). 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.

8.3 Materials and Methods

8.3.1 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.

Table 1. Feed Ingredients and Mash Diet[1] Compositions

Ingredients	2016 Layer Diet[2]	1940 Layer Diet[2]
Corn	940.5	1146.38
Soybean Meal	718.0	232.57
Alfalfa Meal		305.97
Limestone, gr.	145.5	124.2
Coarse limestone	50.0	
Fat	110.0	
Phosphate Mono/D	17.6	
Salt	6.8	5.0
D.L. Methionine	2.9	
T-Premix	1.0	
Sodium Bi-carb	2.0	
Prop Acid 505	1.0	
Choline CL 60%	1.3	4.0
Hy-D 62.5 mg/lb		
Trace Min PMX[3]	1.0	
L-Vitamin PMX[4]	1.0	
.06% Selenium[5]	1.0	
Ronozyme HI P (GT)	0.4	
Total	2000.0	2000.0
	Calculated Analysis	
Protein %	20.8	20.0
ME kcal/kg	2926	1330
Calcium %	4.10	0.90
A. Phos %	0.45	0.42
Lysine %	1.20	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.

8.3.2 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.

8.3.3 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).

8.3.4 Morphometric Analysis

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

8.3.5 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.

8.4 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≤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≤0.05) 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 significance (P≤0.5) interaction between strain and diet for the intestinal measurements.

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 x 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.

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 **Tables 3**, **4**,

and **5**. In the duodenum for the strain effect, hens of the 2016 strain had a significant difference ($P \leq 0.05$) with the villus height, villus tip width, and villus bottom width when compared to hens of the 1940 strain. However, hens of the 1940 strain had significantly ($P \leq 0.05$) 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) having deeper crypt depth and thicker muscularis when compared to hens fed on the 2016 diet. Significant differences ($P \leq 0.05$) of the interaction between strain and diet was observed for each of the parameters measured.

In the jejunum for the strain effect, a slight significance ($P \leq 0.05$) 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) 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 different ($P \leq 0.05$) of a higher villus height when compared to hens of the 1940 strain. Hens fed on the 1940 diet had a significant difference ($P \leq 0.05$) 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 ($P \leq 0.05$) were observed between strain and diet for the all of the parameters measured.

In the ileum for the strain effect, fluctuation between the strains resulted in significance ($P \leq 0.05$). 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) were observed between strain and diet for all of the parameters measured. Significant ($P \leq 0.05$) interactions were observed in the parameters measured.

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.

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	958[a]	186[a]	225[a]	135[b]	142[b]
1940	972[a]	148[b]	191[b]	152[a]	180[a]
SEM	36.1	10.25	11.64	5.71	5.30
p-value	0.052	0.002	0.0004	0.027	0.006
Diet (D)					
2016	997[a]	161[b]	193[b]	148[a]	173[a]
1940	934[a]	174[a]	223[a]	139[b]	148[b]
SEM	36.1	10.23	11.64	5.71	6.30
p-value	0.052	0.028	0.001	0.028	0.009
Interaction					
S x D	0.050	0.009	0.006	0.028	0.007

[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 x 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.

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 (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.

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	0.0570	0.049	0.057	0.034
Interaction						
S x D	0.0003	0.037	0.055	0.040	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

8.5 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.

8.5.1 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., (2021) 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., (2017) 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.

8.5.2 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 (Gu, Y. F., et al., 2021). Most of the digestive and absorptive processes of ingested feeds take place within the intestine (Havenstein G, et al., 1994). Nutrients within the diet could induce morphological differences among the intestinal parts and the intestinal absorptive function of each segment (Laudadio, V. et al., 2012). 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 (Gu, Y. F., et al., 2021). 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.

It is reported that an increase in villus height is a direct indication of improved absorptive function (Birger Svihus, 2014). Longer villi are known to provide increased surface area making them capable of greater absorption of available nutrients (Caspary, W. F. et al., 1992). According to studies from Lauronen et al., (2000), 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 S. Seyyedin et al., (2017) 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., (2015). Maneevan and Yamauchi, (2004) 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 (Xu et al., 2003). 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 A, et al., (2017) and in research conducted by Kelly et al., (1991) 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., (2003); Yamauchi, (2012) 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 (Baurhoo *et al*., 2007; Alizadeh *et al*., 2016). 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 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.

8.5.3 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 (Pinheiro DF, et al., 2004). It has been suggested that internal organ weights undergo modifications, pertaining to size, to accommodate different growth rates (Plavnik I, et al., 1983). 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 (Scanes CG, et al., 2014). 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 (B. Svihus, 2011). 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 (Martínez, Y. et al., 2015) 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 (Nester KE, et al., 1995). 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 JX et al., (2008) that intestinal weight increases with body weight which displayed similar results in this current study.

8.6 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.

8.7 References

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Chapter 9: Conclusion

Domestic animals, in particular, White Leghorns, have provided unique opportunities to explore the genetic basis for phenotypic diversity while being excellent models to better understand evolution research that has taken place over the decades. The White Leghorn, being labeled as one of the most dynamic egg-laying breed, has been selected for overall efficiency in regards to the maximum output of eggs for minimum food intake when compared to its ancestral relatives. Phenotypic differences characterized by various traits including egg production, both external and internal egg quality, age of sexual maturity, feed efficiency, and behavior have been of interest to determine whether domestication and selection have been positively achieved within the industry. More specifically, research centered based on genetics and nutrition has contributed significantly to the layer industry over the past decades. This present research has explored the connection between genetics and nutrient requirements of laying hens from different eras being fed representative diets of those eras during a single production cycle. Our understanding of how nutrition and its influence on measurable factors which have been affected by genetic selection has created a demand for continuous novel research. It is evident that tremendous strides have been implemented to ensure the efficiency and productivity of these birds. Results from this research have demonstrated that genetic selection has contributed to various areas such as pullet development during the different rearing phases for egg production which appeared not to have been altered; the connection between strain difference and maintenance necessities via nutrition and its influence on egg quality, both external and internal, along with overall egg production performance. The results from this research gave insight in the genetic foundation and its involvement in the alteration of the microbiota of both the ileum and ceca that further led to the identification of dominate bacterial taxa were observed. The results from this research also

examined the evaluation of how genetic divergence could potentially played a role in skeletal integrity with relation to the keel, humerus and tibia bones and its effect on overall bone health. Lastly, the results from this research investigated the intestinal histological modifications within the small intestines and its correlation to the availability of the nutrients further detecting the rate of absorption to evaluating nutrient utilization. It has been demonstrated that genetic differences contribute to an important criterion for ongoing selection for improved performance. The significant strain differences of the parameters that were measured in this study potentially could have been affected by the development of the W40 strain being maintained by random mating, therefore creating selection pressure. However, the variation of the strains did not diminish the production cycle of these hens making it evident that strain difference alone did not reflect the outcome of this trial. The data presented in this research provided an insight into how these modifications in selection have contributed to a more evolving industry as well as insight for potential enhancement. There has been little to no data generated available in this area that provides a more precise understanding of how the interplay of genetics and nutrition takes into effect of laying hen from different eras. The research conducted in this present study demonstrated that differences exhibited between strains of laying hens do exist and the need to develop potential sanctions that will cater to a growing industry is essential. With a growing population, it is vital to ensure that supply and demand are equally distributed in order to maximize the potential of the industry. However, in order to meet those demands, it is important to take heed to the past evolution of the layer industry to be able to continue the positive success of the present industry leading to a sustainable future industry. Researchers and collaborators are needed to continue the exploration of the genetic and nutrition advancements made over the decades to improve the intensifications that have already been established.