

**Comparative impact of feeding high fiber diets to modern Lohmann Select  
Leghorn- Lite and Shaver Heritage White Leghorn hens**

by

Alisha Mills (née Wornath- Van Humbeck)

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## **Abstract**

### **COMPARITIVE IMPACT OF FEEDING HIGH FIBER DIETS TO MODERN LOHMANN SELECT LEGHORN- LITE AND SHAVER HERITAGE WHITE LEGHORN HENS**

**Alisha Mills**  
**University of Guelph, 2020**

**Advisor:**  
**Dr. Elijah Kiarie**

The research outlined in this thesis investigated comparative impact of feeding ground (GOH) or unground (UGOH) oat hulls to modern Lohmann Select Leghorn- Lite (LSL-L) and heritage Shaver Heritage White Leghorn (SHWL) on egg production and gastrointestinal physiology. Most responses were independent of breed or diet. The LSL-L hens showed superior egg production to SHWL hens. Feeding oat hulls increased gizzard weight, however, LSL-L gizzard showed a greater response. Yet, oat hulls resulted in poor eggshell quality and energy digestibility linked to lower concentration of nutrients in oat hull diets. Furthermore, GOH fed birds had a lower digestive capacity relative to UGOH suggesting further processing of insoluble fiber may be detrimental. Overall, the data indicated that the modern hen is adaptable to ingestion of structural material rich in insoluble fiber, however, the results need to be confirmed at placement to the end of lay.

## **Dedication**

This thesis is dedicated to my son, Logan James Mills. May you always persevere.

“Blessed is the one who perseveres under trial because, having stood the test, that person will receive the crown of life that the Lord has promised to those who love him.” James 1:12

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# Table of Contents

Abstract .....	ii
Dedication .....	iii
Acknowledgements .....	iv
Table of Contents .....	v
List of Tables .....	vii
List of Figures .....	viii
List of Abbreviations .....	ix
Chapter 1: Literature review .....	1
1.1 Introduction .....	1
1.2 The modern hen .....	3
1.2.1 Evolution of hen genetics .....	3
1.2.2 Evolution of hen housing: rise and fall of cages .....	5
1.2.3 Persistent calcium metabolism insufficiency and the risk of osteoporosis .....	9
1.3 Physiological and nutritional implications of structural material .....	12
1.3.1 Innate need for consumption of structural material .....	12
1.3.2 Feed ingredient processing and particle size .....	12
1.3.3 Dietary fiber .....	15
1.4 The role of dietary fiber on gut development and function in layers .....	17
1.4.1 Physiology and function .....	17
1.4.2 Egg production and quality .....	22
1.4.3 Hen health and welfare .....	24
1.5 Summary and gaps in knowledge .....	26
Chapter 2: Hypothesis and objectives .....	28
Hypothesis .....	28
Overall objective .....	28
Specific objectives .....	28
Chapter 3: Comparative effects of feeding ground or unground oat hulls on feed intake, egg production, eggshell quality and apparent retention of nutrients in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorn hens .....	29
3.1 Abstract .....	29
3.2 Introduction .....	30
3.3 Materials and methods .....	31

3.3.1 Birds and husbandry .....	31
3.3.2 Pre-experiment period.....	32
3.3.3 Experimental procedures .....	32
3.3.4 Measurements and sampling.....	32
3.3.5 Sample processing and laboratory analyses.....	33
3.3.6 Calculations and statistical analysis.....	35
3.4 Results and discussion .....	36
Chapter 4: Comparative effects of feeding ground or unground oat hulls on visceral organs weight and indices of gastrointestinal ecology in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorn hens .....	48
4.1 Abstract.....	48
4.2 Introduction.....	49
4.3 Materials and Methods.....	50
4.3.1 Experimental procedures and sampling .....	50
4.3.2 Sample processing and laboratory analyses.....	50
4.3.3 Calculations and statistical analysis.....	51
4.4 Results and discussion .....	52
Chapter 5: General discussion and conclusions.....	59
Chapter 6: References.....	62

## List of Tables

<b>Table 3.1</b> Composition of control diet, on an as fed basis .....	42
<b>Table 3.2</b> Analyzed chemical composition of oat hulls and experimental diets, % as fed basis .	43
<b>Table 3.3</b> Hen day egg production (HDEP, %) and feed intake in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorn fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days .....	44
<b>Table 3.4</b> Egg weight, Feed conversion rate (FCR) and eggshell characteristics in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorn hens fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days <sup>1</sup> .....	45
<b>Table 3.5</b> Apparent retention of nutrients in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorns fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days .....	46
<b>Table 4.1</b> Gastrointestinal tract and liver weights in Lohmann Select Leghorn-Lite and Shaver Heritage White Leghorns fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days .....	56
<b>Table 4.2</b> Jejunal histomorphology in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorns fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days .....	57
<b>Table 4.3</b> Concentration of short chain fatty acids in ceca digesta and excreta dry matter (DM) in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorns fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days .....	58

## List of Figures

<b>Figure 3.1</b> Particle size distribution in the experimental diets .....	47
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## List of Abbreviations

**AGP**, antimicrobial growth promoters

**AID**, apparent ileal digestibility

**AME**, apparent metabolizable energy

**AR**, apparent retention

**AV**, aviary

**B.C.**, before Christ

**BAB**, Babcock B-300

**BL**, Brown Leghorn

**BW**, body weight

**Ca**, calcium

**CC**, course corn

**CD**, crypt depth

**CF**, crude fat

**CP**, crude protein

**DDGS**, distillers dried grain with solubles

**DM**, dry matter

**EBS**, eggshell breaking strength

**EFC**, Egg Farmers of Canada

**EM**, egg mass

**EST**, eggshell thickness

**EW**, egg weight

**FC**, fine ground corn

**FCR**, feed conversion ratio

**GE**, gross energy

**GI**, gastrointestinal

**GIT**, gastrointestinal tract

**GOH**, ground oat hull

**HDEP**, hen day egg production

**Hrs**, hours

**ISAB**, ISA Brown

**LB**, Lohmann Brown

**LSL-L**, Lohmann Select Leghorn- Lite

**LSL**, Lohmann Select Leghorn

**Min**, minutes

**mRNA**, messenger ribonucleic acid

**N**, nitrogen

**NCWL**, North Carolina White Leghorn

**NDF**, neutral detergent fiber

**OM**, organic matter

**PC**, protein concentrate

**SHWL**, Shaver Heritage White Leghorn

**SM**, shell meal

**UGOH**, Unground oat hull

**VH**, villus height

**VH:CD**, villus height and crypt depth ratio

**Wk**, week

**Wt**, weight

**WW**, whole wheat

## **Chapter 1: Literature review**

### ***1.1 Introduction***

Advances in genetics has produced a hen with a capacity for greater performance with minimal feed input. Specifically, the combined effects of increased egg mass production (number and size), reduced body weight and a lowered maintenance requirement brought about significant improvement in feed utilization efficiency in modern layers (Jones and Anderson, 2013). A review of North Carolina white leghorn (NCWL) performance tests from 1958 to 2011 showed that the average age at 50% production decreased by 34 days, pullet ( a laying hen before reaching sexually maturity) body weight at point of lay dropped from 1.61 to 1.16 kg, mature hen body weight from 2.05 to 1.68 kg, feed conversion improved from 2.90 to 1.99, while egg mass increased from 16.3 to 19.9 kg per hen housed (Anderson et al., 2013). The nutrition of these birds has also evolved over time as a necessity to achieve and sustain genetic potential. In this context, feeding, a major control point of profitability, has evolved and progressed both in terms of understanding digestive physiology and metabolism, and in the more precise evaluation of the quality of dietary raw materials. Feedstuff processing and diet manufacturing have also evolved such that the composition, ingredient choices and diet form have been refined to improve feed intake and efficiency (Kiarie and Mills, 2019). Adoption of practices such as antimicrobial growth promoters and confined housing (e.g. cages) have also contributed to production efficiency in modern poultry operation (Kiarie et al., 2013).

The poultry sector is in transition to adopt alternative housing for layers (litter floors, cage-free) and to reduce the use of sub-therapeutic antimicrobial growth promoters. Antimicrobial use in the poultry industry has become increasingly restricted as a result of recent consumer and public pressure due to perceived impact on human and animal health (Timbermont et al., 2011; Mateos

et al., 2012; M'Sadeq et al., 2015; Kheravii et al., 2018). Coupled with a demand for a cage-free environment, resulting in exposure to excreta, incidences of enteric diseases have risen dramatically in laying hens, reducing production and animal welfare (Oviedo-Rondón, 2019). Strategies targeting nutrition and feeding practices have been proposed as a potential means to mitigate the prevalence of gut diseases, such as necrotic enteritis, caused by reductions in antimicrobials and increased exposure to pathogens (Kheravii et al., 2018). Examples of such strategies include changes to the nutrient composition of the diet such as the addition of insoluble fiber, as well as changes to the physical composition of the diet such as the addition or removal of mechanical processing of ingredients in the feed mill (Kiarie and Mills, 2019).

Most modern poultry feed has become increasingly refined, improving intake and performance. However, highly refined, energy dense feed may not facilitate gut development, limiting the hen's ability to fight pathogenic diseases of the gut and reducing nutrient intake, potentially impacting health and longevity (Svihus, 2011; Kiarie and Mills, 2019). Evidence can be provided to support dietary intervention through the manipulation of feed structure for poultry for the purpose of optimal gastrointestinal health and function. For example, data suggests coarse feed structure (created by grain particle size manipulation, limestone particle size, inclusion of insoluble fiber or access to litter) may improve digestive function and development in poultry (Mateos et al., 2012; Jiménez-Moreno et al., 2013; Xu et al., 2015a). However, much of this research is heavily focused on broiler production. Maintaining egg production and quality in both conventional and enriched housing systems as well as in potentially outdoor, cage-free pathogen-rich environments, despite reductions in the use of antimicrobials has presented challenges for the laying hen industry and is currently under investigation.

Manipulating the structure of the modern hen's diet may influence the gut, aiding in the maintenance of egg quality and skeletal integrity of highly prolific modern strains, despite their rearing environment. In addition, the use of a larger feed particle size and materials such as fiber in the diet may have a cascading effect on other markers of health and welfare. This may include production and behavioural effects such as feather pecking, as well as other aspects of welfare such as incidences of bone deformities and fractures; however, it is still unclear how this approach may specifically influence the modern laying hen. In addition, modern laying hens may respond differently to holistic treatment in the form of nutritional interventions than their lineages as they have been selected for high egg production over many generations under conditions in which they may have had little exposure to pathogens. However, there is also limited data comparing modern, highly selected lines to heritage (non-selected) lines in terms of impact of manipulating feed structure on productivity, gut function, and health, warranting further research.

## ***1.2 The modern hen***

### **1.2.1 Evolution of hen genetics**

Egg production is markedly shaped by genetic selection in laying hens (Silversides et al., 2006). The ancestor of the modern bird, the Red Jungle Fowl of India (or *Gallus ferrugineus*), was about the size of the modern leghorn but only laid approximately 20-26 eggs per year (Buckner and Martin, 1922). Domestication of the modern chicken began in India in the year 3200 B.C. then migrated to Europe in 2000 B.C. and finally reached North America during the pre-Columbian period (pre- 1492) via China and South America (Wood-Gush, 1958). Selection for egg production characteristics began during the agricultural boom in Roman times, anchored on the basic understandings of heredity. During this time, egg production increased considerably before the

domestic hen became a “scavenger of the barnyard”, not returning into the agricultural landscape until the 19<sup>th</sup> century when poultry production intensified once again in Britain (Wood-Gush, 1958). However, at that time, selection was primarily based on fighting ability, affecting characteristics such as body weight and lack of molting (Wood-Gush, 1958). Criteria for selection has been under investigation since this time with early selection based on pigmentation of the leg, as a paler leg often indicated high production due to fat deposition (Blakeslee and Warner, 1915). Beginning in the early 1960s (when a typical leghorn laid just over 100 eggs/year), consumer demand in addition to an overall preference for larger eggs resulted in selection such that a typical leghorn is now producing upwards of 300 eggs a year (Buckner and Martin, 1922; Stratmann et al., 2016).

Genetic advances in combination with improvements in feed ingredient availability and an improved nutritional value have resulted in a modern hen, capable of great production outputs with high efficiency. In fact, in a comparison between modern and heritage leghorns, the modern strains were found to have higher hen day egg production (**HDEP**) complemented by the higher egg mass (**EM**), despite having the lower body weight (Jones et al., 2001). However, modern strains were also found to have higher levels of mortality, which has been found to be true in many studies involving comparisons between modern and heritage hens, indicating an impact of higher production on health and longevity in modern breeds (Jones et al., 2001). Silversides et al. (2006) compared three lines of commercial laying hens including ISA Brown (**ISAB**), Brown Leghorn (**BL**), and Babcock B-300 hens (**BAB**). The authors found BL had lower HDEP than BAB and ISAB; however, no differences in egg weight were observed (Silversides et al., 2006). This was in agreement with other researchers who found that despite differences in HDEP, egg weight did not differ between commercial and heritage breeds (Sosnówka-Czajka et al., 2011).

Intensive breeding and selection of laying hens began with the goal of increasing egg production, resulting in dramatic increases in the number of eggs produced by modern hens (Stratmann et al., 2016). Current selection and breeding programs are more thorough and incorporate many parameters of production, including age of first lay, egg size and number of eggs produced. However these advances are often coupled with a decline in mature hen body weight as a result of reaching sexually maturity earlier, potentially impacting hen health and welfare (Jones et al., 2001; Buitenhuis and Kjaer, 2008; Jones and Anderson, 2013). This may limit nutrient reservoirs, particularly calcium (**Ca**) in the skeletal system that is necessary to meet the demands of higher egg production and could provide an explanation for high mortality rates within modern breeds, necessitating further research for nutritional interventions.

### **1.2.2 Evolution of hen housing: rise and fall of cages**

Although a small number of specialized, commercial egg farms existed in the early part of the 20<sup>th</sup> century, egg production was more often part of the mixed-farming operations that were characteristic of farm agriculture at that time (Pelletier et al., 2018). However, from mid-late 1920, the egg industry entered a period of significant and sustained industrialization (Pelletier et al., 2018). Key advances to the specialization and intensification of egg production were the adoption of cage systems for housing laying hens and improved genetics, as previously discussed. Pioneer research on hen confinement started in the early 20<sup>th</sup> century. For example, the impact of confinement was evaluated at the Ohio Agricultural Research Station to characterize livability and productivity (Kennard, 1928). However, complete indoor confinement would not be realized for commercial egg production at this time, mainly because of lack of complete feed, artificial light and heating (Pelletier et al., 2018). The development of commercial layer diets in the 1920s and the introduction of the Rural Electrification Act of 1936 in the US, which enabled barns to be lit



artificially, revolutionized the egg industry allowing what was previously a seasonal food in temperate climates to be produced year-round (Lee, 1951; Hanke et al., 1974; Freidberg, 2008; Pelletier et al., 2018).

The beginning of modern egg production in larger flocks can be traced back to the first years after World War I (Coles, 1954). However, at the time most of the egg production involved small flocks kept on thousands of farms. Hens were reared in a hybrid or free range system and confinement included deep litter systems in winter (Coles, 1954). However, hen productivity was low in these systems and eggs were often contaminated with zoonotic bacteria such as *salmonella*, with high hen mortality (Coles, 1954; Harry, 1963). However, some farmers were aware that keeping hens away from their droppings reduced mortality dramatically and also kept eggs cleaner (Windhorst, 2017). This led to experimentation with cage systems in the 1930's, however, these experiments were not successful as the birds became crippled and could no longer stand on their own feet within weeks of placement (Windhorst, 2017). The leg problem was later linked to rickets associated with a lack of vitamin D and when hens were fed feed mixed with cod liver oil they could be kept in cages for a whole laying cycle (Windhorst, 2017). This opened the door for development of caged housing for the laying hens (Windhorst, 2017). Further studies established cages as the gold standard for egg production, anchored on food safety. For example, Harry (1963) reported that the shells of eggs from deep litter systems had approximately fifteen times more bacteria and a higher proportion of organisms that could cause potential spoilage than eggs from conventional cage systems. Quarles et al. (1970) reported that litter floor houses had, on average, nine times more bacteria in the air, and twenty to thirty times more aerobic bacteria on the eggshell than wire floor houses.

The industry initially preferred individual cages because they mitigated cannibalism and allowed ease of identification and culling of non-laying hens (Harry, 1963; Quarles et al., 1970). However, due to the cost advantages of housing multiple birds in the same cage, the conventional cage was adapted to house small groups of hens (Harry, 1963; Quarles et al., 1970). Beginning in the 1960s egg production in Canada transitioned from “free-run” (i.e., indoor non-cage) to cage-based production to maximize egg production and feed conversion efficiency (Pelletier et al., 2018). Further development revolved around genetic selection of hens adaptable to group housing in cages while, the integration of artificial incubators facilitated rapid progress in selection for production efficiency (Siegel et al., 2006). Moreover, development of improved management practices for disease prevention such as biosecurity protocols, along with the advent of poultry vaccines, served to improve bird health and reduce losses due to mortality (Pelletier et al., 2018).

Over the years, advancements in housing technology including artificial ventilation systems and climate control, automated feeding, egg collection and manure removal were implemented, thereby reducing labor and allowing for thousands or tens of thousands of birds to be housed in a single barn. Collectively, superior genetics, housing and management technologies have enabled considerable improvements in resource utilization efficiency and the reduction of environmental impacts associated with egg production. The environmental footprint of Canada’s egg production supply chain declined by almost 50% between 1962 and 2012, while egg production increased by 50% (Pelletier, 2018). This same 50-year interval was also marked by declining mortality rates (falling from roughly 13% in the early 1960s to 3.2% at present for pullets and laying hens combined), and by much improved feed conversion efficiencies (Pelletier, 2018).

As the egg industry continued to expand, there was emerging and concurrent public debate regarding the welfare of laying hens (Pelletier et al., 2018). The key criticisms of using

conventional cages for egg production include lack of space and the inability of hens to perform natural behaviors (Harrison, 1964). In defense of cages, the industry argued that they decreased disease and facilitated the provision of clean food and water. Cognizant of evolving issues around hen housing, the Canadian national egg industry association, Egg Farmers of Canada (EFC), mandated the National Farm Animal Care Council in 2012 to develop a new code of practice for laying hens that was released in early 2017 (NFACC, 2017). The code requires all hens be provided nests, perches, scratch mats or foraging material by 2036, to align with similar directives in the European Union. The code also set detailed standards for non-cage systems, including aviaries. Although the codes are not legislated, “when included as part of an assessment program, those who fail to implement requirements may be compelled by industry associations to undertake corrective measures or risk a loss of market options” (NFACC, 2017). As the codes were being developed, the global egg industry was under pressure from consumers and legislative actions over the issue of caged housing. In 2015, the first few North American restaurants, grocers and manufacturers made public, corporate pledges to purchase eggs only from non-cage systems. In February 2016, the EFC announced that no new conventional cage systems could be installed after July 2016, but both enriched cages and non-cage systems would be allowed. Shortly thereafter, the Retail Council of Canada, which comprises most large grocery chains in Canada, committed to sourcing only cage-free eggs by the end of 2025 (Mench et al., 2011). By the end of 2016, an unprecedented number of North American corporations made commitments to cage-free housing which will require new or retrofitted barns for over 200 million hens in the United States and Canada by 2026 (Shields et al., 2017; Pelletier et al., 2018).

As of 2016, Canada housed 98% of its 26 million egg laying hens in conventional, battery cages (NFACC, 2017). With a shift from conventional cages to enriched colony cages and cage-

free operations, there will be an increase in bird activity (scratching, foraging and dust bathing) potentially leading to increased airborne pollutants like dust and ammonia (Nimmermark et al., 2009; Tactacan et al., 2009). Moreover, the chicken gut microbiome harbors several taxa capable of causing significant illnesses in humans, most importantly *Campylobacter* and *Salmonella* and removal of antimicrobial growth promoters (AGP) in conjunction with changes in housing environment could increase risk of contaminating poultry products.

### **1.2.3 Persistent calcium metabolism insufficiency and the risk of osteoporosis**

Appropriate mineral nutrition is critical for optimal production in laying hens, with many trace minerals playing critical roles in countless physiological processes (Mabe et al., 2003; Hafeez et al., 2015). Once consumed by the hen, minerals are transported in the bloodstream, either in complex structures such as lipoproteins or in an ionic form, depending on the properties of the mineral (Hafeez et al., 2015). Due to the low supply of Ca found in most ingredients used in animal feed formulation, additional Ca supplementation, commonly in the form of limestone, is required in the diet to meet the demands of the laying hen. Calcium intake from feed is largely dependent on the amount of feed consumed, in addition to the level of Ca present in the diet formulation, resulting in feed intake being the first limiting factor (Lukic et al., 2011). However, feed intake is complex with factors such as age, environment, temperature, feeding frequency and nutrient composition of the diet all being potential influencers, further increasing the challenge of Ca balance in laying hens (Lukic et al., 2011).

Accompanying increased production is an increase in Ca demand for eggshell formation (Stratmann et al., 2016). Early work showed that 60-75% of Ca in eggshell was derived from dietary sources whereas 25-40% was from skeletal stores. It is not known what proportion of Ca

the modern layer draws from these sources for its daily egg shell formation needs; however, given the low feed intake capacity of the modern hen, the proportion drawn from bone reserves could be higher than from dietary sources (Driggers and Comar, 1949). Moreover, there is diminished capacity for intestinal Ca absorption as the hen ages, which coincides with decreased bone mineral content. All these factors collectively suggest more mineral output in eggshell with less mineral input in the feed and smaller mineral reserves in the skeletal mass (al-Batshan et al., 1994). In addition, age of first lay has decreased as a consequence of selection, influencing the ability of the hen to build up proper Ca stores before sexual maturity, risking bone integrity during laying (Habig and Distl, 2013).

A common disease resulting from insufficient Ca stores in laying hens is osteoporosis. Osteoporosis is associated with selection for early sexual maturity and high egg production (Habig and Distl, 2013). Osteoporosis occurs in laying hens when the structural bone becomes porous, and as a result, weak and brittle (Whitehead and Fleming, 2000). Once a hen becomes osteoporotic, bone mass is reduced as the amount of mineralised structural bone is compromised, leading to the development of fragile bones and increasing the risk of fracture (Whitehead and Fleming, 2000; Habig and Distl, 2013; Rodriguez-Navarro et al., 2018).

Laying hen bone has three distinct components, the cortical and trabecular, making up the structural component of the bone, and the medullary, representing the non-structural component of bone, which is unique to avian species (Stratmann et al., 2016). Medullary bone forms in female laying hens upon the onset of estrogen production at sexual maturity and is responsible for the temporary storage of Ca during egg production in the laying hen (Stratmann et al., 2016). Pullets must deposit Ca into the structural bone before the onset of medullary bone production, as once this critical period has passed, bone remodeling results in the depletion of cortical bone which can

no longer be replenished without molting (Whitehead and Fleming, 2000). Calcium storage is altered once the hen reaches sexual maturity, which limits bone stores and the availability of Ca later in life (Whitehead and Fleming, 2000).

Although 60-75% of Ca needed during egg formation is normally effectively achieved through dietary intake, hens will rely on medullary stores for the remaining 25-40%, and in the case when these stores are depleted and not replenished, will turn to Ca stores in cortical bone, which increases the risk of osteoporosis (Stratmann et al., 2016). Approximately 2 to 3 g of Ca is needed to form the structural component ( $\text{CaCO}_3$ ) of the eggshell, and when this is not available the hen will deplete critical structural bone resulting in increased incidence of bone deformities and fractures (Stratmann et al., 2016). Osteoporosis is often associated with cage layer fatigue, a condition caused by fractures in the third and fourth thoracic vertebrae, causing compression on the spinal cord in laying hens kept in cages (Duncan, 2001; Lay Jr. et al., 2011). Cage layer fatigue is frequently accompanied by an increase in bone fractures and deformities, and can result in an S-shaped deformation within the keel bone (Lay Jr. et al., 2011). Although intensified in caged housing due to lack of exercise, osteoporosis continues to be a concern in all laying hens regardless of environment (Lay Jr. et al., 2011). Due to this, in addition to the complex nature of Ca metabolism within the laying hen, maximizing nutrient absorption through any means possible may help to prevent development of osteoporosis and thus improve the welfare of laying hens (Stratmann et al., 2016).

## ***1.3 Physiological and nutritional implications of structural material***

### **1.3.1 Innate need for consumption of structural material**

Foundational studies more than half a century ago demonstrated that chickens, when offered free choice, will consume a great variety of ingredients (Emmans, 1979). However, these free choice studies also showed that chickens can adjust feed consumption according to needs, and voluntarily consume ingredients considered of low or non-existent nutritional value. More recent research demonstrated chickens' preference for low nutritive but structural feed material had a beneficial impact on overall feed utilization and productivity (Hetland and Svihus, 2001; Hetland et al., 2003, 2005). This beneficial impact was linked to modulation of the gastrointestinal tract, suggesting that poultry require a certain amount of structural feed for proper gut development and functionality. Different feed ingredient and processing approaches have been proposed to not only maximize the digestibility of the contents of the diet but also to increase intake and maximize development and function of the gastrointestinal tract (Kiarie and Mills, 2019). Some of the strategies proposed include the incorporation of fiber (Jørgensen et al., 1996; Hetland and Svihus, 2001) and/or the addition of enzymes into the diet (Kiarie et al., 2013, 2017; Sanchez et al., 2019), as well as mechanical and heat processing of both complete feed and ingredients (Moritz et al., 2005; González-Alvarado et al., 2008).

### **1.3.2 Feed ingredient processing and particle size**

Poultry species are largely dependent on a variety of endogenous enzymes for their dietary needs. Therefore, one of the most important factors that determines feed utilization is dietary particle size and the resulting effects on actions of endogenous enzymes (Kiarie and Mills, 2019). Cereal grains are primary energy sources and they require processing before or after mixing with

other diet components. Particle size reduction often includes a grinding step with a hammer or roller mill to facilitate further processing (e.g., mixing, pelleting, extrusion, expansion). Feed structure refers to the internal binding strength and size of ingredients and can be noted as macro or microstructure (Svihus, 2011). For the refinement of feeding strategies and processing techniques it is necessary to better understand the relationship between particle size, and the development and function of the gastrointestinal tract (Hetland et al., 2005; Mateos et al., 2012). Once ingredients are ground and thoroughly mixed, they are further processed to crumbles. This is a common practice to increase feed intake by the introduction of a secondary structure or macrostructure to the diet (Svihus, 2011). Once a feed pellet is ingested by the bird and exposed to the digestive tract secretions, the pellet begins to dissolve, revealing the microstructure. Researchers have found that the macrostructure of feed does not affect gizzard development (Svihus, 2011), but relative gizzard size is reduced when birds are fed a pellet diet rather than mash diets. This is most likely due to the pelleting process resulting in a finer microstructure of ingredients (Engberg et al., 2002; Amerah et al., 2007a; Kiarie and Mills, 2019).

The manipulation of feed structure influences gastrointestinal functioning through enhancing gizzard activity, increasing retention time, reducing digesta pH and diversifying the bacterial profile (Kiarie and Mills, 2019). For example, researchers conducted an experiment to determine the effects of coarse and finely ground corn fed to broilers on performance, litter quality, organ weight and apparent ileal digestibility (**AID**) (Xu et al., 2017). The effect of processing was determined using coarse (**CC**) and finely ground corn (**FC**) with the CC (1.15 mm) produced using a roller mill to achieve more uniform sized particles, and FC using a hammer-mill to produce finer particles (0.27 mm) (Xu et al., 2017). The utilization of 50% CC had a positive effect on feed intake, body weight and feed conversion, in addition to decreasing litter moisture, nitrogen and



pH. The authors suggested the better nutrient utilization was due to enhanced gastrointestinal function as the inclusion of CC resulted in stimulation of the gizzard, increased digesta retention, an increased reverse peristalsis aiding the reduction of digesta pH, increasing nutrient absorption (Xu et al., 2015b, 2017). The utilization of CC reduced production costs by 9.47 cents per metric ton (Xu et al., 2017), which is notable as feed cost is the primary expense in poultry production.

Although processed feed is beneficial in stimulating the gastrointestinal (**GI**) tract in poultry species, research in layers has focused primarily on limestone particle size. For the full effect of particle size on the gizzard to be realized, particles must be no less than 1.0 mm in size (Rao et al., 1992). Large limestone particles (> 1.0mm), rather than fine (<1.0 mm) have been recommended for laying hen diets (Skřivan et al., 2010). Depending on hen age and stage of production, current recommendations suggest an ideal limestone particle size ranging between 1.40 and 5.60 mm (Witt et al., 2009). Self-selection of larger particle sizes by laying hens has been reported (Molnár et al., 2018). One experiment determined the self-selection of coarse shell meal (**SM**), protein concentrate (**PC**), and whole wheat (**WW**) by 24 wk old laying hens, with the authors finding an increased consumption of all three diets prior to active eggshell formation (Hetland et al., 2003). In a larger scale experiment using laying hens aged 16- 68 wks, birds were given an experimental diet containing 40% WW fed as pellets. Birds fed the WW had a tendency ( $P = 0.07$ ) for a lower feed conversion ratio (**FCR**), better plumage, and increased gizzard weight and gizzard contents (Hetland et al., 2003). The influence of feed processed through a roller or hammer mill as mash or thermal processing via expander on mineral retention was investigated in 19 wk old Lohmann Browns (Hafeez et al., 2015). Despite feed produced using the hammer mill having a negative effect on digestibility, mineral concentrations in the egg were comparable among

treatments providing evidence that influencing the microstructure of the feed can positively influence digestion without reducing egg quality (Hafeez et al., 2015).

### **1.3.3 Dietary fiber**

Dietary fiber can be divided into two major categories: soluble and insoluble fibers. Although both fiber types can be beneficial to health and digestion in some way, they have contrasting qualities that affect poultry differently (Knudsen, 2014). Processing of fiber can also influence its properties particularly due to the reduction in particle size (Hetland et al., 2004).

#### *1.3.3.1 Soluble fiber*

Soluble fiber is dissolvable in water, often forming a viscous, gel like substance within the digestive tract, increasing the water-holding capacity of the digesta and potentially leading to challenges such as wet litter (Kay, 2018). Common examples of soluble fiber ingredients in animal feed include: flaxseed, oats, pectin, beet pulp, rice bran, and barley (Abdel-Hafeez et al., 2018; Leung et al., 2018; Thanabalan et al., 2020). Soluble fiber can be used as an energy source in poultry feed and may have some benefits as it is often fermentable and can be used as a food source for gut microflora (Kay, 2018; Leung et al., 2018). However, feed ingredients that are rich in viscous, water-soluble fiber result in poor poultry performance because of increased intestinal digesta viscosity, often inhibiting the digestion of major macromolecules such as starch, protein and fat (Bedford and Schulze, 1998; Kiarie et al., 2014; Kay, 2018).

#### *1.3.3.2 Insoluble fiber*

Insoluble or non-water-soluble fiber does not dissolve in water and mostly aids in digestion through its bulking effect on the diet; however it can also act as a pre-biotic for short chain fatty

acid production (Kay, 2018). Common sources of insoluble fiber in animal feed include lignified vegetable material such as hulls and straws in addition to materials available through other sources such as litter material made from by-products of wood processing (Kalmendal et al., 2013; Leung et al., 2018). Insoluble fiber can change the structure of the diet, influencing the gastrointestinal system similar to the addition of coarse particle sizes (Mateos et al., 2012; Xu et al., 2017). Feeding insoluble fiber to poultry is beneficial to the development of the gizzard (Hetland and Svihus, 2001), as it stimulates gizzard functioning (Hetland et al., 2003). The feeding of insoluble fiber increases digesta retention in addition to stimulating the production of secretions by the proventriculus, aiding in digestion (Duke, 1986; Hetland et al., 2005).

#### *1.3.3.3 Sources of dietary fiber*

Wide ranges of dietary fiber sources are available, and many have been investigated for use in the production of high fiber diets (Abdel-Hafeez et al., 2018; Leung et al., 2018). Common sources of dietary fiber are diverse in terms of availability and include seed hulls, by-products of human food production, sugarcane bagasse, a by-product of sugar production, and distillers dried grain with solubles (**DDGS**), a by-product of the ethanol industry (Kiarie and Nyachoti, 2009; Walugembe et al., 2014). The use of by-products high in dietary fiber can result in a reduction in feed costs, as these ingredients may be less expensive than other commonly used feed ingredients such as corn and soy (Kiarie and Nyachoti, 2009; Walugembe et al., 2014). Other sources of fiber that have been investigated for use within the poultry industry include wheat straw, flax meal, whole grains, and litter materials such as wood shavings and recycled paper (Leung et al., 2018).

## ***1.4 The role of dietary fiber on gut development and function in layers***

As discussed previously, dietary fiber has the potential to influence water-holding capacity, digesta viscosity, fermentation, and stool quality in the laying hen (Mateos et al., 2012). Poultry ingest dietary fiber from two main sources: feed and fibrous litter material (when available) (Kalmendal et al., 2013). Fiber was often referred to as a dietary diluent with no nutritional benefits, sometimes even considered to be an anti-nutritional factor due to its negative impacts on nutrient digestibility, production, and voluntary feed intake (Angkanaporn et al., 1994; Mateos et al., 2012; Walugembe et al., 2014; Kiarie et al., 2017). However, bedding materials are rich in insoluble fiber and when consumed, it may affect hen nutrition and productivity. For example, broilers, turkeys and layers reared on the floor have been shown to ingest litter, which may be a significant source of insoluble fiber (Mateos et al., 2012). Similarly, layers in modified cages with dustbathing material will sometimes consume the available litter (Hetland et al., 2003). Related to the effects of fiber on gastrointestinal health, the use of high fiber in broiler diets has gained attention in the past several years as a result of the ban of the use of antibiotics as growth promoters in feed in many countries, prompting researchers to search for alternative strategies to combat enteric disease (Mateos et al., 2012). Although less studied, the use of fiber to promote gastrointestinal health is relevant to the laying hen industry, as antimicrobials are being removed from feed and cage- free housing environments may result in a higher exposure to pathogens.

### **1.4.1 Physiology and function**

Organ physiology and function can be manipulated using nutrition with many of the strategies discussed previously. These strategies, including processing and the inclusion of fiber to enhance feed structure, have potential to enhance development of the gastrointestinal tract

(GIT). Well-developed organs can also increase in functionality, contributing to other benefits such as increased feed intake, improved egg shell quality and better animal health and welfare (Rogel et al., 1987; Jørgensen et al., 1996; Moritz et al., 2005; Gracia et al., 2009).

#### *1.4.1.1 Gizzard*

The gizzard is an important organ in the poultry digestive system as it plays an integral part in digestion by reducing digesta particle size, participating in the chemical degradation of nutrients, and controlling the flow of feed through particle retention and neurological signaling (Svihus, 2011). The gizzard is comprised of two thick, opposing lateral muscles and two thinner, anterior and posterior muscles that allow for a rotary and crushing motion with contraction, promoting mechanical digestion (Svihus, 2011). Chemical digestion is accomplished with cooperation of the proventriculus, a digestive organ located anterior to the gizzard that secretes pepsinogen and hydrochloric acid among other digestive enzymes (Svihus, 2011). A duodenal contraction results in a contraction of the thick muscles in the gizzard, which causes a reflux of material from the gizzard back into the proventriculus. This then allows further secretions to be added, and upon relaxation of the thick gizzard muscles the proventriculus will contract and the digesta will then be allowed back into the gizzard (Svihus, 2011). The avian proventriculus has a relatively small compartmental volume, which allows for the majority of the chemical digestion to take place in the gizzard. The average retention time for a standard commercial diet with few structural components in the proventriculus/gizzard varies between 30 and 60 minutes (Amerah et al., 2007a; Svihus, 2011, 2014; Goodarzi Boroojeni et al., 2016). The gizzard selectively retains larger particles for further digestion; small particles have a shorter retention time of 30 minutes, while a high fiber diet can stimulate the gizzard to slow the retention time up to two hours (Amerah et al., 2007a; Svihus, 2011).

The gizzard is a highly versatile organ, fluctuating in size and volume in response to dietary changes. A significant enlargement of the organ has been observed upon the introduction of large structural components to the diet in the form of hulls, wood shavings and large cereal particles (Svihus, 2011). Upon the introduction of whole wheat into the diet of broiler chickens, a significant change in gizzard size occurred in birds as young as seven days old (Biggs and Parsons, 2009). A study on quails documented a size increase in the gizzard upon feeding a high fiber diet for 14 days, and a similar decrease after changing to a low fiber diet for 14 days (Starck, 1999). Many more studies support the ability of the foregut to adapt to changes in diet structure, and effects on gastrointestinal development have been shown using numerous fiber sources including whole grain, wood shavings, oat hulls and fibrous by-products such as sugarcane bagasse (Biggs and Parsons, 2009; Svihus, 2011; Jiménez-Moreno et al., 2013; Kheravii et al., 2017, 2018). Effects of the addition of structural components such as fibrous products have been shown to be dramatic, with one study finding the gizzard nearly doubling in size after this nutritional intervention (Sacranie et al., 2012).

#### *1.4.1.2 Nutrient digestion and absorption*

Two areas of focus to boost digestion include maximizing retention time and the incorporation of processing to manipulate particle size (Fritz et al., 2011). Retention time is important for the digestion of plant-based feedstuffs as it provides more access to symbiotic microorganisms able to break down plant cell walls (Fritz et al., 2011). The gizzard is critical for the control of gastroduodenal refluxes influencing the passage rate of digesta. The addition of coarse particles into the diet stimulates gastroduodenal reflux, presumably through the effects it may have on the gizzard (Hetland et al., 2003). Different factors that influence nutrient digestion and absorption include the type of cereal, method of processing, and source of fiber (Jiménez-

Moreno et al., 2009). The positive effects of fiber were greater in younger birds, and both oat hulls and soy hulls were more beneficial than a control, while a rice base performed better than corn (Jiménez-Moreno et al., 2009). Meanwhile, oat hulls improved nutrient retention compared to sugar beet pulp (Jiménez-Moreno et al., 2009).

Pancreatic enzyme activity can have dramatic effects on digestion (Jiménez-Moreno et al., 2009). Research supports the retention of larger sized limestone particles of low solubility (*in vitro*; 30 to 50%) being retained in the gizzard for longer periods of time increasing *in vivo* solubility up to 94% (Wang et al., 2014). It was shown that an increased amount of calcium was solubilized from sources containing larger particles during a 25-h period (Rao and Roland, 1989). Therefore, when larger pieces of a Ca source such as limestone or oyster shell are consumed, they are retained within the gut for longer periods of time, as they do not readily pass through the gizzard. Thus increasing the solubility of Ca ions in the feed and maximizing absorption (Wang et al., 2014). In addition, longer retention times increases Ca uptake, reducing the impact of Ca need during active eggshell formation and calcification (Wang et al., 2014). Likewise, the addition of dietary fiber can amplify the retention time of feed in the GIT (Kheravii et al., 2018).

#### *1.4.1.3 Microbial activity*

In addition to a large and well-developed gizzard, the poultry GIT depends on enzyme secretions, which may increase nutrient digestibility and therefore the propagation of a beneficial gut microbiota (Amerah et al., 2007b; Svihus, 2011; Mateos et al., 2012; Xu et al., 2015a; Kheravii et al., 2018). Within the chicken digestive system, there is a mucus layer that assists in protecting the digestive organs from physical damage, chemical deterioration and bacterial infection (Kheravii et al., 2018). Goblet cells lining the intestinal mucosa are responsible for producing mucin in the gut, which is the primary glycoprotein responsible for the formation of mucus

(Kheravii et al., 2018). Mucin production is affected by the diet (Hussein et al., 2017). Inclusion of dietary fiber increases the acidity of mucin, which allows it to be more effective in resisting pathogenic bacteria, reducing incidence of disease, improving animal welfare and health (Hussein et al., 2017). In addition, insoluble fiber has been linked to the upregulation of mRNA expression of mucin 2 in the jejunum, which is important for mucin production and digestive tract health in chickens (Hussein et al., 2017; Kheravii et al., 2018).

Feed particle size and added dietary fiber play important roles in influencing gut microflora. Feed retention in upper digestive tract organs such as the proventriculus and gizzard is important for the exposure to digestive enzymes and low pH which helps kill potentially pathogenic bacteria and prevents it from reaching the hindgut (Kheravii et al., 2018). When chickens are fed fine particles, the digesta moves through the digestive system too quickly to be properly exposed to the gizzard and proventriculus, resulting in the presence of undigested material in the upper small intestine (Kiarie and Mills, 2019). This can then act as a substrate and increases the potential for pathogenic bacteria to proliferate in the lower digestive tract or hindgut, causing health problems. There was an increased presence of beneficial caecal bacteria such as *Lactobacillus* and *Bifidobacteria* spp. in the GIT, when chickens are fed particles size with a mean diameter greater than 1000  $\mu\text{m}$  (Kheravii et al., 2018). This coincided with a decrease in potentially harmful bacteria such as *Clostridium*, *Campylobacter*, *Escherichia* and *Salmonella* (Kheravii et al., 2018). Dietary fiber use predisposes the bird to a favorable environment for the generation of beneficial bacteria, acting as a substrate in the production of favourable bacteria while hindering the proliferation of potentially harmful pathogenic species. Therefore, the use of insoluble fiber in the diet can help to reduce the incidence of enteric diseases, not only through its ability to regulate gastrointestinal development and function but also due to its influence on gut microflora (Kheravii



et al., 2018). Fiber can act as a prebiotic, with its breakdown creating by-products suitable to serve as an energy source for commensal bacteria (Van Immerseel et al., 2004; Dahiya et al., 2006; Tellez et al., 2006; Kheravii et al., 2018). The addition of fiber in the diet may not only provide physiological benefits but can also contribute to maintenance energy with the production of short chain fatty acids (SCFA), a product of fiber fermentation within caeca of the bird (Kiarie et al., 2014; Leung et al., 2018). Response to fiber inclusion can depend on many factors such as the existing physiological and health status of the bird, in addition to the measure and source of dietary fiber used in the composition of the diet (Svihus et al., 2013b; van Krimpen and de Jong, 2014).

#### **1.4.2 Egg production and quality**

Without appropriate mineral concentrations, there will be a high number of defective eggshells (Mueller, 1956). Fibrous materials resulting in lower energy diets were studied as a nutritional means to improve egg quality. Results of these studies showed a reduction in body weight with the more fibrous diets however, no impact was seen on egg production or shell thickness (Mueller, 1956). The eggshell is one of the most important features of the egg, due to its role in providing a structural shape as well as ensuring that the contents of the egg are kept free from contamination (Samiullah et al., 2014). Shell defects increase the risk of microbes entering the egg, causing contamination and severely impacting egg quality (Samiullah et al., 2014). There have been efforts to reduce shell defects through genetic selection, careful environmental considerations, and improved diet formulation (Wolc et al., 2012). In a study conducted to evaluate egg quality characteristics of commercial cage- housed vs. free- range flocks from 25 to 75 weeks of age, researchers found that eggs from cage-housed birds had higher egg weight, shell weight, and shell thickness, indicating a need to improve egg quality from free-range flocks (Samiullah et

al., 2014). In addition, eggs from the free-range hens had greater microbial load than those from the cage-housed hens (Samiullah et al., 2014).

Another important consideration in egg quality is hen diet. As mentioned previously, hens require a large amount of Ca to produce eggs. In a study that tested various sources of Ca in layer hen diets, researchers found that egg quality was not significantly affected but egg weight was highest in a diet formulated with 50% fine limestone and 50% large limestone (Tunç and Cufadar, 2015). The mineral content in the hens' bone was also measured and larger dietary sources of calcium such as limestone, oyster shell, and eggshell resulted in higher mineral content in the tibia, which contains medullary bone important for calcium storage in laying hens (Tunç and Cufadar, 2015). The properties of large sources of dietary calcium are advantageous to the hens by slowing their passage through the digestive tract. Larger particles allow the hens to utilize dietary calcium at night, when egg formation predominantly occurs. The presence of dietary calcium also decreased the mobilization of calcium stores from the bone.

Fiber intake can also have an effect on mineral metabolism in hens, therefore adding another consideration for calcium uptake and interior and exterior egg quality. A study was conducted which compared two sources of insoluble fiber on two different strains of laying hens. Insoluble fiber in the form of 3% ground straw pellets in the feed and crumbled straw pellets in the litter bath was tested with Lohmann Brown (**LB**) and Lohmann Select Leghorn (**LSL**) strains (Kalmendal et al., 2013). Straw pellets in the feed caused a reduction in eggshell thickness in LB but not LSL; this could be attributed to higher consumption of crumbled straw pellets in the litter than just the 3% added straw in the feed pellets. There were no notable effects to production performance, egg quality, or feather cover, however it may be concluded that litter is an important source of insoluble fiber to laying hens when compared to fiber added to the diet alone as it more

readily allows for self-regulation of the amount of fiber ingested by the chicken (Kalmendal et al., 2013).

### **1.4.3 Hen health and welfare**

#### *1.4.3.1 Footpad dermatitis, hock burns and odour emissions (ammonia)*

An important consideration for animal welfare is litter quality as it can be associated with a number of health concerns including footpad dermatitis, hock burns and exposure to ammonia (De Jong et al., 2014; Kheravii et al., 2018). The issue has been studied more extensively in broilers as damp litter has been known to compromise FCR however, due to the increased demand for cage-free eggs, there is a need to address litter conditions in the layer industry as well (De Jong et al., 2014). Therefore, controlling litter moisture through proper husbandry and nutrition, may advance animal welfare, increase production, and reduce the environmental impact of poultry production (Kheravii et al., 2018). Introduction of a more structural feed is one of the nutritional strategies that can be used to manipulate litter quality. When the effects of fine versus coarsely ground corn in the diets of broiler chickens were examined, the addition of coarsely ground corn significantly lowered litter moisture, N, and ammonia concentrations (Xu et al., 2017). It is unclear whether the addition of coarser material alone can influence litter quality and therefore animal welfare, therefore warranting further study into the matter (Carré et al., 2002; Xu et al., 2017; Kheravii et al., 2018).

#### *1.4.3.2 Feather pecking*

Not only is selection thought to influence production parameters such as onset of lay, as well as number and quality of eggs produced, but it is also thought to influence behaviour traits as well, with increased incidence of pecking behaviour noted to be a correlated trait with the selection for increased egg production and early maturation (Buitenhuis and Kjaer, 2008). Therefore, it is

not surprising that incidences of feather pecking have increased in modern strains of laying hens (Giersberg et al., 2020). Feather pecking is a notable issue among laying hens with many hypotheses regarding the cause, including nutrient deficiencies, lack of stimulation, or boredom (Buitenhuis and Kjaer, 2008).

Varying results have been reported regarding the use of insoluble fiber as a nutrition strategy to reduce feather pecking in laying hens, however most report additional fiber to be beneficial (Rodenburg et al., 2013). Some studies reported that feeding insoluble fiber reduced the incidence of feather pecking, controlled cannibalism, and resulted in healthier plumage however, the reasoning for this is not well understood (Bears et al., 1940; Rodenburg et al., 2013; Giersberg et al., 2020). Feather pecking has been positively associated with feather eating in laying hens, indicating a dietary deficiency (Harlander-Matauschek and Feise, 2009). Feather eating can decrease digesta passage rate, similar to insoluble fiber (Harlander-Matauschek and Feise, 2009). Overall, the feeding of insoluble fiber seems to have a beneficial effect on welfare in terms of feather pecking with most authors reporting positive effects, supporting the hypothesis that one cause of feather pecking may be the result of a lack of fiber or coarse material in the diet and results as the hen is attempting to access this material (Harlander-Matauschek and Feise, 2009; Rodenburg et al., 2013).

#### *1.4.3.3 Bone health*

As previously discussed, laying hens are especially susceptible to osteoporosis, bone fractures and structural bone loss due to the high demand on their calcium deposits (Whitehead and Fleming, 2000). When a bone is fractured and subsequently healed it can form a deformity (Fleming et al., 2004). Deformities are indicative of trauma during the life of the hen where fractures may have occurred during transport etc. (Fleming et al., 2004). Keel deformities are

more common in laying hens than in pullets (Fleming et al., 2004), indicating that bone mass deteriorates with age (Fleming et al., 2004). Bone fractures are a serious welfare concern (Whitehead and Fleming, 2000). Another consideration in some laying hen strains is insufficient breast muscle to support the keel, leaving it more susceptible to fracture or deformity (Harlander-Matauschek et al., 2015). Keel bone fractures are also linked to lower egg production and increased mortality (Stratmann et al., 2016). Genetic selection can alleviate fractures and deformities in keel structure in hens, however nutrition to increase bone mineralization may be able to control this welfare concern as well (Harlander-Matauschek et al., 2015; Stratmann et al., 2016). Manipulating feed structure improves nutrients digestion and absorption, however, there is limited research whether feed structure affects bone health in laying hens.

### ***1.5 Summary and gaps in knowledge***

Advances in genetics, housing and management have produced modern hens with greater productivity than ever before. Specifically, the combined effects of increased egg mass and production (number and size), reduced body weight, lower feed intake, and lower maintenance requirements have improved feed utilization efficiency in modern layers. The diets have also evolved such that the composition, ingredients and processing have been refined to improve intake. However, the egg industry is in transition due to restriction on use of antimicrobial growth promoters and changes in housing systems from conventional cages to alternative systems including enriched cages and cage-free systems. A feature of alternative housing is access to litter and/or scratching materials, most of which are rich in insoluble fiber. These materials influence many physiological attributes and processes, with implications on productivity and gastrointestinal health and function in poultry. However, most of the published data focuses on broiler chickens and it is not fully understood how ingestion of insoluble fiber affects egg production, egg quality,

and indices of gut health in modern laying hens. Characterizing the impact of ingestion of insoluble material on egg production, egg quality, and nutrient utilization is therefore timely as the industry transitions to alternative housing systems. Moreover, responses of ingesting insoluble fiber may differ between modern strains and unselected heritage birds.

## **Chapter 2: Hypothesis and objectives**

### ***Hypothesis***

Ingestion of insoluble fiber in the form of ground or unground oat hulls will stimulate gastrointestinal physiology in modern and heritage strains without negative consequences on egg production or quality.

### ***Overall objective***

Compare impact of feeding ground or unground oat hulls to Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorn on gastrointestinal physiology and egg production.

### ***Specific objectives***

1. Comparative effects of feeding ground or unground oat hulls on feed intake, egg production, eggshell quality and apparent retention of nutrients in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorn hens
2. Comparative effects of feeding ground or unground oat hulls on visceral organ weights and indices of gastrointestinal ecology in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorn hens

## **Chapter 3: Comparative effects of feeding ground or unground oat hulls on feed intake, egg production, eggshell quality and apparent retention of nutrients in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorn hens**

### ***3.1 Abstract***

The impact of feeding ground (**GOH**) or unground (**UGOH**) oat hulls on feed intake, production, eggshell quality and apparent retention (**AR**) of nutrients was investigated in 57-wk old Lohmann Select Leghorn-Lite (**LSL-L**) and 44-wk old Shaver Heritage White Leghorn (**SHWL**) hens. Diets included: a control, or control with either GOH or UGOH at 80/20 kg control/kg oat hull as fed. All diets had TiO<sub>2</sub> as an indigestible marker. A total of 288 birds (144/breed) were placed within breed in cages (6 hens/cage, n=8) and fed for 28 days. Hen day egg production (**HDEP**) and feed intake were recorded. Egg quality (egg weight, **EW**, eggshell thickness, **EST** and breaking strength, **EBS**) was determined on eggs laid on days 26-28 and excreta samples were collected on days 26-28 for AR. There was an interaction ( $P<0.01$ ) between diet and breed on HDEP in weeks 1-2 such that the feeding of oat hulls reduced HDEP in LSL-L but not in SHWL. There was no ( $P>0.05$ ) effect of diet on HDEP in weeks 3-4. Egg weight was higher ( $P<0.01$ ) for LSL-L compared with SHWL. An interaction between diet and breed ( $P<0.01$ ) was seen on EST and EBS; EST was lower in LSL-L birds fed GOH and in SHWL birds fed UGOH relative to respective control-fed birds. Generally, LSL-L had higher ( $P<0.01$ ) EST and EBS than SHWL birds. Birds fed oat hulls had lower ( $P<0.01$ ) apparent metabolizable energy (**AME**) relative to the control and birds fed UGOH had higher AME (2,962 vs. 2,758 kcal/kg DM,  $P <0.01$ ) than birds fed GOH. In summary, the selected line had better performance than the heritage line. By week 3 there was no effect of diet on HDEP due to oat hulls, however poor eggshell quality and nutrient utilization was evident. Feeding UGOH improved eggshell and AME



relative to GOH but was inferior when compared to the control diet.

### ***3.2 Introduction***

The introduction of structural components into the feed is well known to influence the physiology of the gut and can yield many benefits to the modern laying hen such as improvements to the digestion and absorption of nutrients, potentially resulting in improved egg quality (Hetland et al., 2005). A common practice to manipulate feed structure is the addition of insoluble fiber. Other practices used to manipulate feed structure include processing of feed ingredients to influence macrostructure through practices such as steam pelleting (Hetland et al., 2005; Guo and Kim, 2012; Kiarie and Mills, 2019). The inclusion of fiber in the diet of broiler chickens aids the absorption of nutrients (Barekatin et al., 2017), with most studies demonstrating that the addition of moderate amounts of fiber improved nutrient utilization (Amerah et al., 2007b; Mateos et al., 2012; Barekatin et al., 2017).

Concerns regarding the addition of insoluble fiber to the diet include a reduction in feed intake due to the bulking effect of fiber (Jiménez-Moreno et al., 2009; Mateos et al., 2012). This can be a major concern to producers as feed intake can affect feed efficiency, a major factor in reducing the cost of poultry production (De Verdal et al., 2011). However, the addition of insoluble fiber can have positive effects. Many of these positive effects may be attributed to the effect fiber has on the upper gastrointestinal tract of the bird and the resulting impact this has on digestion (Mateos et al., 2012). These effects may include reducing gizzard pH and increasing the action of pancreatic enzymes, both of which may be attributed to a reduction in passage rate (Mateos et al., 2012). For example, addition of 3% oat hulls or soy hulls improved nutrient retention in broilers (Jiménez-Moreno et al., 2009). It has been found that the inclusion of oat hulls consistently improves nutrient digestion in broilers (González-Alvarado et al., 2010). Despite this, evidence of

the effect of added fiber on laying hens is lacking and breed specific effects and influence of selection in modern breeds remains unclear, warranting further study. The objective of this chapter was to compare effects of feeding ground or unground oat hulls on feed intake, egg production, eggshell quality and apparent retention of nutrients in LSL-L and SHWL. We hypothesized that feeding oat hulls would increase feed intake and improve apparent retention of nutrients and eggshell quality as a result of the insoluble fiber present. We also predicted this effect to be more dramatic in birds fed UGOH when compared to birds fed GOH. In addition, it was expected that LSL-L would respond differently to the addition of insoluble fiber due to selection.

### ***3.3 Materials and methods***

The use of animals was approved by the University of Guelph's Animal Care Committee (AUP#3634). Birds were cared for according to guidelines provided by the Canadian Council on Animal Care (CCAC, 2009).

#### **3.3.1 Birds and husbandry**

Two strains of laying hens were procured for comparison between a modern and heritage breed, including 144 SHWL and 144 LSL-L aged 44 and 57 weeks, respectively. Birds were housed in conventional style, battery cage housing at the Arkell Poultry Research Station meeting guidelines provided by the National Farm Animal Care Council (NFACC, 2017). Room temperature was controlled throughout the experiment and remained at 20°C with birds exposed to 14 hours of light per day at 60% intensity (20 lux). A total of 48 cages (24" x 26") were used to house all 288 birds with 6 hens/cage, placed by breed. A two-tier conventional housing system was used with rows of 12 cages in length. Each housing unit contained two water nipples as well as a feed trough 24" in length.

### **3.3.2 Pre-experiment period**

To acclimatize birds, a one-week pre-experimental period was carried out in which all birds were fed on an *ad libitum* basis, a standard commercial layer-breeder mash diet provided by Floradale Feed Mill Limited (Table 3.1). During this period, production data was collected for the basis of experimental diet allocation. Daily egg production (number of eggs present/cage) was recorded and averaged over the entirety of the pre-experimental period with experimental diets assigned equally among groups of birds varying in production within breed to eliminate bias. All birds not assigned to an experimental unit were necropsied by manual cervical dislocation for organ baseline data reported in Chapter 4, resulting in 5 hens/cage starting the experimental period.

### **3.3.3 Experimental procedures**

At the conclusion of the pre-experimental period, birds were assigned one of three diets consisting of either control (standard layer-breeder mash provided by Floradale Feed Mill Limited) or a mixture of control and unprocessed, UGOH or processed, GOH at a ratio of 80/20 kg control diet/kg oat hull, as fed. Ground oat hulls were processed twice to ensure adequate grinding via a hammer mill at the Arkell Feed Mill located at the Arkell Research Station. TiO<sub>2</sub> was used as an indigestible marker and was added to all diets at a level of 0.5%. Diets were assigned to eight replicate cages of either SHWL or LSL-L hens outlining a 2 x 3 factorial experiment. Birds were hand fed experimental diets once per day, with *ad libitum* access to the feed for a period of 28 days, while also having free access to water.

### **3.3.4 Measurements and sampling**

Feed intake was measured weekly and egg production by cage was recorded daily. Eggs were visually examined by barn staff for the presence of any cracks, dirt, feces or foreign material.

Eggs were considered dirty if more than 1/16 of the total shell surface was covered with dirt, feces or foreign material or more than 1/32 if in a localized area (USDA, 2000). All eggs laid on days 26, 27 and 28 were individually labeled by cage and immediately analyzed for egg weight and eggshell quality characteristics (thickness and breaking strength). Excreta samples were taken on days 25, 26, 27 and 28 for measurement of apparent retention of nutrients and excreta dry matter (reported in Chapter 4). Briefly, plastic mats were placed under the cages at 08:00 on collection days, the mats were subsequently removed after a period of 6 hours and excreta free from feed and feathers was collected into plastic bags and stored frozen at -20°C until required for analyses. At the end of the experiment all birds were necropsied for measurements reported in Chapter 4.

### **3.3.5 Sample processing and laboratory analyses**

Eggs collected on days 26, 27, and 28 were analyzed for individual egg weight, as well as shell thickness and strength according to (Mwaniki et al., 2018). After determining individual egg weight, eggshell thickness was determined in mm using an eggshell thickness gauge (ESTG-1, ORKA Food Technology Ltd., West Bountiful, Utah, USA). Briefly, a small drop of ultrasound gel was placed in the center the egg and distributed using a cotton swab. Next the egg was placed gel side down in the cradle of the unit, with thickness then being displayed in mm and recorded. For determination of eggshell strength, a Force reader was used to give readings in kg force (**kgf**) required to crack eggshell (ORKA Food Technology Ltd., West Bountiful, Utah, USA). Briefly, each egg was placed vertically, blunt side up, in the cradle of the machine. Once turned on, the machine applied mechanical force to the egg, determining the amount of force required to break the egg. This measurement was then displayed on the unit and was recorded before disposal of the egg.

To prepare excreta samples for analyses, samples were defrosted for a period of 72 hrs at room temperature. Once thawed, the excreta samples were pooled by cage, weighed and air dried at 60°C for 24 hrs. Dried excreta were weighed and ground into a fine powder using a coffee grinder and used for analyses of dry matter (**DM**), ash, titanium, calcium (**Ca**), gross energy (**GE**), nitrogen (**N**), and neutral detergent fiber (**NDF**) according to (AOAC, 2005). Experimental diets and oat hulls were ground and analyzed for the same variables as the dried excreta, plus crude fat (**CF**). All sample analyses were performed in duplicate. Dry matter was determined in a forced air oven set at 100°C for a period of 24 hrs. Next, the sample was placed in a muffle furnace for 12 hrs and heated to 500°C for ash determination. Samples were wet-acid digested with nitric and perchloric acid mixture (AOAC, 2005; method 990.08) and Ca concentrations read on an inductively coupled plasma mass spectrometer (Varian Inc, Palo Alto, CA, USA). The N content was determined using the Kjeldahl method, first by digesting in a heating block for a period of 1 hr and subsequently titrated using the Kjeltac™ 8200 (Foss, Hilleroed, Denmark). Then, crude protein (**CP**) content was calculated from N values by multiplying by a conversion factor of 6.25. Gross energy was determined at the University of Manitoba using an adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) using benzoic acid as the calibration standard. NDF contents were determined using methods described by (Van Soest et al., 1991) using an Ankom 200 Fiber Analyzer (Ankom Technology, Fairport, NY) (1991). A UV spectrophotometer was used to measure titanium content according to the method described by (Myers et al., 2004). Crude fat percentage was determined using the ANKOM XT 20 Extractor (Ankom Technology, Fairport, NY). Feed samples and with oat hulls were subjected to feed particle analyses (mean and standard deviation) as described by (Baker and Herrman, 2002).

### 3.3.6 Calculations and statistical analysis

Every two weeks, hen day egg production (HDEP, %) was calculated as total number of eggs laid per cage divided by the number of hens per cage. Intake of gross energy and Ca was calculated based on calculated feed intake and analyzed dietary concentrations. Egg weight (g/bird) was calculated as total egg weight per cage divided by the number of hens housed per cage. The egg mass was calculated as HDEP multiplied by egg weight. Every two weeks, feed intake per cage was divided by fourteen and the number of hens per cage to calculate daily feed intake per day per bird (g/bird). Feed conversion ratio (**FCR**) was calculated by dividing feed intake by egg mass corresponding to days 26-28. The apparent retention of components was calculated according to (Kiarie et al., 2014) using the following equation:

$$\begin{aligned} & \text{retention, \%} \\ & = \left[ \frac{(\text{feed intake} \times \text{component}_{\text{diet}}) - (\text{excreta output} \times \text{component}_{\text{excreta}})}{(\text{feed intake} \times \text{component}_{\text{diet}})} \right] \times 100. \end{aligned}$$

Statistical analyses were performed using JMP®, V13.1 (SAS Institute Inc., Cary, NC). Data was subjected to a two-way ANOVA with fixed effects of diet, breed, and the interaction between diet and breed with mean differences subsequently determined using Student's t test for main effects.

### ***3.4 Results and discussion***

The analyzed experimental diet composition is shown in Table 3.2. Due to the dilution of the control diet with fiber, there were reductions in many of the major nutrients analyzed including CP, Ca, and CF, as oat hulls did not represent a significant source of these nutrients. However, GE only varied slightly between diets. Still, the addition of oat hulls increased fiber (NDF) by more than 2-fold (Table 3.2). Mean particle size and SD of the control, GOH, and UGOH feeds were  $267\pm 11.7$ ,  $277\pm 6.1$  and  $276\pm 8.0\mu\text{m}$ , respectively (Table 3.2). Particle size distribution graphs can be found in Figure 3.1.

The data for feed intake is shown in Table 3.3. There was an interaction ( $P<0.01$ ) between breed and diet on feed intake in the first two weeks of the experiment, with both GOH-fed LSL-L and UGOH-fed LSL-L birds having lower feed intake than control fed LSL-L birds; however, the opposite was observed in SHWL birds. Among the dietary factors, dietary energy concentration is the biggest influencer of feed intake as birds will consume feed to satisfy their energy requirements (NRC, 1994; Robinson and Kiarie, 2019). Given the analyzed gross energy content in the diets (Table 3.2), it appears this phenomenon applied for SHWL birds but not the LSL-L birds. To increase intake, much of the feed offered to modern poultry breeds is refined (Svihus, 2011), which may explain why the modern strain was unable to adapt as readily to the dietary dilution with fiber as indicated by their significant reduction in feed intake, compared to LSL-L fed control and SHWL hens during the first two weeks of the experiment. This lower feed intake in the LSL-L birds fed the GOH and UGOH diets was accompanied by lower egg production during the first two weeks, an effect not observed in the SHWL birds. Over the past several decades, laying hen diets increasingly lacked structural feed components (Svihus, 2011), perhaps limiting hens' physiological ability to adapt the foregut to fiber compared to heritage lines, who may have been

more exposed to structural dietary components (Svihus, 2011). However, this effect was confined to the first two weeks as there were no effects ( $P>0.05$ ) on feed and energy intake in either breed during the later weeks of the experiment (weeks 3-4), indicating that, once initially introduced into the diet of the laying hen, the addition of oat hull whether ground or unground, does not influence feed intake. The lack of treatment effects on feed intake in weeks 3 and 4 may indicate alterations in digestibility and nutrient absorption in this experiment (Wen et al., 2015). This may be relevant when hens have access to litter, as is becoming more prevalent with the recent increase in free-run systems within the laying hen industry. Overall, the breed effect was such that SHWL birds ate more feed than LSL-L, perhaps indicative of larger gut capacity.

There was an interaction ( $P<0.01$ ) between diet and breed on HDEP in weeks 1 and 2 such that the oat hulls reduced HDEP in LSL-L but not in SHWL (Table 3.3). The SHWL birds increased feed intake immediately when fed a high fiber diet, which may explain why HDEP was not affected. Because LSL-L had higher HDEP than SHWL, their nutrient demand is higher, resulting in a higher demand for nutrients to sustain production (NRC, 1994). The SHWL birds were able to meet their nutrient demand to maintain production. There was no ( $P>0.05$ ) diet by breed interaction on HDEP in weeks 3 and 4, with the main diet effect of hens fed GOH showing a lower HDEP ( $P<0.01$ ) compared to hens fed other diets. This may indicate an effect of processing and macrostructure on the ability of the foregut to adapt quickly to changes in feed structure (Hetland et al., 2003). In addition, there was no diet effect on feed intake during weeks 3 and 4, indicating the bird's ability to acclimatize to a high fiber diet. The reduction in HDEP in GOH birds may have resulted as the gut acclimated to the additional fiber. However, our results show that once acclimated, the addition of insoluble fiber in the form of oat hull does not influence



production. Nevertheless, this emphasizes the adaptability of the heritage breed in comparison to the modern hen.

Another value for HDEP was calculated using eggs collected on days 26 to 28 and is shown in Table 3.4 in addition to feed intake, energy and Ca intake, egg weight and mass, and FCR. In general, there was no ( $P>0.05$ ) interaction between breed and diet on these parameters. HDEP, egg weight and mass, and FCR were higher ( $P<0.01$ ) for LSL-L compared to SHWL (Table 3.4). This was expected as LSL-L have been selected for these traits (Anderson et al., 2013). The SHWL birds produced smaller eggs, which may have lessened the nutrient demand of the hen making it easier to adapt to the change in diet. Although there was no effect of diet on feed intake, a diet effect ( $P<0.001$ ) was noted for Ca intake; birds fed GOH consumed less Ca intake than either control- or UGOH-fed birds. This was somewhat surprising and could be attributed to inaccuracies in Ca analyses resulting in lower assayed Ca in GOH diet. An interaction between diet and breed ( $P<0.01$ ) was observed for EST and EBS (Table 3.4). EST was lower in LSL-L birds fed GOH and in SHWL birds fed UGOH relative to respective control-fed birds. EBS was highest in LSL-L hens fed control compared to GOH or UGOH, or SHWL fed any diet. This may not necessarily indicate that fiber had negative effect on eggshell quality but may be due to lower dietary Ca concentration in oat hulls diets. However, independent of diets, LSL-L had higher ( $P<0.01$ ) EST and EBS than SHWL birds perhaps an indication that modern birds have been selected to sustain superior egg quality.

There was no ( $P>0.05$ ) interaction between breed and diet on AR of nutrients and energy (Table 3.5), suggesting that response of fiber addition was independent of genetic background. The diet effects on AR of DM was such that control-fed birds had higher ( $P<0.001$ ) AR of DM

than birds fed oat hulls; however, birds fed GOH had lower ( $P<0.001$ ) AR of DM than birds fed UGOH. Partitioning DM into ash and organic matter (**OM**), indicated that birds fed UGOH had higher ( $P=0.028$ ) AR of ash than either control- or GOH-fed birds, whilst birds fed control had higher ( $P<0.001$ ) AR of OM than birds fed oat hulls. With respect to AR of ash, there was no ( $P=0.178$ ) effect of diet on AR of Ca, which suggests that the observed higher retention of ash was not reflective of increased Ca retention due to UGOH. Addition of oat hulls reduced dietary Ca concentration by 14 and 4.4% for GOH and UGOH diets respectively relative to the control. If retention of Ca for oat hull fed birds had been higher it might have been argued birds fed GOH and UGOH increased efficiency of Ca utilization when faced with dietary deficiency as has been reported in hens when fed diets lower in Ca concentration (Khanal et al., 2019). However, poor eggshell characteristics in hens fed oat hulls (Table 3.4) indicated that Ca intake was still insufficient in the OH diets. Better (+11%,  $P=0.009$ ) AR of Ca was observed for LSL-L hens relative to SHWL, which suggests genetic advances have resulted in hens being more efficient in digesting Ca and corresponded with improved indices (EST and EBS) of eggshell quality.

Birds fed control and UGOH diets had higher ( $P=0.0005$ ) retention of nitrogen than birds fed the GOH diet. Similar nitrogen retention of birds fed UGOH to the control suggests that the addition of structural materials improved crude protein and amino acid utilization, as has been demonstrated in broilers (Xu et al., 2015a, 2017). It has been suggested that the addition of diet structure either through particle size manipulation or insoluble fiber results in a more developed gizzard and subsequently increased nutrient utilization, particularly for crude protein/amino acids (Hetland et al., 2003, 2005; Svihus, 2011; Barekatin et al., 2017). When the diet contains structural components, digestive function improves through increased retention time, lower pH, and better grinding, all mechanisms thought to improve nutrient utilization (Svihus et al., 2013a;

Kheravii et al., 2018). Nir et al., (1995) reported that a greater coarseness of feed increased the relative gizzard weight, whereas Amerah et al., (2007b) suggested gizzard stimulation was due to the length of time that the coarse particles resided in it. In the present study, improved AR of nitrogen in birds fed UGOH diet is relevant from an efficiency point of view as this diet had 18% lower crude protein than the control diet (Table 3.2). Reduced crude protein and amino acids utilization is known to reduce egg size (Veens et al., 2009; Robinson and Kiarie, 2019). In laying hens, poor crude protein (amino acids) availability (digestibility) is linked with reduced egg weight (Leeson and Summers, 2005). In this context, it is hard to pinpoint why birds fed GOH had lower nitrogen retention because this was not reflected in lower egg mass. Analysis of body composition may be able to provide more clarity into this concept and should be considered in further studies. Moreover, as shown in Table 3.2, the particle size of GOH diet was comparable with the UGOH diet, however due to the variation in SD, particle size may still play a role.

Oat hulls reduced ( $P < 0.001$ ) AR of gross energy relative to the control; specifically, birds fed GOH and UGOH had 15.2 and 10.1%, respectively lower AR of gross energy than control fed birds. Consequently, birds fed oat hulls had lower ( $P < 0.01$ ) AME relative to the control, however, birds fed UGOH had higher AME (2,962 vs. 2,758 kcal/kg DM,  $P < 0.01$ ) than birds fed GOH. Birds fed UGOH had similar AR of NDF and nitrogen to the control. The data suggests that the lower AR of gross energy in birds fed UGOH relative to control was due to fat and starch retention. However, fat and starch retention were not determined in the present study. The differences between GOH and UGOH on AR of gross energy and NDF, might indicate that grinding insoluble fiber has negative effects on nutrient digestibility. The effects of structural material passage in the gizzard of 68-week old laying hens was investigated by incorporating ground and unground oat hulls in commercial layer diet (Hetland et al., 2005). The diets with oat hulls were formulated by

mixing the control diet with either fine or coarse oat hulls at a ratio 1:10. The authors observed that 50% of the ingested oat hulls from the coarse oat hull diet and 90% of the ingested oat hulls from fine oat hull diet had passed the gizzard after 2 hours post-feeding. However, no oat hulls were found in birds fed fine oat hulls diet after 48 h, whereas as much as 30% of the ingested oat hulls were found in the gizzard of birds fed the coarse oat hull diet (Hetland et al., 2005). Increased gizzard retention in birds fed UGOH may have resulted in increased grinding and exposure to digestive fluids, causing higher nutrient retention. As the current study used oat hull diets with comparable particle sizes, it is plausible that the holding of food in the gizzard depends on its insoluble fiber contents (Hetland et al., 2005). It is also plausible a different mechanism might have been at play. Increased digesta passage rate in poultry is often associated with fiber solubility (Kiarie et al., 2017). Although fiber solubility was not measured in the present study, it has been shown that processing feed ingredients containing high insoluble fiber increased fiber solubility (de Vries et al., 2012). In this context, increased gizzard passage rate in birds fed GOH and perhaps increased digesta viscosity might have reduced nutrients digestibility relative to birds fed UGOH diets. Therefore, to exercise the greatest effect of insoluble fiber in the form of oat hulls further processing is not required.

**Table 3.1** Composition of control diet, on an as fed basis

Item	Amount, %
Corn	55.5
Soybean meal, 47%	16.6
Limestone	9.5
Pork meal	7.0
Wheat shorts	5.1
Corn gluten, 60%	2.5
Tallow	2.5
Monocalcium phosphate	0.55
Salt	0.3
Vitamin premix <sup>1</sup>	0.3
Alimet liquid, 88	0.1
Choline chloride	0.05
Calculated provisions	
AME, kcal/kg	2,866.30
Crude protein, %	17.85
SID Lys, %	0.76
SID Met, %	0.34
TSAA, %	0.58
Thr, %	0.54
Try, %	0.18
Ca, %	4.22
Av. P, %	0.44
Na, %	0.18
Cl, %	0.27

<sup>1</sup>Vitamin mineral premix provided per kilogram of diet: vitamin A, 15 KIU; vitamin D3, 4.5 KIU; vitamin E, 60 IU; vitamin B12, 37.5 mcg; biotin, 225 mcg; menadione, 375 mg; thiamine, 3.75 mg; riboflavin, 14.25 mg; pantothenic acid, 24 mg; pyridoxine, 6.75 mg; niacin, 75 mg; folic acid, 3.75 mg; Fe, 210 mg; Mn, 120 mg; Zn, 118 mg; Cu, 12.7 mg and I, 1.50 mg.

The control was mashed through the hammer mill before mixing with oat hulls and titanium.

**Table 3.2** Analyzed chemical composition of oat hulls and experimental diets, % as fed basis

Item	Oat hulls	Experimental diets		
		Control	GOH	UGOH
Dry matter, %	90.64	92.39	91.85	91.37
Gross energy, kcal/kg	3,991	3,683	3,622	3,655
Crude protein, %	5.49	18.42	15.49	15.04
Crude fat, %	1.68	5.47	4.45	4.49
Neutral detergent fiber, %	75.8	8.80	18.72	18.47
Calcium, %	0.10	4.12	3.53	3.94
Mean particle size, $\mu\text{m}$	293.0	267.0	276.6	276.1
Particle size SD, $\mu\text{m}$	11.67	10.69	6.07	7.80

GOH, ground oat hulls

UGOH, unground oat hulls

**Table 3.3** Hen day egg production (HDEP, %) and feed intake in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorns fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days

Breed	Diet	Feed intake, g/bird/day			Hen day egg production, (HDEP, %)		
		Week 1-2	Week 3-4	Week 1-4	Week 1-2	Week 3-4	Week 1-4
LSL-L	Control	117.2 <sub>b</sub>	111.0	114.1 <sub>bc</sub>	94.1 <sub>a</sub>	90.4	92.2 <sub>a</sub>
LSL-L	GOH	99.0 <sub>c</sub>	110.3	104.7 <sub>c</sub>	73.0 <sub>b</sub>	83.9	78.5 <sub>c</sub>
LSL-L	UGOH	100.2 <sub>c</sub>	110.7	105.4 <sub>c</sub>	77.1 <sub>b</sub>	85.5	81.3 <sub>b</sub>
SHWL	Control	118.2 <sub>b</sub>	109.8	114.0 <sub>bc</sub>	57.0 <sub>c</sub>	69.8	63.4 <sub>cd</sub>
SHWL	GOH	133.8 <sub>a</sub>	112.2	123.0 <sub>ab</sub>	55.7 <sub>c</sub>	60.4	58.0 <sub>d</sub>
SHWL	UGOH	140.0 <sub>a</sub>	110.9	125.5 <sub>a</sub>	57.8 <sub>c</sub>	67.9	62.9 <sub>cd</sub>
	SEM	5.185	3.114	3.565	2.295	1.848	1.501
Breed	LSL-L	105.5 <sub>b</sub>	110.7	108.0 <sub>b</sub>	81.4 <sub>a</sub>	86.6 <sub>a</sub>	84.0 <sub>a</sub>
Breed	SHWL	130.7 <sub>a</sub>	111.0	120.8 <sub>a</sub>	56.9 <sub>b</sub>	66.0 <sub>b</sub>	61.4 <sub>b</sub>
	SEM	2.994	1.798	2.058	1.325	1.067	0.867
Diet ID	Control	117.7	110.4	114.1	75.5 <sub>a</sub>	80.1 <sub>a</sub>	77.8 <sub>a</sub>
Diet ID	GOH	116.4	111.3	113.8	64.4 <sub>b</sub>	72.1 <sub>b</sub>	68.3 <sub>c</sub>
Diet ID	UGOH	120.1	110.8	115.4	67.5 <sub>b</sub>	76.7 <sub>a</sub>	72.1 <sub>b</sub>
	SEM	3.667	2.202	2.521	1.623	1.307	1.061
Probabilities							
Breed		<0.01	0.906	<0.01	<0.01	<0.01	<0.01
Diet		0.770	0.962	0.887	<0.01	<0.01	<0.01
Breed x Diet		<0.01	0.879	0.012	<0.01	0.281	<0.01

GOH, ground oat hulls

HDEP, hen day egg production

LSL-L, Lohmann Select Leghorn-Lite

SHWL, Shaver Heritage White Leghorn

UGOH, unground oat hulls

Within factor of analysis (breed, diet and interaction), means assigned different letters are significantly different, P<0.05.

**Table 3.4** Egg weight, Feed conversion rate (FCR) and eggshell characteristics in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorns fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days<sup>1</sup>

Breed	Diet	Egg			Feed and nutrients intake			FCR	Shell characteristics	
		HDEP	Weight, g	Mass, g	Feed, g/d	Energy, kcal/d	Ca, g/d		EST, mm	EBS, kgf
LSL-L	Control	85.0	63.9	54.1	103.9	382.7	4.281	2.020	0.407 <sub>a</sub>	4.24 <sub>a</sub>
LSL-L	GOH	85.8	62.9	54.2	103.1	373.4	3.639	2.028	0.395 <sub>bc</sub>	3.66 <sub>c</sub>
LSL-L	UGOH	85.8	62.9	54.1	108.5	396.6	4.257	2.131	0.403 <sub>ab</sub>	3.90 <sub>b</sub>
SHWL	Control	71.7	60.2	43.1	106.8	393.5	4.402	2.656	0.401 <sub>ab</sub>	3.35 <sub>d</sub>
SHWL	GOH	71.7	59.8	42.7	111.6	404.3	3.940	2.919	0.397 <sub>b</sub>	3.30 <sub>d</sub>
SHWL	UGOH	68.3	61.7	42.1	106.9	390.7	4.212	2.706	0.387 <sub>c</sub>	3.26 <sub>d</sub>
	SEM	3.869	0.829	2.468	2.183	8.002	0.087	0.163	0.003	0.077
Breed	LSL	85.6 <sub>a</sub>	63.2 <sub>a</sub>	54.1 <sub>a</sub>	105.2	384.2	4.059	2.060 <sub>b</sub>	0.402 <sub>a</sub>	3.93 <sub>a</sub>
Breed	SHWL	70.6 <sub>b</sub>	60.6 <sub>b</sub>	42.6 <sub>b</sub>	108.5	396.2	4.185	2.761 <sub>a</sub>	0.395 <sub>b</sub>	3.30 <sub>b</sub>
	SEM	2.234	0.479	1.425	1.260	4.620	0.050	0.094	0.002	0.044
Diet	Control	78.3	62.0	48.6	105.4	388.1	4.341 <sub>a</sub>	2.338	0.404 <sub>a</sub>	3.79 <sub>a</sub>
Diet	GOH	78.8	61.3	48.5	107.4	388.9	3.790 <sub>b</sub>	2.474	0.396 <sub>b</sub>	3.48 <sub>b</sub>
Diet	UGOH	77.1	62.3	48.1	107.7	393.6	4.234 <sub>a</sub>	2.419	0.395 <sub>b</sub>	3.58 <sub>b</sub>
	SEM	2.735	0.586	1.745	1.544	5.659	0.062	0.115	0.002	0.055
Probabilities										
Breed		<0.001	<0.001	<0.001	0.0672	0.070	0.079	<0.001	0.014	0.029
Diet		0.904	0.484	0.980	0.516	0.754	<0.001	0.705	<0.001	<0.001
Breed x Diet		0.850	0.306	0.983	0.070	0.074	0.143	0.590	<0.001	<0.001

EBS, eggshell breaking strength

EST, eggshell thickness

FCR, feed conversion rate

GOH, ground oat hulls

HDEP, hen day egg production

LSL-L, Lohmann Select Leghorn-Lite

SHWL, Shaver Heritage White Leghorn

UGOH, unground oat hulls

<sup>1</sup>Measurements taken the last three days of the experiment (d 26-28).

Within factor of analysis (breed, diet and interaction), means assigned different letters are significantly different, P<0.05.



**Table 3.5** Apparent retention of nutrients in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorns fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days

Treatment		Apparent retention, %							kcal/kg DM
		DM	Ash	OM	Ca	NDF	CP	GE	AME
Breed	Diet								
LSL-L	Control	74.4	47.3	74.1	49.8	39.5	48.7	81.7	3255.0
LSL-L	GOH	64.4	47.8	62.4	56.8	22.1	42.9	69.7	2750.2
LSL-L	UGOH	67.5	52.0	64.0	55.7	34.9	46.7	73.2	2925.8
SHWL	Control	75.6	44.6	76.2	46.1	42.2	54.1	83.1	3311.1
SHWL	GOH	63.8	42.4	62.5	47.7	28.6	45.9	70.1	2765.8
SHWL	UGOH	68.7	49.9	61.3	49.8	44.4	56.0	75.0	2998.7
	SEM	0.901	2.257	1.376	2.798	2.745	1.864	0.758	30.106
Breed	LSL-L	68.8	49.0	66.9	54.1 <sub>a</sub>	32.2 <sub>b</sub>	46.1 <sub>b</sub>	74.9	2977.0
Breed	SHWL	69.4	45.6	66.7	47.9 <sub>b</sub>	38.4 <sub>a</sub>	52.0 <sub>a</sub>	76.1	3025.2
	SEM	0.520	1.305	0.795	1.616	1.585	1.077	0.438	17.386
Diet	Control	75.0 <sub>a</sub>	45.9 <sub>b</sub>	75.2 <sub>a</sub>	47.9	40.9 <sub>a</sub>	51.4 <sub>a</sub>	82.4 <sub>a</sub>	3283.0 <sub>a</sub>
Diet	GOH	64.1 <sub>c</sub>	45.1 <sub>b</sub>	62.5 <sub>b</sub>	52.2	25.4 <sub>b</sub>	44.4 <sub>b</sub>	69.9 <sub>c</sub>	2758.0 <sub>c</sub>
Diet	UGOH	68.1 <sub>b</sub>	50.9 <sub>a</sub>	62.7 <sub>b</sub>	52.8	39.7 <sub>a</sub>	51.3 <sub>a</sub>	74.1 <sub>b</sub>	2962.3 <sub>b</sub>
Breed	Diet	0.637	1.597	0.973	1.979	1.941	1.318	0.830	21.292
Probabilities									
Breed		0.395	0.071	0.886	0.009	0.008	0.0004	0.058	0.057
Diet		<0.0001	0.028	<0.001	0.178	<0.0001	0.0005	<0.0001	<0.0001
Breed x Diet		0.510	0.748	0.222	0.629	0.460	0.235	0.624	0.618

AME, apparent metabolizable energy

CP, crude protein

DM, dry matter

FCR, feed conversion rate

GE, gross energy

GOH, ground oat hulls

HDEP, hen day egg production

LSL-L, Lohmann Select Leghorn-Lite

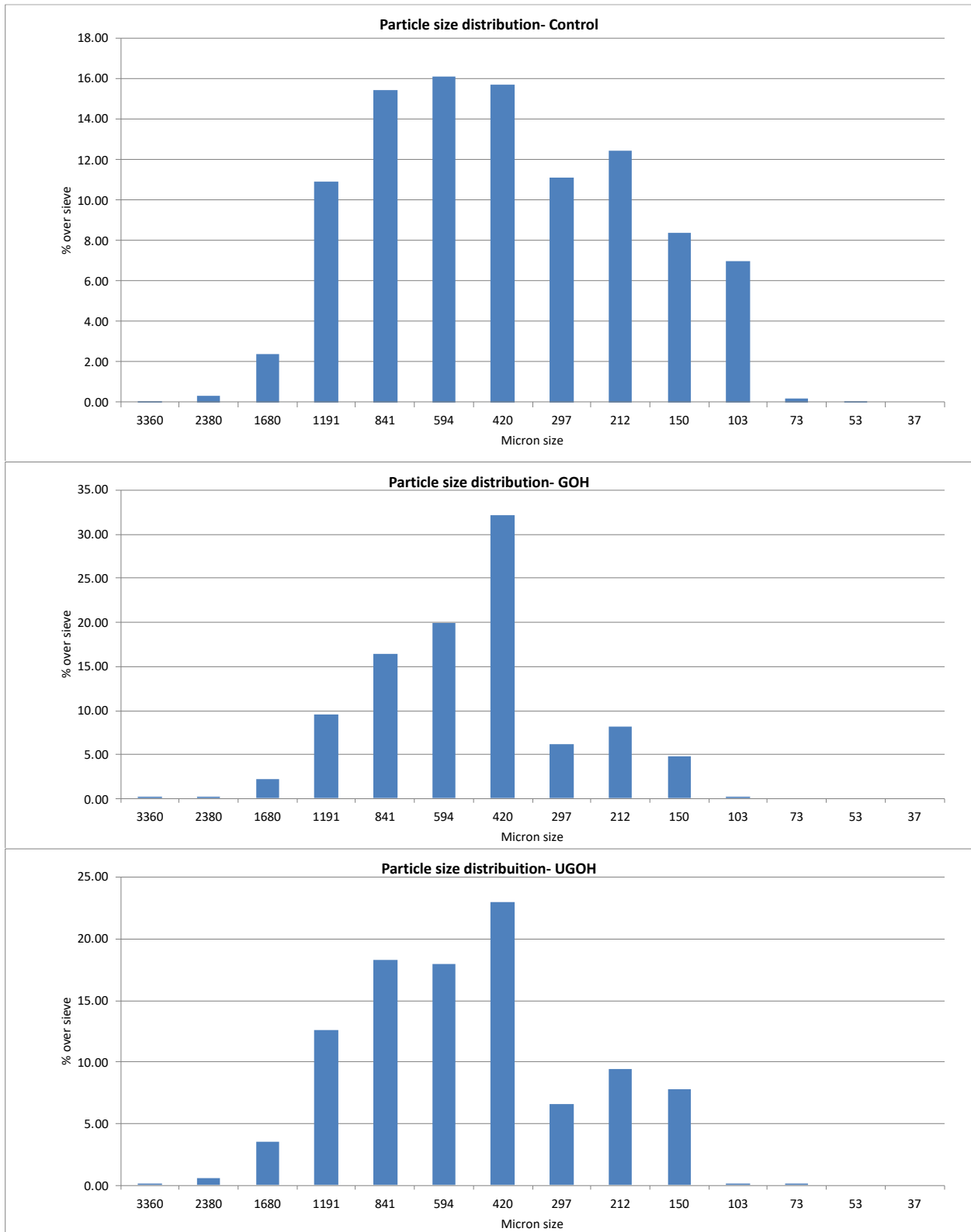
OM, organic matter

SHWL, Shaver Heritage White Leghorn

UGOH, unground oat hulls

Within factor of analysis (breed, diet and interaction), means assigned different letters are significantly different, P<0.05.

**Figure 3.1** Particle size distribution in the experimental diets



## **Chapter 4: Comparative effects of feeding ground or unground oat hulls on visceral organs weight and indices of gastrointestinal ecology in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorn hens**

### ***4.1 Abstract***

The present study evaluated effects of feeding a control diet or a control diet with ground (**GOH**) or unground (**UGOH**) oat hulls at a ratio of 80/20 kg/kg as fed, to 57-wk Lohmann Select Leghorn-Lite (**LSL-L**) and 44-wk Shaver Heritage White Leghorns (**SHWL**) on select physiological measurements. A total of 288 birds were placed by breed (6 hens/cage) and allocated to diets (n=8). One hen per cage was sacrificed immediately prior to treatment application, one week after placement in cages for baseline organ weights. Water and feed were given *ad libitum* throughout the 28-d trial. Excreta samples were collected on days 25-28 for excreta dry matter (**DM**) and on day 28, all birds were euthanized and dissected for organ weights, jejunal tissue for histomorphology and/or ceca digesta samples for short-chain fatty acid (**SCFA**). LSL-L hens had heavier proventriculus than SHWL ( $P < 0.01$ ; 3.7 vs. 3.3 g/kg live BW), and SHWL had greater gizzard weight than LSL-L ( $P < 0.01$ ; 17.0 vs. 13.4 g/kg live BW). Gizzard weight change was affected by a breed by diet interaction; in this context, LSL-L birds receiving oat hulls had the greatest ( $P < 0.01$ ) gizzard weight increase compared to the LSL-L control or SHWL fed any diet. An interaction ( $P < 0.05$ ) was found for total SCFA such that LSL-L hens fed fiber had a lower total SCFA concentration than LSL-L hens fed control, or SHWL fed any diet. Breed effects ( $P = 0.011$ ) were also seen on jejunal crypt depth (**CD**) but not on villi height (**VH**). CD was greater in SHWL than LSL-L, contributing to a lower ( $P < 0.01$ ) villous height and crypt depth ratio (**VH:CD**). In conclusion, high insoluble fiber with or without processing altered SCFA production and intestinal morphology. Furthermore, the data showed that, the modern layer can adjust the gastrointestinal tract (**GIT**) in response to fibrous feed more than predecessors.

## ***4.2 Introduction***

The addition of insoluble fiber is beneficial to the development of the gizzard (Hetland and Svihus, 2001). The use of oat hulls specifically has been shown to increase gizzard size in many studies (Svihus, 2011; Barekatain et al., 2017; Sacranie et al., 2017). Hetland et al. (2003) found the gizzard to increase in size when insoluble fiber was added to the diet. Effects seen on the gizzard and other organs may be related to increases in digesta retention when a source of insoluble fiber is present; this may also result in a production of additional secretions by the proventriculus, benefiting digestion (Duke, 1986; Hetland et al., 2005). The gizzard is highly adaptable to dietary changes and has the ability to fluctuate in both size and volume (Svihus, 2011). This is especially significant when large structural feed ingredients such as hulls, wood shavings, and large cereal particles are added into the diet. Significant enlargements of the gizzard have been observed, with gizzard size nearly doubling in some studies (Svihus, 2011; Sacranie et al., 2017). However, much of the aforementioned work was with broilers, and limited research has been reported in laying hens whose feed intake is significantly less. Moreover, there is no record of comparative responses of feeding insoluble fiber to a modern and heritage breed.

Another way the ingestion of a diet including insoluble fiber may influence the digestive tract is through effects on the small intestine and mucin production (Hussein et al., 2017; Kheravii et al., 2018). This can benefit the laying hen by modulating intestinal mucosal structure and microbial activity, which has become increasingly important due to changes in housing and antimicrobial use in the laying hen industry. Fiber fermentation in the ceca can also contribute to maintenance energy as a result of SCFA production (Kiarie et al., 2014; Leung et al., 2018). The aim of this study was to investigate the effects of feeding ground or unground oat hulls on visceral

organ weights and indices of gastrointestinal ecology in Lochmann Select Leghorn- Lite and Shaver Heritage White Leghorn hens.

### ***4.3 Materials and Methods***

#### **4.3.1 Experimental procedures and sampling**

The experimental procedures are presented in Chapter 3. 144 SHWL and 144 LSL-L aged 44 and 57 weeks, were used. Briefly, one hen per cage was weighed and sacrificed prior to commencement of experimental feeding for baseline organ weights. At the conclusion of the pre-experimental period, birds were assigned one of three diets consisting of either control (standard layer-breeder mash provided by Floradale Feed Mill Limited) or a mixture of control and unprocessed, UGOH or processed, GOH at a ratio of 80/20 kg control/kg oat hull, as fed. Excreta samples were collected on days 25-28 and subsequently frozen at -20° C for determination of excreta dry matter. At the end of the trial, two birds per cage were weighed and sacrificed via manual cervical dislocation for gastrointestinal and liver samples, with the remaining birds sacrificed to access caecal digesta for SCFA determination.

#### **4.3.2 Sample processing and laboratory analyses**

Gizzards were cut latterly using a scalpel, emptied of contents, rinsed with saline solution, patted dry with paper towel, and subsequently weighed. A similar procedure was followed for proventriculus. Small intestine was removed, flushed with saline, and weighed. All weights were recorded using a calibrated scale, accurate to 0.01g. The small intestines were further dissected for jejunum histomorphology samples, which were immediately fixed in formalin for a period of 48 hrs. Cross sections of tissue were then prepared from the fixed samples by embedding in paraffin

wax, sectioning into 5µm segments and staining with hematoxylin and eosin. Villus height and crypt depth were recorded based on an average of 5 villous-crypt structures per sample under observation of a Leica DMR microscope (Leica Microsystems, Wetzlay, Germany). The VH and CD were used to calculate the VH:CD.

Ceca digesta was collected and stored at -20°C for determination of SCFA concentration according to Leung et al. (2018). Specifically, the concentrations of SCFA that were analyzed included lactic, formic, acetic, propionic, isobutyric and n-butyric concentrations according to procedures described by Leung et al. (2018). Briefly, the digesta was thawed and approximately 0.1 g of the digesta was resuspended with 1 mL 0.005N H<sub>2</sub>SO<sub>4</sub> (1:10, wt/vol) in a microcentrifuge tube. The tube was vortexed until sample was completely dissolved. Then samples were centrifuged at 11,000 × g for 15 min. After centrifuging, 400 µL of supernatant was transferred into a high- pressure liquid chromatography (HPLC) vial and 400 µL of 0.005N H<sub>2</sub>SO<sub>4</sub> buffer was added. The resulting digesta fluid was then assayed for SCFA by using HPLC (Hewlett Packard 1100, Germany) with Rezex ROA-Organic Acid LC column, 300×7.8mm from Phenomenex and Refractive Index detector at 40°C (Agilent 1260 Infinity RID from Agilent Technologies, Germany). Twenty microliters of the resulting sample was injected into the column, with a column temperature of 60°C and mobile phase of 0.005N H<sub>2</sub>SO<sub>4</sub> buffer at 0.5mL/min isocratic for 35 min. The detector was heated to 40°C. Excreta DM content was determined by placement in an oven at 80°C for 48 h according to (Mohammadigheisar et al., 2019).

#### **4.3.3 Calculations and statistical analysis**

Organ weights (liver, proventriculus, gizzard, small intestine) were standardized by individual live bird BW. Organ change index was calculated by dividing the final (day 28) organ

weight (g/kg live BW) by baseline organ weight (g/kg live BW). Data was subjected to two-way ANOVA in JMP 13.1 with fixed effects of diet, breed and their two-way interaction. Significance was considered at  $P < 0.05$ , and trends,  $0.05 < P < 0.10$ , were discussed.

#### ***4.4 Results and discussion***

The data for organ weights is shown in Table 4.1. There was no interaction ( $P > 0.05$ ) between breed and diet for proventriculus, gizzard, small intestine or liver weights. Moreover, there was no ( $P > 0.05$ ) breed or diet effect on small intestine and liver weights. Gizzard weight was affected by both diet and breed and weight of proventriculus was affected by breed only ( $P < 0.01$ ). The LSL-L hens had heavier proventriculus than SHWL ( $P < 0.1$ ; 3.7 vs. 3.3 g/kg live BW), and SHWL had a greater gizzard weight than LSL-L ( $P < 0.01$ ; 17.0 vs. 13.4 g/kg live BW). Birds fed oat hulls had more than 1.7 times heavier ( $P < 0.01$ ) gizzards than birds fed the control diet (Table 4.1). This observation agrees with previous studies indicating that gizzard size may increase substantially when structural components are added to the diet, sometimes increasing to more than double the original size (Amerah et al., 2007b; Svihus, 2014). However, birds fed UGOH had heavier gizzards (approximately 10% heavier,  $P < 0.01$ ) than birds fed GOH. This observation indicates further processing of feed ingredients may have negative effects on gizzard development (Kiarie and Mills, 2019). However, mean particle size analyses did not reveal obvious differences between GOH and UGOH; although, the mean particle size standard deviation was higher in UGOH (8.0 vs. 6.1  $\mu\text{m}$ ) than GOH. Larger deviation of particle size in UGOH diet suggested presence of smaller/finer particles and/or larger/coarser particles, the later may help explain heavier gizzard weights in birds fed this diet. Nir et al. (1995) reported that a greater coarseness

of feed increased the relative gizzard weight, whereas Amerah et al. (2007b) suggested gizzard stimulation was due to the length of time that the coarse particles resided in it.

Upon analysis of baseline data (data not shown), the gizzard was the only organ with notable differences between modern LSL-L and heritage SHWL hens, with SHWL having a 52% heavier gizzard ( $P < 0.01$ ; 12.3 vs. 8.1 g/kg live BW). This was expected as the modern hen has been selected based on a response to a heavily refined diet (Svihus et al., 2004; Hetland et al., 2005). To further understand the impact of feeding structural materials on organ weights, organ change index was calculated based on baseline data of the cage (Table 4.1). Only gizzard exhibited a breed by diet interaction on organ change index. The LSL-L hen receiving oat hulls had the greatest ( $P < 0.01$ ) gizzard weight increase when compared to the LSL-L fed control or any of the SHWL. Specifically, the indices for control, GOH and UGOH were 1.01, 1.83 and 2.10, respectively for LSL-L and corresponding indices for SHWL were 0.91, 1.55 and 1.66, respectively. This further demonstrates that ingestion of structural material can almost double the size of the gizzard. The LSL-L hens also had higher indices for proventriculus ( $P < 0.01$ ), small intestine ( $P = 0.03$ ), and a tendency ( $P = 0.08$ ) for livers to have a higher index than SHWL, however no diet effects were seen.

There was no ( $P > 0.05$ ) breed by diet interaction or diet effect on jejunal VH, CD and their ratio (VH:CD) (Table 4.2). Although there was no breed effect on VH, a breed effect ( $P = 0.011$ ) was seen on CD, with SHWL showing deeper CD, contributing to a lower ( $P < 0.01$ ) VH:CD ratio. Other studies have reported no effects of ingestion of structural feed material on small intestine weight and jejunal histomorphology in poultry (Kiarie and Mills, 2019). Poultry do not have teeth and the gizzard has an important additional function of grinding feed material. The gizzard contains strongly myelinated muscles and has a koilin layer that aids in the grinding process



(Svihus, 2014). Therefore, because of gizzard grinding, particles reaching the small intestine have no relationship with feed particle size and /or structural properties, therefore, the impact of feed structure on small intestine and ceca physiology is minimal (Kiarie and Mills, 2019). A tendency for higher jejunal VH:CD ( $P=0.06$ ) was observed for birds fed GOH diet relative to hens fed UGOH or the control diet. The VH:CD is an indicator for digestive and absorptive capacity, and a higher ratio indicates more mature enterocytes and lower enterocytes turnover/apoptosis (Svihus, 2014; Pluske, 2016).

An interaction between breed and diet was observed for ceca digesta concentration of both acetic ( $P=0.015$ ) and total SCFA ( $P=0.04$ ; summation of lactic, acetic, propionic, isobutyric and n-butyric acids) (Table 4.3). In this context, LSL-L hens fed oat hulls had lower acetic and total SCFA concentrations than the LSL-L hens fed the control diet, or SHWL fed any diet (Table 4.3). Furthermore, a tendency ( $P=0.08$ ) for a diet effect was noted for concentration of butyric acid with birds fed oat hulls showing lower butyric acid than the control-fed birds. These observations suggested that addition of oat hulls, which are rich in insoluble fiber, reduced the amount of fermentable materials entering the ceca in LSL-L birds. In contrast, addition of oat hulls in the diet of broiler breeders increased the concentration of ceca digesta SCFA (Leung et al., 2018). The LSL-L hens had lower SCFA concentrations than SHWL (101 vs. 127  $\mu\text{mol/g}$ ,  $P<0.01$ ), with SHWL exhibiting lower propionic ( $P<0.01$ ), but higher iso-butyric ( $P<0.01$ ) and n-butyric acid concentrations than LSL-L ( $P<0.01$ ). Generally, higher concentrations of total SCFA in the ceca digesta of the SHWL indicated a larger flow of fermentable substrates. The gastrointestinal microbiome is influenced by diet composition and can be related to nutrient absorption and intestinal health (Jamroz et al., 2001; Kiarie et al., 2013). Microbial fermentation of complex polysaccharides results in metabolite production aiding in the production of maintenance energy

in the bird. In fact, in broilers about 8% of required maintenance energy can be achieved through SCFA production (Józefiak et al., 2004). An effect of diet ( $P < 0.01$ ) was seen on excreta DM such that high fiber feeds had higher DM%, with a breed effect ( $P < 0.01$ ) of higher DM% in LSL-L. This can be beneficial to the bird's health as it can reduce wet litter, affecting pathogen proliferation and disease spread. Therefore, it can be concluded that high fiber feed in addition to selection can impact health in laying hens.

**Table 4.1** Gastrointestinal tract and liver weights in Lohmann Select Leghorn-Lite and Shaver Heritage White Leghorns fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days

Breed	Diet	Organ weight, g/kg live BW				Ratio of final organ weight to baseline organ weight			
		Gizzard	Prove*	Small intestine	Liver	Gizzard	Prove*	Small intestine	Liver
LSL-L	Control	8.21	3.50	18.23	22.04	1.013 <sub>d</sub>	1.027	1.049	0.961
LSL-L	GOH	14.86	3.75	20.01	20.16	1.833 <sub>b</sub>	1.098	1.151	0.878
LSL-L	UGOH	17.05	3.84	20.40	21.17	2.103 <sub>a</sub>	1.127	1.174	0.923
SHWL	Control	11.29	3.33	19.41	21.49	0.914 <sub>d</sub>	0.969	1.077	0.900
SHWL	GOH	19.14	3.21	18.05	20.75	1.550 <sub>c</sub>	0.932	1.001	0.869
SHWL	UGOH	20.47	3.36	18.19	20.53	1.658 <sub>c</sub>	0.977	1.009	0.860
	SEM	0.473	0.138	0.907	0.693	0.046	0.040	0.051	0.030
Main									
Breed	LSL-L	13.37 <sub>b</sub>	3.70 <sub>a</sub>	19.55	21.12	1.649 <sub>a</sub>	1.084 <sub>a</sub>	1.125 <sub>a</sub>	0.921
Breed	SHWL	16.97 <sub>a</sub>	3.30 <sub>b</sub>	18.55	20.92	1.374 <sub>b</sub>	0.959 <sub>b</sub>	1.029 <sub>b</sub>	0.877
	SEM	0.273	0.080	0.524	0.400	0.027	0.023	0.029	0.017
Main									
Diet ID	Control	9.75 <sub>c</sub>	3.42	18.82	21.76	0.964 <sub>c</sub>	0.998	1.063	0.930
Diet ID	GOH	17.00 <sub>b</sub>	3.48	19.03	20.45	1.691 <sub>b</sub>	1.015	1.076	0.874
Diet ID	UGOH	18.76 <sub>a</sub>	3.60	19.30	20.85	1.881 <sub>a</sub>	1.052	1.092	0.891
	SEM	0.334	0.098	0.641	0.490	0.033	0.028	0.036	0.021
Probabilities									
	Breed	<0.01	<0.01	0.186	0.724	<0.01	0.001	0.027	0.078
	Diet	<0.01	0.398	0.871	0.165	<0.01	0.397	0.854	0.163
	Breed x Diet	0.430	0.365	0.127	0.616	<0.01	0.364	0.122	0.602

\*Proventriculus

BW, body weight

GOH, ground oat hulls

LSL-L, Lohmann Select Leghorn-Lite

SHWL, Shaver Heritage White Leghorn

UGOH, unground oat hulls

Within factor of analysis (breed, diet and interaction), means assigned different letters are significantly different, P<0.05.

**Table 4.2** Jejunal histomorphology in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorns fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days

Breed	Diet	Villi height (VH), $\mu\text{m}$	Crypt depth (CD), $\mu\text{m}$	VH:CD
LSL-L	Control	1119.1	221.7	5.08
LSL-L	GOH	1103.6	205.0	5.47
LSL-L	UGOH	1055.7	217.6	4.95
SHWL	Control	1040.2	235.9	4.28
SHWL	GOH	1116.7	231.4	4.87
SHWL	UGOH	1088.3	239.6	4.56
	SEM	56.2	9.8	0.220
Main				
Breed	LSL-L	1092.8	214.8 <sub>b</sub>	5.17 <sub>a</sub>
Breed	SHWL	1081.7	235.6 <sub>a</sub>	4.57 <sub>b</sub>
	SEM	32.5	5.7	0.127
Main				
Diet	Control	1079.6	228.8	4.68
Diet	GOH	1110.1	218.2	5.17
Diet	UGOH	1072.0	228.6	4.75
	SEM	39.78	6.94	0.156
Probabilities				
	Breed	0.810	0.011	0.001
	Diet	0.773	0.472	0.064
	Breed x Diet	0.573	0.815	0.640

CD, crypt depth

GOH, ground oat hulls

LSL-L, Lohmann Select Leghorn-Lite

SHWL, Shaver Heritage White Leghorn

UGOH, unground oat hulls

VH, villi height

VH:CD, villi height/ crypt depth ratio

Within factor of analysis (breed, diet and interaction), means assigned different letters are significantly different,  $P < 0.05$ .

**Table 4.3** Concentration of short chain fatty acids in ceca digesta and excreta dry matter (DM) in Lohmann Select Leghorn- Lite and Shaver Heritage White Leghorns fed diets containing ground (GOH) and unground (UGOH) oat hulls for 28 days

		Short chain fatty acids, $\mu\text{mol/g}$ of wet digesta						Excreta DM, %
		Lactic	Acetic	Propionic	Iso-butyric	n-butyric	Total SCFA*	
Breed	Diet							
LSL-L	Control	2.96	79.70 <sub>a</sub>	21.45	4.53	14.79	123.43 <sub>a</sub>	27.05
LSL-L	GOH	3.55	55.56 <sub>b</sub>	17.05	2.99	10.17	89.32 <sub>b</sub>	32.10
LSL-L	UGOH	3.72	55.19 <sub>b</sub>	16.06	3.32	10.91	89.20 <sub>b</sub>	31.61
SHWL	Control	5.07	78.69 <sub>a</sub>	30.10	2.46	14.52	130.84 <sub>a</sub>	26.53
SHWL	GOH	3.73	77.10 <sub>a</sub>	30.81	1.61	14.63	127.89 <sub>a</sub>	30.81
SHWL	UGOH	3.64	73.34 <sub>a</sub>	29.09	1.81	14.06	121.93 <sub>a</sub>	30.07
	SEM	0.584	3.968	1.986	0.612	1.106	6.313	0.462
Main								
Breed	LSL-L	3.41	63.49 <sub>b</sub>	18.19 <sub>b</sub>	3.61 <sub>b</sub>	11.96 <sub>b</sub>	100.65 <sub>b</sub>	30.26
Breed	SHWL	4.15	76.38 <sub>a</sub>	30.00 <sub>a</sub>	1.96 <sub>a</sub>	14.40 <sub>a</sub>	126.89 <sub>a</sub>	29.14
	SEM	0.337	2.291	1.147	0.353	0.638	3.645	0.267
Main								
Diet	Control	4.02	79.20 <sub>a</sub>	25.77	3.49	14.66	127.14 <sub>a</sub>	26.79 <sub>b</sub>
Diet	GOH	3.64	66.33 <sub>b</sub>	23.93	2.30	12.40	108.60 <sub>b</sub>	31.46 <sub>a</sub>
Diet	UGOH	3.68	64.27 <sub>b</sub>	22.58	2.56	12.48	105.57 <sub>b</sub>	30.84 <sub>a</sub>
	SEM	0.413	2.805	1.404	0.432	0.782	4.464	0.327
Probabilities								
	Breed	0.128	<0.01	<0.01	<0.01	<0.01	<0.01	<0.0001
	Diet	0.776	0.001	0.282	0.135	0.081	0.003	0.005
	Breed x Diet	0.136	0.015	0.388	0.833	0.100	0.041	0.515

\*Summation of concentration of lactic, acetic, propionic, iso-butyric and n-butyric acid DM, dry matter

GOH, ground oat hulls

LSL-L, Lohmann Select Leghorn-Lite

SCFA, short chain fatty acid

SHWL, Shaver Heritage White Leghorn

UGOH, unground oat hulls

Within factor of analysis (breed, diet and interaction), means assigned different letters are significantly different,  $P < 0.05$ .

## **Chapter 5: General discussion and conclusions**

The egg industry in Canada and much of the world is in transition due to restrictions on the use of antimicrobial growth promoters and changes in housing systems from conventional cages to alternative systems including enriched cage and cage-free systems. A feature of alternative housing is access to litter and/or scratching materials, most of which are rich in insoluble fiber. Chickens have an innate nature of voluntary consumption of non-nutritive structural materials. These materials influence many physiological attributes and processes with implication on productivity and gastrointestinal health and function in poultry. However, most of the published literature focused on broiler chickens and it is not fully understood how ingestion of insoluble fiber affects egg production, egg quality and indices of gut health in modern laying hens. Characterizing the impact of ingestion of insoluble material on egg production, egg quality and nutrient utilization is therefore timely as the industry transitions to alternative housing systems. Moreover, responses of ingesting insoluble fiber may differ between the highly selected modern laying hen and unselected heritage birds.

Research described in this thesis sought to characterize the impact of adding oat hulls, a source insoluble fiber (Knudsen, 2014), in laying hen diets. Chapter 3 investigated comparative impact of oat hulls on feed intake, egg production, egg quality, and nutrient digestibility in a modern breed, Lohmann Select Leghorn-Lite (**LSL-L**), and a heritage breed, Shaver Heritage White Leghorn (**SHWL**). Chapter 4 characterized comparative effects of diets fed in Chapter 3 on aspects of gastrointestinal physiology. Modern hens are bred for high egg production and high efficiency (Jones et al., 2001; Anderson et al., 2013) and a constant challenge for nutritionists is to design diets to maximize nutrient intake and utilization (Leeson and Summers, 2005). Although oat hulls depressed feed intake and egg production in LSL-L birds in the first two weeks, these

hens showed adaptability to fibrous feed by week 3 of exposure. This may be especially relevant in alternative housing systems. Moreover, egg production of hens fed unground oat hull (**UGOH**) was comparable to the control-fed hens in weeks 3 and 4 suggesting nutrient consumption supported adequate egg production. The poor eggshell characteristics in hens fed oat hulls implied Calcium (**Ca**) was still deficient in hens fed oat hulls. Overall, the breed effect was such that SHWL birds ate more feed than LSL-L. However, the higher feed intake in SHWL birds did not translate to increased egg production, egg quality or a better feed conversion ratio (**FCR**). Moreover, better Ca retention observed for LSL-L hens relative to SHWL suggested efficient Ca utilization and corresponded with observed improved indices of eggshell quality seen in LSL-L hens. These observations not only indicated genetic superiority of the modern breed in terms of productivity but also digestive efficiency.

It has been suggested that the addition of diet structure either through particle size manipulation or insoluble fiber in poultry results in a more developed gizzard (Hetland et al., 2003, 2005; Svihus, 2011; Barekatin et al., 2017). Similarly, independent of breed, oat hulls almost doubled gizzard weight in the present study (Chapter 4). Relative to baseline values, LSL-L birds had a greater change in gizzard size over the 28-day period of feeding oat hulls. When the diet contains structural components, the increased gizzard grinding action increases muscle mass (Svihus, 2011). Greater gizzard size has been linked to improved digestive function through increased retention time, lower pH, and better grinding (Hetland et al., 2005). These mechanisms are thought to improve nutrient utilization (Svihus et al., 2013a; Kheravii et al., 2018). Improved nitrogen retention in birds fed the UGOH diet is relevant from an efficiency point of view as this diet had 18% lower crude protein. Consequently, birds fed UGOH had comparable hen day egg production (**HDEP**) in weeks 3- 4 and egg mass to control fed hens. However, oat hulls reduced

energy utilization, indicating enhanced gizzard function did not completely compensate for the dilution of apparent metabolizable energy for birds fed the oat hull diets. The noted differences between ground and unground oat hulls suggested increased gizzard retention in birds fed UGOH might have resulted in increased grinding and exposure to digestive fluids and consequently higher nutrient retention. It is also possible birds fed the ground oat hull (**GOH**) diet experienced an increased gizzard passage rate, and perhaps increased digesta viscosity might have reduced nutrient digestibility relative to birds fed UGOH diets (Kiarie et al., 2017). LSL-L hens fed oat hulls had a lower total short chain fatty acid (**SCFA**) concentration than the LSL-L hens fed the control diet, or SHWL hens fed any diet. These observations suggest that insoluble fiber reduced the amount of fermentable materials entering the ceca in LSL-L birds. In conclusion, the data indicated that the modern hen is adaptable to ingestion of structural material rich in insoluble fiber.



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