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# The effect of high and low dietary fiber diets on the performance of two lines of chickens with divergent growth rates

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**The effect of high and low dietary fiber diets on the performance of two lines of chickens  
with divergent growth rates**

by

**Muhammed Walugembe**

A thesis submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Animal Science

Program of Study Committee:

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## TABLE OF CONTENTS

LIST OF FIGURES	iv
LIST OF TABLES	v
ACKNOWLEDGEMENTS	vi
ABSTRACT	viii
CHAPTER 1. GENERAL INTRODUCTION	1
Thesis Organization	2
CHAPTER 2. REVIEW OF LITERATURE	3
Corn Dried Distillers Grains with Solubles (DDGS)	3
Nutrient composition and value of DDGS	7
Wheat Bran	11
Nutrient composition and value of wheat bran	12
Classification of Fiber	15
Nutritional and Biological Effects of Dietary Fiber	16
Chicken Ceca Microbiology	19
Short Chain Fatty Acids in the Chicken Ceca	21
Literature Cited	23
CHAPTER 3. EFFECTS OF HIGH FIBER INGREDIENTS ON THE PERFORMANCE, METABOLIZABLE ENERGY AND FIBER DIGESTIBILITY OF BROILER AND LAYER CHICKS	37
Abstract	37
Introduction	38
Material and Methods	40
Results	43
Discussion	44
Acknowledgements	47
Literature Cited	48
Figures and Tables	52

CHAPTER 4. EFFECTS OF DIETARY FIBER ON CECAL SHORT CHAIN FATTY ACID CONCENTRATION AND MICROBIAL COMMUNITY OF BROILER AND LAYER CHICKS	57
Abstract	57
Introduction	58
Materials and Methods	60
Results	65
Discussion	66
Acknowledgements	70
Literature cited	71
Figures and Tables	76
CHAPTER 5. GENERAL CONCLUSIONS	82

**LIST OF FIGURES**

- Figure 3.1.** Effect of higher and lower dietary fiber on average daily gain in broiler and layer chicks for the two feeding periods. 55
- Figure 3.2.** Effect of higher and lower dietary fiber on feed efficiency in broiler and layer chicks for the two feeding periods. 56
- Figure 4.1.** Effect of increasing dietary fiber on acetic and total short chain fatty concentration in broiler and layer chicks at 21d of age 79
- Figure 4.2.** Pairwise plots of the 3 dimensional nonmetric multidimensional scaling (nMDS) ordination of cecal microbial communities from *MspI* blue (Mblue) dye of higher (red open triangles) and lower (black open circles) fiber diets showing the overall microbial community pattern. 80
- Figure 4.3.** Pairwise plots of the 3 dimensional nonmetric multidimensional scaling (nMDS) ordination of cecal microbial communities from *MspI* green (Mgreen) dye of broiler (black open circles) and layer (red open triangles) chick lines showing the overall microbial community pattern. 81

**LIST OF TABLES**

<b>Table 1.1.</b> Composition of wheat bran.	14
<b>Table 3.1</b> Ingredient composition and nutrient content broiler starter diets fed from 1-21d of age.	52
<b>Table 3.2.</b> Effect of high and low fiber diets in broiler and layer chicks from 1 to 12 and 1 to 21 d of age on average daily gain (ADG), average daily feed intake (ADFI) and mortality corrected feed efficiency (FE).	53
<b>Table 3.3.</b> Effect of increasing dietary fiber from DDGS and wheat bran in broiler and layer chicks fed from 1 to 21 d on nitrogen corrected Apparent Metabolizable Energy (AMEn), Ileal and apparent total tract digestibility.	54
<b>Table 4.1.</b> Ingredient composition and nutrient content of broiler starter diets fed from 1-21d of age.	76
<b>Table 4.2.</b> Effect of increasing dietary fiber on short chain fatty acid (SCFA) concentration in broiler and layer chicks at 21 d of age	77
<b>Table 4.3.</b> Two-way PermANOVA of cecal microbial communities associated with diet and line for the two restriction enzymes	78

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**ABSTRACT**

An experiment was designed to determine the effects of feeding high dietary fiber on the performance, cecal short chain fatty acid (SCFA) concentration and cecal microbial ecology of broiler and layer chicks. All diets were formulated to meet or exceed NRC (1994) standards for starter chicks with the lower fiber diet based on corn-soybean meal (SBM) and the higher fiber diet formulated using corn-SBM-dried distillers grains with solubles (DDGS) and wheat bran. The higher fiber diet contained 60.0 g/kg of both DDGS and wheat bran from 1-12 d and 80.0 g/kg of both DDGS and wheat bran from 13-21 d. The experiment utilized a completely randomized design with a 2 x 2 factorial arrangement of treatments consisting of two dietary concentrations of fiber (lower and higher fiber) and two chicken lines (broiler and layer) fed from 1 to 21 d. The Ross 308 broiler chicks and Hy-line W36 male chicks were housed in battery cages in an environmentally controlled room with *ad libitum* access to feed and water. Average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (FE) were calculated over the 1-12 and 1-21 d feeding periods. Excreta samples were collected at the end of the experiment to determine nitrogen corrected apparent metabolizable energy (AMEn) and Neutral Detergent fiber (aNDF) digestibility. Cecal samples were collected at the end of the experiment to determine SCFA concentration and microbial ecology.

The results indicate that the higher dietary fiber diet significantly reduced broiler ADG for the 1-12 d and 1-21 d periods, but had no effect on layer chick ADG resulting in a significant interaction ( $P \leq 0.01$ ). Neutral detergent fiber digestibility was higher in layer chicks in comparison to broiler chicks regardless of the diet ( $P < 0.01$ ), and higher dietary fiber concentration resulted in increased ileal ( $P < 0.01$ ) and total tract ( $P < 0.02$ ) aNDF disappearance, across both lines. Although aNDF disappearance was altered between line and diet, AMEn was

not different ( $P>0.41$ ), suggesting that dietary energy was not limiting growth performance. Cecal short chain fatty acid analysis showed an increase in the concentration of acetic acid ( $P=0.02$ ) and propionic acid ( $P<0.01$ ) in broiler chicks in comparison to layer chicks and increasing dietary fiber resulted in a significant reduction of butyric acid ( $P=0.03$ ). There was variation in the cecal microbial communities as a result of chick line and diet modification. These results suggest that high fiber feed ingredients are suitable substitutes for supplementation in layer chick starter diets but not in broiler chicks.

Key words: broiler, layer, dietary fiber, performance

## CHAPTER 1. GENERAL INTRODUCTION

With the increasing price of corn and oil, the poultry industry is increasing the use of available feed ingredients high in dietary fiber in order to reduce production costs. The increase in ethanol production, particularly in the United States, has led to increased production of corn dried distillers grains with solubles (DDGS) (Renewable Fuel Standard, 2013). Corn DDGS has become an important feed ingredients in poultry diets, which together with other feed ingredients such as wheat bran are relatively high in dietary fiber, cost effective alternatives. This thesis aims to determine the effects of high fiber diets formulated using corn DDGS and wheat bran on broiler and layer chick performance, nutrient digestibility, ceca short chain fatty acids (SCFA) content as well as cecal microbiota characterization.

The hypotheses were:

- 1) Increasing dietary fiber by use of high fiber ingredients in diet would improve performance altering nutrient utilization and cecal microbiota populations in both broiler and layer chicks resulting in maintenance of performance at least cost. Dietary fiber would provide energy to the ceca commensal microbiota to facilitate microbiota growth. The increase in bacterial growth would improve fiber digestibility as a result of bacterial metabolism leading to increased production of SCFA.
- 2) Broiler and layer chicks utilize dietary fiber ingredients through similar mechanisms, but layer chicks are more efficient compared to broiler chicks resulting in differences in the performance of the two chicken lines.

The objectives of the thesis were to determine the effects of dietary fiber on metabolizable energy (ME) and fiber fermentation on the performance of birds and to evaluate whether DDGS and wheat bran combinations in chick diets were viable feed ingredients, and to determine cecal fermentation and microbial ecology due to line and dietary fiber differences.

### **Thesis Organization**

This thesis consists of a review of the current literature and two manuscript chapters. Chapter 1 is a general introduction while Chapter 2 covers the entire literature review that addresses the detailed overview of the two high fiber ingredients, corn dried distillers grains with solubles and wheat bran, and their implications to poultry nutrition. Chapter 3 consists of a manuscript that describes the effects of high dietary fiber on the performance of broiler and layer chicks. The high fiber diet was formulated by the addition of dried distillers grains with solubles and wheat bran to the corn-soy bean meal (SBM) base while the low fiber diet was based on a corn-SBM. The diets were formulated to be isocaloric and met or exceeded NRC requirements. Chapter 4 of the thesis is the second and final manuscript that reports the effects of high dietary fiber on SCFA concentration and cecal microbial ecology of broiler and layer chicks. Chapter 5 reviews the findings and conclusions and implications of this thesis.

## **CHAPTER 2. REVIEW OF LITERATURE**

Optimum production at least cost is a major concern to the poultry industry. Corn and soybean meal have been used as basal feed ingredients in poultry feed formulation for decades. However, high corn grain prices have resulted in the use of least cost high fiber feed ingredients such as dried distillers grains with solubles, a primary by-product of corn fermentation. Although use of high fiber feed ingredients in poultry rations may be beneficial from a cost standpoint, it could have detrimental effects to poultry performance. The effects may range from performance, digestibility, product quality and other related parameters. As more focus may be directed towards use of dietary fiber to lower costs, more research should be performed to address the appropriate inclusion levels of different feed ingredients. Fiber in feed ingredients may affect cecal microbial population, nutrient digestibility, and volatile fatty acid production. Interactions of these effects can affect bird performance.

Diet is an important aspect of animal production, and different bird species or lines have different nutrient requirements depending on age, genetic background and environment as well as the health status of the birds. Thus, nutritionists are faced with a challenge of formulating diets with the available feed ingredients, but also having to mitigate the resulting diet effects to achieve optimum bird production.

### **Corn Dried Distillers Grains with Solubles (DDGS)**

The increase in ethanol production in the United States is expected to increase in the future (RFS, 2013). With the increasing corn fermentation to produce ethanol, DDGS will

saturate the local market making them the least cost feed ingredients to replace corn and soybean meal in poultry rations (Adam, 2008).

Several research experiments have been conducted to investigate the most appropriate DDGS inclusion levels in poultry diets. Dried distillers grains with solubles may be less costly, but other nutrition concerns such as nutrient quality and variability in both physical and chemical characteristics should be considered when considering the use of DDGS in poultry feeding. The composition of DDGS has changed over time with a progressive change in quality and nutrient digestibility from the 1960's to present. There has been a change in the feedstock grain used for DDGS production. In the 1960's, DDGS was a by-product of alcohol production mainly from wheat, rye and barley. Today it is mostly produced from corn or sorghum, particularly in the United States. The use of corn for ethanol production increased from 6% in 2000 to 24% in 2008 and is expected to increase from 30 to 35% over the next ten years (Westcott, 2009). Research has shown that corn DDGS produced from "new generation" ethanol plants (built after 1995) were greater in fat and digestible energy content compared to the DDGS produced from "old generation" ethanol plants (Spiehs et al., 2002).

Although DDGS have been used in the formulation of poultry diets for decades (Runnels, 1966; Waldroup et al, 1981), variability in nutrient contents and digestibility is still observed (Cromwell et al., 1993; Noll et al., 2001). In the early years, Runnels (1966) performed three computer-based experiments to determine DDGS biological value in comparison to soybean meal. The experiment was not successful in determining the DDGS appropriate nutrient parameters in computer based formulations, but results showed that up to 200 g/kg DDGS inclusion would be suitable for broiler chick performance. Waldroup et al. (1981) used up to 250 g/kg DDGS in two trials; one with variable energy and the second trial was supplemented with

fat to attain a fixed (13.34 MJ/kg) energy level. A reduction in body weight and feed efficiency (gain to feed) for the variable energy regime was observed when up to 150 g/kg of DDGS was included in the diet. The fixed energy regime had no effect on body weight nor feed efficiency for up to 250 g/kg DDGS inclusion. The authors of the two aforementioned early experiments did not state the source of the DDGS.

Recent research focused at maximum inclusion rate of corn DDGS and involved use of 0, 60, 120 and 180 g/kg DDGS inclusion rates in broiler starter, grower and finisher diets (Lumpkins et al., 2004). The inclusion rate of 180 g/kg had adverse effects on performance and was not recommended in broiler starter diets. Wang et al. (2007) formulated broiler diets based on digestible amino acids at 0, 50, 100, 150, 200 and 250 g/kg DDGS to evaluate birds' performance. Results indicated that inclusion rate of 250 g/kg had no effect on growth rate, but chicks had a decreased feed conversion in comparison to the control treatment. In addition, use of 250 g/kg DDGS in broiler diets led to a reduction in both dressing percentage and breast weight.

Early laying hen experiments showed that DDGS could be incorporated at 50-200 g/kg in diets without having significant impact on egg production and egg weight (Matterson et al., 1966; Harms et al., 1969; Jensen, 1974). Recent research that focused at maximum inclusion rate of DDGS in high nutrient dense diets reported that 100-120 g/kg DDGS was the maximum inclusion in laying hen diets. The use of 150 g/kg DDGS was suitable for commercial diets. However, low density diets resulted in reduced production when DDGS were included at 150 g/kg in the diet (Lumpkins et al., 2005). Diet formulation was based on total amino acid basis and this could have resulted in lysine deficiency when DDGS were increased to 150 g/kg inclusion. Other laying hen experiments were formulated with more than 150 g/kg DDGS

inclusion. Results indicated that 150 g/kg DDGS diets decreased egg weight and 200 g/kg DDGS decreased hens body weight gain (Masa'deh and Scheideler, 2008). Diets were again formulated on total amino basis and this led to a deficiency in lysine. There were no effects on egg production, feed intake, and specific gravity and haugh units at 250 g/kg DDGS inclusion. High inclusion rates have been reported to decrease performance. Feed intake, egg production, egg weight, egg mass and feed efficiency were adversely affected with 500 g/kg DDGS inclusion during a twelve week period of a laying hen experiment (Sun et al., 2012). In addition, use of 500 g/kg DDGS is also reported to alter the chemical composition and nutritional components of the egg yolk (Sun et al., 2013). Increasing the concentration of essential amino acids such as lysine and methionine mitigates the negative effects of high DDGS inclusion. Reformulation of diets to increase concentration of both lysine and methionine reduced differences among dietary treatments, as 500 g/kg DDGS greatly improved performance (Sun et al., 2012).

There has been little or no research published recently regarding the use of corn DDGS in pullets or layer chicks' diets. In this regard, research studies need to be conducted to investigate the most appropriate DDGS inclusion rates in both pullets and chicks. Early studies tested distillers grains and solubles (DGS) in pullet diets and concluded that use of DGS would improve growth rate in pullets in comparison to the pullets that were feed on a corn-soy bean based diet and meat scraps (Insko, 1949). Two lines , New Hampshire and Barred Rocks, were used in both the pullet and production periods of the experiment. A reduction in average body weight was observed in DGS fed birds compared to the hens fed on meat scraps, and combination of DGS and meat scrap was a better combination for optimum growth. A more recent study tested the use of varying inclusion rates in pullet diets. In this experiment, Single Comb White Leghorn chicks were fed diets containing 0, 25, 50, 75, 100, or 125 g/kg DDGS



from 1 to 16 weeks of age. The results of the study showed no difference in the overall pullet body weight and pullets that were fed 125 g/kg DDGS had greater body weight than other dietary treatments at 14, 15, and 16 weeks of age (Masa'deh et al., 2012). However, feeding 125 g/kg did not have economic benefit compared with feeding the corn-soybean based diet.

An early experiment investigated the use of corn DDGS in broiler breeder replacement pullets (Crouch, 1966). The study involved use of 350 broilers breeders that were assigned to three dietary treatments and were fed for 9-25 weeks of age. Diet one contained a corn-soybean meal, diet two contained corn and 370 g/kg DDGS, and diet three contained soybean meal and ground milo. All the three diets were formulated to contain 150g/kg crude protein 7.0, 4.0, and 7.6 g/kg lysine respectively. Results showed that pullets fed diet two had better feed conversion ratio, higher egg production and greater egg weight compared to pullets fed diets one and three.

### **Nutrient composition and value of DDGS**

Although DDGS have been used for decades for poultry nutrition, there are several concerns that limit their use in poultry diets. Nutrient variability has been one of the major limiting factors for use of DDGS in poultry diets. Addition of solubles, composition of corn and drying conditions may be among the factors that contribute to variability of nutrients. Noll et al. (2007) reported that addition of solubles had a great effect on the DDGS particle size, color, fat content as well as mineral content. The study conducted in cooperation with ethanol plants in Minnesota involved producing batches of DDGS with different levels of solubles added to the wet grains. Solubles (syrup) were added at 0, 30, 60 and 100% to mash (wet grain). Large particle sizes were observed in the batch that contained the 100% solubles addition. The fat content, nitrogen corrected total metabolizable energy (TMEn) content, and ash increased with

increased solubles addition. Levels of minerals such as magnesium, phosphorus, sodium, potassium, sulfur and chloride increased when solubles addition were increased. There was a negative correlation between true amino acids digestibility coefficients of the essential amino acids with addition of solubles. In contrast to Spiels et al. (2002), 118 DDGS samples from 10 ethanol plants were collected in Minnesota and South Dakota across three years. Results showed a variation between 5% and 10% for dry matter, crude protein, crude fat, and crude fiber and calculated metabolizable energy. High variability occurred in total methionine and lysine content (coefficient of variation 13.6 and 17.3 respectively). Fastinger et al. (2006) reported that 5 samples from 5 plants varied in total lysine digestibility from 0.48 to 0.78% and their lysine digestibility ranged between 38.6% - 63%. Methionine and lysine are the first and second most limiting amino acids in poultry diets with methionine ranging from 0.41% to 0.65% (Evonik, 2005; Spiels, 2002; Fastinger et al., 2006), thus digestibility of these amino acids needs great consideration. Methionine digestibility ranges from 85 to 92% (Martinez-Amezcuca et al., 2005). According to a study by Pahm et al. (2008) methionine digestibility ranged from 83.6 to 88.8%. These values were close to those reported by Martinez-Amezcuca et al. (2005).

DDGS nutrient composition and characteristics vary among different plants. The origin of the corn contributes to nutrient variation in DDGS. According to Reese and Lewis (1989), corn that was produced in the year 1988 in Nebraska varied in lysine concentration (0.22 to 0.32%), crude protein (7.8 to 10%), and phosphorus content (0.24 to 0.34%). Moreover, studies conducted at the University of Minnesota (Noll et al., 2003; Ergul et al., 2003) reported that DDGS sources varied in protein, fat, and ash contents. Variations in amino acid contents among the different sources were observed with the exception of serine. Lysine, cysteine and tryptophan contents were the most variable with coefficients of variations; 11.2%, 11.3% and 11.1%

respectively. Amino acids like lysine, threonine, arginine, and cysteine varied in digestibility coefficients (71, 72, 93 and 77% respectively). Lysine had high variation (0.38 to 0.65%) in true digestibility. Fastinger et al. (2006) and Batal and Dale (2006) reported that DDGS true amino acid digestibility may vary in different batches within the same plant as well as among different plants. Heating during DDGS processing may damage lysine (Bregendahl, 2008), and a decrease in lysine content and its digestibility is likely to happen (Cromwell et al., 1993). The color of DDGS has been considered as one of the parameters or a rough guide to assess DDGS quality, particularly the availability of lysine. Darkening of DDGS indicates heat damage and may subsequently decrease amino acid digestibility (Cronwell et al., 1993; Batal and Dale, 2006). Maillard reactions due to heating cause darkening of DDGS, lysine reacts with reducing sugars rendering the availability of lysine (Bregendahl, 2008). Ergul et al. (2003) reported that light color DDGS have high lysine digestibility (80%) while dark color DDGS have lower lysine digestibility (60%).

### **Mineral Content of DDGS**

Mineral content of DDGS is an important aspect to consider before inclusion in poultry diets. An evaluation to assess the mineral content of 12 DDGS samples, collected from the Mid-west region of the United States reported that sodium content of DDGS ranged from 0.09 to 0.12% (Batal and Dale, 2003). It was not clear on what could be the source of sodium. Batal and Dale (2003) indicated that the addition of solubles during processing from other manufacturing streams contributed to the increase in sodium content in the final product. Most samples had low sodium content with exception of one plant that had sodium varying from 0.39 to 0.44%. Related

studies conducted at University of Minnesota reported a variation in mineral content among different DDGS sources (Ergul et al., 2003; Noll et al., 2003).

Dried distillers grains with solubles are considered a good source of non-phytate phosphorus (Ergul et al., 2003; Noll et al., 2003). Studies have shown that DDGS contain a great concentration of non-phytate phosphorus with high. The concentration varies from 0.59 and 0.95% greater than corn which varies from 0.17 to 0.29% (NRC, 1994 Spiehs et al 2002; Batal and Dale, 2003; Martinez Amazcua et al., 2006). Thus, inclusion of DDGS in poultry diets can contribute to available phosphorus because poultry are unable to utilize phytate phosphorus in corn due to lack of the phytase enzyme (McCuaig et al., 1972).

Dried distillers grains with solubles have been reported to contain other minerals such as calcium and pigments such as xanthophyll. Similar to sodium, there is a greater variation of calcium concentration in DDGS (Parsons et al., 2006).

### **Metabolizable energy content**

Several studies have been conducted in United States ethanol plants to determine DDGS energy content. The energy content of DDGS is commonly evaluated using the precision-fed rooster assay that was developed by Sibbald (1976; 1986). The Lumpkins et al. (2004) broiler feeding study reported that DDGS contained 12.15 MJ/kg, but in the subsequent study, 11.74 MJ/kg TMEn was reported in a laying hen study (Lumpkins et al., 2005). Batal and Dale conducted a study to determine the TMEn of 17 samples that were collected from 6 plants. The values of TMEn varied between 10.42 and 13.35 MJ/kg. These values are close to the TMEn estimated values in the NRC (1994) of 10.38 MJ/kg for DDGS on 86% dry matter (DM) basis and 9 % fat. Another study was conducted for 5 samples from 5 different plants. Fastinger et al.

(2006) reported that TMEn content of 5 samples ranged from 10.39 to 12.75 MJ/kg. However, in a study which evaluated many samples a larger variation among samples was reported. The results showed that the TMEn value was  $11.98 \pm 1.87$  MJ/kg (Parsons et al., 2006). Because DDGS has a significant amount of energy it has been suggested to have a potential use in poultry feed. In a review paper, Waldroup et al. (2007) suggested a DDGS nutrient matrix using summarized results from different publications, addressing the potential of DDGS in poultry feed.

### **Wheat Bran**

Wheat bran is one of the by-products from wheat processing into flour that could be used in poultry feed (Hemey et al 2007). With DDGS receiving great attention from both poultry and livestock industries, such as the dairy and beef industries (Ham et al., 1994; Powers et al., 1995), it is speculated that an elevation in price of DDGS may occur. It would be useful to the poultry industry to utilize wheat bran, in addition to other by-products as long as production is not compromised.

Wheat bran is a by-product made by dry milling of wheat to produce flour. It comprises of small amounts of wheat kernel, endosperm and the outer layers (Hoseney, 1994). Depending on the method of processing and the wheat variety, wheat bran consists of 50% wheat offals and 10 to 19% wheat kernel (Hassan et al., 2008; Prikhodho et al., 2009). Wheat bran has been considered a palatable feed ingredient and appropriate for feeding livestock (Piccioni, 1965; Fuller, 2004), but may not be included in poultry diets in appreciable amounts. Research has shown that wheat bran inclusion in poultry diets is usually kept low, especially in cases where calculations are based on least cost formulation. However, high inclusion rates could be used in

layer molt diets (Soe et al., 2009). Wheat bran may be used in high levels in layer diets, but lower levels are used in broilers diets (Soe et al., 2009; Boudouma, 2010b). Use of high rates (>13%) has been reported to reduce feed intake in broilers (Boudouma, 2010b). According to Ali et al. (2008), use of 300 g/kg wheat bran in broiler starter, grower and finisher diets did not have adverse effects on body weight gain and feed conversion from 7-45 d period, but its inclusion in broiler starter diets decreased body weight gain in the 7-14 d experimental period. However, some beneficial effects on phosphorus, globulin, and plasma antioxidant capacity were noted. Wheat bran inclusion in the diet significantly increased plasma antioxidants, globulin levels, and decreased abdominal fat and cholesterol levels.

There is little to no recently published research about the use of wheat bran in layer chicks or pullet diets. Therefore, experiments need to be conducted to investigate the most appropriate inclusion rates of wheat bran in layer chicks and pullet diets. In early studies which used lower amounts reported that inclusion of up to 150 or 250 g/kg wheat bran in ground whole grains mash was found to cause rapid growth and early sexual maturity (Taylor and Lerner, 1939). However, other research used wheat bran in pullet diets to reduce feed intake and delay sexual maturity. Summers et al. (1991) reported that pullets that were fed wheat bran until 1% production and those fed wheat bran for two weeks prior to production the period (20 weeks) lost around 200 g of body weight.

### **Nutrient composition and value of wheat bran**

Wheat bran nutritive value is highly variable irrespective of the origin. A wide range of products are sold under the name wheat bran which is a composite material made up of three discrete layers formed from several histological tissues. The issues are divided into inner and

outer pericarp, seed coat, hyaline layer and aleurone layer (Antoine et al., 2002). Like other animal feed ingredients, the variation in wheat bran composition has been attributed to differences in variety, maturity, soil conditions and climate, management as well as processing factors (Safdar et al., 2009).

Composition of both micro and macronutrients depends on wheat cultivar and processing technique. The mineral content is a true reflection of the quantity of bran in wheat (Safdar et al., 2009). Wheat bran is a good source of protein, carbohydrate, minerals, vitamins and bioactive compounds such as betaine and choline (Slavin, 2003). In addition, wheat bran contains other important components like carotenoids, phenolic acid, phytosterols, lignans, phytic acid, and phenolic acid (Slavin, 1999; Zhou and Yu, 2004; Buri et al., 2004; Esposito et al., 2005). The phenolic nature of lignin in the wheat bran fiber may inhibit enzyme activity (Fahey et al., 1993). But the significant amount of betain (1505.6mg/100g) is known to decrease carcass fat, protect intestinal cells from coccidia. infection, choline and methionine sparing, and improve performance (Kidd et al., 1997; Kettunen et al., 2001; Zeisel et al., 2003; Fetterer et al., 2003).

**Table 1.1.** Composition of wheat bran (Adapted from Palmarola-Adrados et al., 2005)

<b>Component</b>	<b>g/100g Wheat bran</b>
i) Non-Starch polysaccharide	
Glucan	10.5
Xylan	18.3
Arabinan	10.1
Galactan	1.1
ii) Starch	34
iii) Klason lignin	5
iv) Crude protein	13.5
Total	92.5

Wheat bran is rich in dietary fiber containing 50.4% DM neutral detergent fiber (NDF), 16.7% DM acid detergent fiber (ADF) and lower amounts, 4% DM of acid detergent lignin (ADL) (Hill et al., 1960). The high fiber content in wheat bran limits its utilization in monogastric diets, particularly poultry due to ineffective fiber fermentation. Wheat bran contains pentosans, a non-starch polysaccharide thought to be anti-nutritive to poultry and causes a depression in nutrient utilization and subsequently leads to poor growth (Choct et al., 1992). Starch and fiber are inversely correlated, high dietary fiber in wheat bran adds bulk to the feed, but causes a reduction in metabolizable energy compared to other cereal products such as corn, barley or sorghum (NRC, 1994; Boudouma, 2010a). Early reports showed that wheat bran contained low ME of 6.72MJ/kg (Hill et al., 1960), but later experiments reported that wheat bran ME was lower with values varying from 5.26 to 5.44 MJ/kg (Allen, 1989 and NRC, 1994). The low fat content of wheat bran (3-5% DM) contributes to low ME. This low fat concentration is beneficial during diet formulation because wheat bran contains a very stable heat-stable lipase which causes hydrolytic rancidity and is active in finely ground wheat bran (Allen et al., 1994).



On the other hand, wheat bran is a good source of active phytase, particularly in the ungerminated grain which is beneficial to the availability of phosphorus in poultry (Mollgaard, 1946; Abernethy et al., 1973; Cavalcanti and Behnke, 2004). Early research reported that phytase in wheat bran leads to an improvement in the animal phytate phosphorus utilization (McGillivray, 1978). Feeding chicks diets that contain wheat bran enables them to hydrolyze phytate better compared to the corn-soybean meal fed chicks (Ballam et al., 1984). Research conducted in poultry showed that wheat bran phytase increased phosphorus utilization and growth rate in broilers (Cavalcanti and Behnke, 2004) and turkeys (Roberson et al., 2005). However, this phytase is said to be heat labile and the activity is reduced during processing.

### **Classification of Fiber**

Dietary fiber is typically plant cell wall materials that are not digested by endogenous enzymes (Theander et al., 1993; Theander et al., 1994; Turner and Lupton, 2011). The plant cell wall is comprised of non-starch polysaccharides (NSP) and lignin. Classification of polysaccharides based on differences in solubility places them into the following categories: cellulose, hemicellulose, pectin and lignin (Albersheim et al., 1984). However, composition and physiological activity is not fully reflected in this kind of classification. Plant cell walls also contain glycoproteins, glycolipids and polyphenols. The composition of these components depends on different plant species and on plant stage of maturity (Smiths and Annison, 1996).

With differences in carbohydrate digestion and utilization, early research invented methods that classified dietary fiber into neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude fiber (CF). NDF measures hemicellulose, cellulose and lignin, ADF estimates all cell wall cellulose and lignin. Crude fiber includes a portion of hemicellulose, cellulose, and

lignin (Van Soest, 1963; Van Soest and Wine, 1967). It is difficult to precisely define dietary fiber. Fiber has historically been defined as the balance between nutritional significance and availability of adequate analytical methods, thus adapting the definition to the analytical procedure instead of the physiological effect of the fiber fractions (Bach Knudsen, 2001; Lupton, 2010). Dietary fiber consists of diverse polymers with variability in physiochemical properties that result in differences in ion exchange capacity, viscosity, bulking, and fermentation in the GIT when included in the diet. It is not easy to measure dietary fiber, but differentiating it into soluble and insoluble components helps to explain the physiological effects of fiber (Mertens, 2003; Newman et al., 1992). Insoluble dietary fiber is not degraded by microbial fermentation and could increase fecal output. In poultry intake, excretion, and degradation of fiber (NDF, ADF or CF) vary among different species. Ning et al. (2003) reported that intake, excretion and degradation of dietary fiber were high for five and seven months old emus compared to turkeys and chickens of a similar age. The authors suggested that the difference could be attributed to the well-developed ceca in the emus as opposed to turkeys and chickens. The results also showed that most fiber degradation was reported to occur in the ceca. Wang et al. (2007) reported that NDF and ADF degradation was better in the ceca compared to ileum and rectum portions of the GIT. Degradation of fiber also increases with age in poultry. Emus, turkeys, and chickens of seven months degraded NDF and ADF much better than the five month old birds (Ning et al., 2003).

### **Nutritional and Biological Effects of Dietary Fiber**

Use of feed ingredients high in dietary fiber in poultry nutrition has generally been discouraged due to the negative effects exerted on nutrient utilization and performance such as decrease in body weight gain and feed conversion. It is important to note that fiber in

monogastric diets is mainly utilized in the hind gut (i.e. ceca, rectum and the colon). Feeding animals diets high in dietary fiber, particularly soluble fiber alters the rate of fecal passage, microbiota, metabolites, and efficacy of digestion (Bach Knudsen, 2001). Commensal bacteria in the large intestine utilize fiber as a source of energy. An increase in the energy supply increases microbial metabolism and microbial population growth. Bacteria produce short chain fatty acids such as acetate, butyrate and propionate during the metabolism causing increased bacterial populations. This results in further production of short chain fatty acids. There has been limited inclusion of dietary fiber in poultry diets, particularly chickens, due to its negative effects on nutrient digestion. Villamide and San Juan (1998) reported that the true metabolizable energy (TME) of sunflower seed meal with variations in NDF and ADF had a negative correlation with NDF, ADF, CF, hemicellulose, cellulose, and lignin.

In previous years, a great focus was directed towards the effects of soluble fiber to digestibility of feed. Soluble fiber is known to increase viscosity in the small intestine (Choct et al., 1996), and subsequently inhibits digestion and absorption. The rate of digesta passage is reduced, feed intake is decreased, creating favorable conditions for proliferation of microbes in the intestine (Smiths and Annison, 1996; Choct et al., 1996; Langhout, 1998). Large amounts of fermentation were observed in some experiments when chickens were fed diets containing soluble NSP (Choct et al., 1996; Langhout, 1998). Hence, the ability of microorganisms to ferment dietary fiber is correlated to the amount and type of dietary fiber components in the diet.

Insoluble fiber in monogastric diets has for long been considered as diluent of nutrients (Edwards, 1995). The little or no degradation of insoluble fiber in chickens results in increased bulk of digest in the intestinal tract. This makes its effect on microbial population quite insignificant (Krogdahl, 1986; Choct et al., 1996; Langhout, 1998). Since diets high in insoluble

fiber contain low energy, birds tend to increase feed consumption as a way to compensate for the reduced nutrient concentration in feed (Hill and Dansky, 1954). Feed ingredients high in insoluble fiber cause an increase in the bulk of the digesta that eventually leads to fast digesta passage through the GIT, unless the animal has a large digestive system capacity. This effect has been reported to improve digestibility (Krogdahl, 1986; Rogel et al., 1987a,b). There are suggestions that fiber decreases nutrient digestion because it encapsulates nutrients into the plant cell causing a reduction in the activity of digestive enzymes. In some cases, some fiber sources may cause pancreatic enlargement, leading to an increase in secretions (Kratzer et al., 1967). Insoluble fiber has been reported to have some beneficial effects. Improvement in digestibility of starch from wheat and potatoes was observed when oat hulls were included in chicken diets (Rogel et al., 1987a, b). Some experiments have shown that as long as insoluble fiber is included in poultry diets at moderate concentrations, performance of birds will not be affected despite the fact that the nutrient concentration of the diet is reduced (Hetland and Svihus, 2001; Hetland et al., 2002). However, the mechanism of formulating diets with moderate levels of insoluble fiber is not well known (Hetland and Svihus, 2001).

Fiber in chicken diets is also reported to influence the behavior of birds by reducing cannibalism as birds spend more time eating than pecking each other (Hughes and Duncan, 1972). Other experiments conducted to determine the effect of dietary fiber on bird behavior reported that birds fed diets containing oats do not cannibalize each other (Bears et al., 1940; Wahlstrom et al., 1998). Oats are high in NSP (20-25%), with the highest dietary fiber proportion (85-90%) being insoluble (Fincher and Stone, 1986). Choct and Hartini (2003) found that birds fed a high fiber diet with 20% of NSP during the pre-lay (17-20 weeks) and early lay (21-24 weeks) periods had reduced mortality due to cannibalism compared to birds fed a

commercial diet. Hence, fiber in diets may as well reduce pecking in chicks or young birds causing a reduction in pecking that could result in to mortality.

### **Chicken Ceca Microbiology**

Chicken intestinal microbiota have been studied since the 1940s (Shapiro and Sarles, 1949), but it was not until the 1970s that comprehensive characterization studies were conducted to culture as many microbiota as possible. Early experiments involved culturing of bacteria from the ceca and results revealed that the ceca harbored complex microbiota (Barnes et al., 1972; Salanitro et al., 1974; Mead and Adam, 1975; Barnes, 1979). Maintenance of strict anaerobic conditions during bacterial isolation and biochemical differentiation presented the major technical challenge. The failure of chicken microbiota to grow under laboratory conditions limited the ability of investigators to address specific genetic detail. Molecular techniques have been used in the recent studies to address cecal microbial ecology. These approaches provided an accurate measure of cecal microbial diversity (Zhu et al., 2002). However, these molecular techniques have some limitations including the possibility that the DNA isolation, amplification and cloning could in some way favor specific species of bacteria. Although other parts of the chicken gastro-intestinal tract such as the ileum may be important microbiota sites, the ceca remains the most beneficial GIT part with largest number of bacteria in the chickens (Barnes et al., 1972, 1973; Barnes 1979; Jamroz et al., 1998) that is responsible for non-starch polysaccharide degradation. Ceca contain a diversity of microbiota, with some bacterial species such as *Campylobacter* and *S. enterica* considered as potential pathogens in humans (Barrow et al., 1987; Doyle, 1991; Engberg et al., 2002). It is estimated that one gram of cecal content contains  $10^{11}$  bacteria (Mead, 1997). According to Barnes (1979), more than 200 different

bacterial species were isolated and divided into dominating, sub-dominating and temporary populations. Different factors such as diet, age as well as health status of the birds influence the establishment of a particular bacterial colony in the ceca (Barnes et al., 1972; Barnes 1979). In healthy chickens, bacteria like the *Enterococcus spp*, *Lactobacillus spp*, and *Enterobacteriaceae spp* predominate the ceca during the first days of life, while *Eubacterium spp* and *Bacteriodes spp* establish themselves after the subsequently two weeks of life. Obligate anaerobic microflora dominate from 7 days of age onwards (Barnes et al., 1972; Salanitro et al., 1974; Mead et al., 1975; Mead, 1997). *Enterobacteriaceae* population decreases due to increase in ceca short chain fatty acids. Other studies have reported variety of bacterial species to be dominating the ceca. Barnes et al. (1972) reported that ceca consist mainly of gram negative non-spore bacteria. Mead and Adam (1975) report gram positive non-spore bacteria while Salanitro et al. (1974) reports a mixture of both gram negative and gram positive to be dominating. Culturable cecal bacterial flora, gram positive cocci were found numerous, while prominent populations of *Eubacteria* and *Clostridium spp* were observed in previous studies (Barnes et al., 1972; Barnes 1979; Mead, 1989; Rolfe, 2000). Some species have been reported to have positive impacts in the colon, as *Bafidobacterium*, together with *Lactobacilli* are beneficial bacteria and play good role in inhibiting pathogens (Gibson and Roberfroid, 1995). Use of the 16S rRNA gene to characterize ceca microbiota addresses many genus and species, but still does little to reveal comprehensive details of all ceca microbiota species.

### **Short Chain Fatty Acids in the Chicken Ceca**

The ceca are paired blind pouches which together with the colon and rectum form hind gut of the chicken. Ceca account for 1% of the chicken body weight (Redig, 1989). According to a Steven and Hume (1998) study that involved measuring relative lengths of different intestinal parts in 644 specimens, from 24 orders, 51 families, 124 genera of birds, well developed ceca were present in granivores and species whose diet primarily contained high fiber and chitin levels. Although it is not absolute, there is a relationship between ceca development and the diets of birds. Research has speculated that the ceca have several functions: water absorption, microbial degradation of some soluble carbohydrates, microbial synthesis of vitamins, degradation of nitrogenous products, as well as cholesterol digestion and absorption (Coates et al., 1968; McNab, 1973; Tortuero et al., 1975; Goldstein, 1989; Jorgensen et al., 1996; Jamroz et al., 2002). The role of the ceca in chickens is not well known, but previous experiments have reported that the ceca accounts for about 4% of the metabolizable energy, derived from SCFA (Persia et al., 2002; Sugahara et al., 2004). In chickens, the ceca microbiota ferment undigested carbohydrates, such as non-starch polysaccharides, (NSP), to short chain fatty acids and some gases (Marounek et al., 1999; Jamroz et al., 2002). The SCFA produced as a result of fermentation include; acetate, butyrate, propionate, lactate, valerate, and isovalerate (Jamroz et al., 1998). In chickens, concentration of these SCFA increase from very low levels at day 1 to high levels at day 15, after which they then stabilize (Van der Wielen et al., 2000). The concentration of these products will depend on the composition of the diet. Feeding chickens with a plant protein based diet resulted in the production of high levels of SCFA compared to when birds were fed on normal protein based diet (Tsukahara and Ushida, 2000).

In conclusion, both DDGS and wheat bran are good sources of nutrients for poultry diets. Dried distillers grains with solubles is a good source of energy in particular and previous research has recommended it as a good feed ingredient, with up to 100 and 150 g/kg in broiler and layer diets, respectively. The two feed ingredients, DDGS and wheat bran vary in nutrient concentrations and there is not sufficient research regarding the appropriate inclusion levels in poultry diets. Further studies with high inclusions or a combination of the two feed ingredients should be performed to investigate the optimal inclusion rates. Little or no research about the effects of feeding wheat bran or DDGS in laying chicks or pullets has been published recently. Research needs to be performed to investigate the inclusion levels of both ingredients in layer chicks and pullets.



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**CHAPTER 3. EFFECTS OF HIGH FIBER INGREDIENTS ON THE PERFORMANCE,  
METABOLIZABLE ENERGY AND FIBER DIGESTIBILITY OF BROILER AND  
LAYER CHICKS**

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**Abstract**

An experiment was conducted to evaluate the effects of feeding diets with various fiber contents on the performance of broiler and layer chicks. The lower fiber diet was based on a traditional corn-soybean meal (SBM) diet and the higher fiber diet was formulated by the addition of corn dried distillers grains with solubles (DDGS) and wheat bran to the corn-SBM base. The diets were isocaloric and were formulated to meet or exceed NRC requirements. Two lines of male chicks, Ross 308 broilers and Hy-Line W36 layers, were randomly assigned to treatments with 11 replicates of 8 chicks for each of the 4 treatments. The evaluation criteria consisted of average daily gain (ADG), average daily feed intake (ADFI), feed efficiency (FE), nitrogen corrected apparent metabolizable energy (AMEn) and neutral detergent fiber (aNDF) digestibility. The higher dietary fiber diet significantly reduced broiler ADG ( $P \leq 0.01$ ) for the 1-12 d and 1-21 d periods but had no effect on layer chick ADG resulting in a significant interaction. Increasing dietary fiber had no significant effects on ADFI ( $P > 0.05$ ) for the 1-12 d and 1-21 d periods. Although broiler chicks had better feed efficiency compared to layer chicks, dietary fiber did not affect feed efficiency in broiler and layer chicks for either feeding period.

Neutral detergent fiber digestibility was higher in layer chicks than in broiler chicks regardless of diet ( $P \leq 0.01$ ), and higher dietary fiber concentration resulted in increased ileal ( $P \leq 0.01$ ) and total tract ( $P \leq 0.02$ ) aNDF disappearance, across both lines. Apparent metabolizable energy was not different ( $P = 0.41$ ) between lines or dietary fiber content. These results suggest that layer chicks are able to better utilize feed ingredients rich in fiber content compared to broiler chicks, possibly due to the decreased growth rate and feed consumption of layer chicks in comparison to broiler chicks.

Key words: DDGS, wheat bran, broiler, layer, performance

### **Introduction**

The recent increase in the price of corn and oil has led to an interest in dietary fiber as a method to reduce feed costs in poultry diets. According to the Renewable Fuels Standard, ethanol production is expected to increase through 2022, with corn based ethanol capped at about 15 billion gallons by 2015 (Renewable fuel standard, 2013). In reality, the 15 billion gallon mark was nearly achieved in 2011 and maintained or slightly lower in 2012. It appears that biofuel demand for corn will continue to pressure corn prices therefore, lower cost corn and wheat by-products have the potential to provide economical alternatives to traditional US feed ingredients in poultry (Waldroup et al., 1981; Shalash et al., 2009a). It is important to understand the effects of these alternative dietary feed ingredients on bird performance to allow for proper formulation into current poultry diets. Fiber has been regarded as nutrient diluent in poultry (Angkanaporn et al., 1994), and higher fiber in feed ingredients has been shown to have negative effects on digestion and absorption of nutrients in chickens (Krogdahl, 1986). However, previous



experiments have indicated that performance does not decrease when feed ingredients high in fiber are included at moderate levels to both layer and broiler diets despite the reduction in nutrient concentration of the diet (Hetland and Svihus, 2001; Hetland et al., 2002).

In the past, poultry diets in the USA have been formulated with low fiber concentrations because the addition of higher fiber ingredients has decreased feed efficiency (Longe and Ogedegbe, 1989). Dried distillers dried grains with solubles (DDGS) have been used in poultry diets for decades and were included in low levels in early broiler experiments (Day et al., 1972). Later experiments reported that DDGS could be incorporated at 250 g/kg in the diet without detrimental effects, if metabolizable energy is kept constant (Waldroup et al., 1981). Current ethanol production has resulted in a new DDGS product generated from corn and early work with corn DDGS has focused on broiler chicks. The use of 120 g/kg DDGS in broiler diets during the starter period resulted in reduced performance (Dale and Batal, 2003), but up to 80 g/kg DDGS during the starter phase had no effect on broiler performance through 14 or 28 d of age (Loar II et al., 2010). Other reports resulted in acceptable performance with the use of DDGS at a concentration of 60 g/kg in broiler starter diets and 120 to 180 g/kg in broiler grower and finisher diets respectively (Lumpkins et al., 2004). Wheat bran was fed at 150 g/kg of the diet with negative effects on bird performance (Donkoh et al., 1999).

There have been several experiments involving the use of higher fiber ingredients in adult laying hens' diets and 50-200 g/kg of DDGS have been suggested to have no effects on production (Matterson et al., 1974; Lumpkins et al., 2005; Roberson et al., 2005). However, there are few reports concerning the use of high fiber ingredients in layer chicks. Masa'deh et al., (2012) fed layer chicks up to 125.0 g/kg of corn DDGS without negative performance results.

Previous experiments have utilized a single high fiber ingredient, but few have concentrated on a combination of high fiber ingredients. In this experiment, two high fiber feed ingredients (DDGS and wheat bran) were included. The objective was to evaluate the performance, metabolizable energy and neutral detergent fiber (NDF) digestibility of broilers and layer chicks fed corn-soybean diet or higher fiber corn-soybean – DDGS- wheat bran based diet over a 21 d feeding period.

### **Material and Methods**

All animal procedures were approved by the Institutional Animal Care and Use Committee of Iowa State University before the start of the experiment.

#### **Dietary treatments**

Starter diets for both broiler and layer chicks were formulated to be isocaloric and meet or exceed National Research Council (1994) poultry nutrient recommendations. Birds were fed one of the two diets; lower fiber diet based on corn-soy bean meal (SBM) and the higher fiber diet formulated based on corn- SBM-DDGS -wheat bran to contain 60.0 g/kg of both DDGS and wheat bran fed from 1-12 d and 80.0 g/kg of both DDGS and wheat bran fed from 13-21 d (Table 3.1). Birds had an *ad-libitum* access to feed and water throughout the experimental period. Titanium dioxide, an inert marker, was added to all diets at a rate of 2500 mg/kg to determine nitrogen corrected apparent metabolizable energy (AMEn) and neutral detergent fiber (aNDF) digestibility.

## **Birds and experimental design**

A total of 250 male Ross 308 broiler chicks (Aviagen Group, Huntsville., AL) and 250 male Hy-line W36 (Hy-Line International, Dallas Center, IA) layer chicks were secured from separate commercial hatcheries and transported to the ISU Poultry Research and Teaching Unit. On d 1, chicks were individually weighed, sorted by weight, wing banded and assigned by body weight to battery cages to minimize differences in mean treatment bodyweight of groups at the start of the experiment. Broiler and layer chicks were assigned in different battery cages in a completely randomized design. Chicks were maintained in raised wire battery cages (432cm<sup>2</sup>/chick) with continuous light in an environmentally controlled room where all chicks had initial access to a temperature of 32 °C that was gradually decreased to 26 °C in the last week of the experiment.

Treatments were arranged as a 2 x 2 factorial with two chicken lines (broiler and layer) and two dietary fiber concentrations (lower fiber and higher fiber). Each of the 4 treatments consisted of 11 experimental units of 8 chicks, resulting in 88 total chicks per treatment.

## **Data and sample collection**

Feed intake was determined by cage as the difference between feed offered and refused over the 1-12 d and 1-21 d periods and expressed as average daily feed intake (ADFI) in g per chick. Body weight gain was determined over 1-12 and 1-21 d periods expressed as average daily gain (ADG) in g per chick. Mortality corrected feed efficiency (FE) was expressed as body weight gain in g per kg of feed intake. The FE was corrected for mortality by subtracting the estimated feed intake of dead birds from the feed supplied to their respective pens. All chicks were euthanized by carbon dioxide asphyxiation on d 21 for collection of ileal contents. The

ileum was defined as the region between Meckel's diverticulum and the ileo-cecal junction. All ileal samples were pooled by cage. Clean excreta samples, free of feed or feathers, were collected over the 19 to 21 d period. Both ileal and excreta samples were stored frozen at -20 °C for later ileal aNDF analysis and excreta aNDF and AMEn analysis.

### **Chemical analyses**

Excreta and ileal samples were oven dried at 65°C for 3 d (AOAC, 2005), ground to pass a 1.0 mm sieve screen (Brinkmann Instruments Inc., Westbury, NY), and stored in airtight plastic containers for the determination of AMEn and aNDF disappearance. Feed samples were dried in a conventional oven at 100°C for 24 h (Yamato Scientific America Inc., Santa Clara, CA) and were ground to pass a 0.5 mm screen. Excreta and diet nitrogen (N) concentration was determined by the according to the AOAC (1995) using a Leco nitrogen/protein determinant (model FP-2000, Leco Corp., St. Joseph, MI) and adiabatic oxygen bomb calorimeter (Parr Instrument Co., Moline, IL) was used to determine gross energy. Titanium in both the feed and excreta was determined to calculate AMEn and aNDF disappearance (Leone, 1973). All samples (feed, excreta and ileal contents) were analyzed in duplicate. The Scott et al. (1982) equation was used to calculate AMEn, with one modification; chromic oxide was replaced with titanium dioxide as the inert dietary marker.

Ileal contents, feed, and excreta samples were analyzed for neutral detergent fiber using the method of Ankom Technology (2006 b) with amylase enzyme addition. Approximately 0.45-0.50 g of oven dried ground samples were weighed into pre-weighed filter bags, heat sealed and closed within 4mm of the top to encapsulate the sample. Feed sample bags were pre-treated with acetone to extract fat. The sample bags were then placed on each of eight suspender trays and

were rotated at 120 degrees in relation to the tray below. An empty bag was included in each run to determine the blank bag correction. Hot water ( $> 70\text{ }^{\circ}\text{C}$ ) was turned on, and 20 g of sodium sulfite plus 4.0 ml of alpha-amylase were added. 8.0 ml of alpha-amylase was diluted to a volume of 250 ml and poured into the attached amylase dispenser assembly to port B on the instrument. This amylase solution was added automatically by the ANKOM2000 instrument (Ankom Technology, Macedon, NY) during the first and second rinse. When NDF extraction and rinsing procedures were complete, excess water was gently pressed out of the sample bags and soaked in acetone for 3-5 minutes. Bags were air dried on a wire screen to remove acetone and then completely dried in an oven at  $102\text{ }^{\circ}\text{C}$  for 4 hours after which they were directly placed in collapsible desiccant pouch and flattened to remove air before measuring the final bag weights.

### **Statistical Analysis**

Statistical analysis was carried out using a two way ANOVA as a 2 x 2 factorial for a completely randomized design using GLM procedure in SAS (versions 9.3, 2012). Battery cage was defined as the experimental unit. Tukey's Honestly Significant Difference test was used to find difference among treatment means and significance was considered at  $P\leq 0.05$ .

### **Results**

There were no interactions ( $P>0.05$ ) between bird line and dietary fiber for ADFI, but as expected, broiler chicks consumed significantly more feed over 1-12 d and 1-21 d periods in comparison to layer chicks ( $P<0.01$ ; Table 3.2). Dietary fiber had no effect on ADFI in either line of birds ( $P>0.05$ ). There were significant interactions in ADG over both the 1-12 d ( $P=0.01$ )

and 1-21 d ( $P=0.02$ ) periods as higher dietary fiber resulted in a significant decrease in ADG for the broiler chicks, but did not have significant effects on layer chicks (Figure 1). Overall mortality was low and not dependent on diet or line. The broilers fed lower fiber, higher fiber, layers fed lower fiber and higher fiber treatments resulted in 1, 2, 1 and 0 mortalities, respectively. Over both the 1-12 and 1-21 d periods the significant ( $P=0.04$ ) and near significant ( $P=0.06$ ) interactions in FE are due to a non-significant decrease in FE in broiler chicks fed higher fiber diets and a subsequent non-significant increase in FE in layer chicks fed higher fiber diets (Figure 2).

Increasing dietary fiber significantly increased ileal ( $P<0.01$ ) and total tract ( $P=0.02$ ) aNDF disappearance regardless of bird line. Layer chicks had a significantly higher ( $P=0.01$ ) total tract aNDF disappearance in comparison to broiler chicks (Table 3.3). Although there were differences in aNDF disappearance, there were no significant main effects or interactions between line and dietary fiber for AMEn ( $P>0.41$ ).

## Discussion

As expected, broilers consumed more feed, grew at a faster rate and converted feed intake to body weight gain more efficiently than layer chicks regardless of dietary treatment. Interestingly, the higher fiber diet supported maximal ADG in the layer chicks, but resulted in reduced ADG in broiler chicks. In agreement with previous reports (Donkoh et al., 1999; Loar et al., 2010; Lumpkins et al., 2004;), broiler chicks' body weight gain was reduced at higher concentrations of high fiber dietary ingredients. A possible explanation for the reduced performance could be that inclusion of DDGS in broiler diets. A greater than 50 g/kg DDGS increases the rate of feed passage and reduces mean retention time, providing less time for the

digestion of nutrients (Rochell, 2012). In contrast to broiler chicks, layer chick ADG was not different between low or higher fiber diets. This response is again consistent with previous research in that 125g/kg of DDGS did not alter layer chick weight gain, feed intake or feed efficiency (Masa'deh et al. (2012). These results are especially interesting as there were no differences in feed intake in either broiler or layer chicks with the increased dietary fiber. This change in body weight gain without alterations in feed intake results in the interactions between bird line and dietary fiber content as broiler chick FE is slightly reduced by higher dietary fiber content and layer chick FE is slightly increased by higher fiber content. A possible explanation for the reduced performance could include higher total intake of high fiber feed ingredients in the broiler chicks resulting in reduced ADG. Fiber has been considered as a nutrient diluent in broiler diets that does not affect digestion and absorption of nutrients (Edwards, 1995), however, that observation is only reported for moderate levels of fiber (Hetland and Svihus, 2001; Hetland et al., 2002). Higher fiber concentrations in chick diets can have negative effects on nutrient digestion and absorption (Krogdahl, 1986) and may subsequently affect performance as seen in the ADG response of the broiler chicks in the current experiment.

Neutral detergent fiber measures the plant cell wall structural components and thus estimates hemicellulose, cellulose and lignin (Van Soest and Wine, 1967). The current experiment indicates that layer chicks had better total tract aNDF digestion than broiler chicks. This might be due to a relatively longer intestinal length in layer chicks compared to broiler chicks, increasing aNDF disappearance in layer chicks (Kaminiska, 1979). Another factor favoring increased aNDF disappearance is that reduced feed intake has been shown to reduce digesta passage rate and improve digestion and possibly fermentation (Tenoeschate et al., 1993). In both broiler and layer chicks increased dietary fiber resulted in increased ileal and total track

aNDF disappearance. This could possibly be due to the increased dietary fiber decreasing passage rate or mean transit time allowing for increased fermentation of dietary fiber and increased aNDF disappearance (Langhout, 1998). There does not appear to be large differences between aNDF disappearance at either the ileal or total tract level although there were differences in significance between measurement locations.

Although there were significant differences in aNDF disappearance between both chicken lines and dietary fiber content, there were no significant differences observed in the AMEn. This is not altogether surprising as diets were balanced with oil to maintain isocaloric content. Increases in dietary oil have also been associated with reduced intestinal transit or increased diet retention rates and could have also increased aNDF disappearance (Sibbald et al., 1962). Kaminiska, (1979) reported that Leghorn chicks derive between 1-7% more AME from a diet than broilers chicks and attributed this difference to Leghorn chicks having increased gizzard weights and longer intestines, relative to body weight. Current data from modern broiler and layer genetics may suggest that this is no longer the case as both lines extracted similar amounts of energy from a common diet.

In conclusion, feed intake was not altered in either broiler or layer chicks by the inclusion of two high fiber feed ingredients into the dietary formulation. Although feed intake was not different between either line, broiler chicks had reduced ADG with the high fiber ingredients while the layer chicks performed equally well regardless of dietary fiber. These responses cannot be completely attributed to fiber fermentation as layer chicks had increased aNDF disappearance and higher fiber diets stimulated aNDF disappearance. Although aNDF disappearance was altered by line and diet, AMEn was not altered in this experiment. Although



the mechanism is yet unclear, it appears that layer chicks may tolerate higher concentrations of high fiber dietary ingredients in comparison to faster growing broiler chicks.

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### Figures and Tables

**Table 3.1. Ingredient composition and nutrient content broiler starter diets fed from 1-21d of age.**

Ingredient Composition (g/kg)	Low fiber	High fiber	High fiber
	1-21d	1-12d	13-21d
Corn	609.9	505.6	470.8
Soybean Meal 48% CP	307.6	271.8	259.9
Dried Distillers Grains with Solubles	0.0	60.0	80.0
Wheat Bran	0.0	60.0	80.0
Meat and Bone Meal (Porcine)	20.0	20.0	20.0
Animal Vegetable blend oil	24.5	45.6	52.6
Salt	2.9	2.6	2.5
DL Methionine	1.7	1.7	1.7
Limestone	10.1	10.8	11.1
Dicalcium Phosphate	13.5	12.1	11.6
Choline Chloride 60	1.0	1.0	1.0
Vitamin and Mineral Premix <sup>a</sup>	6.25	6.25	6.25
Titanium Dioxide	2.5	2.5	2.5
-----			
Calculated Composition (g/kg)			
Crude Protein	210	210	210
Metabolizable Energy (MJ/Kg)	12.98	12.98	12.98
Calcium	10.0	10.0	10.0
Non-Phytate Phosphorus	4.5	4.5	4.5
Crude Fat	53.4	76.7	84.5
Digestible Methionine + Cysteine	8.5	8.5	8.5
Digestible Lysine	11.2	10.7	10.5
Digestible Threonine	8.1	7.9	7.9
-----			
Analyzed Composition (g/kg)			
Crude Protein	203	208	210
Neutral Detergent Fiber	100.2	140.2	149.7
Acid Detergent Fiber	24.5	37.5	42.5

<sup>a</sup> Provided per kg of Diet: Selenium-250 µg; Vitamin A-8,250 IU; Vitamin D<sub>3</sub>-2,750 IU; Vitamin A-17.9 IU; Menadione 1.1 mg; Vitamin B<sub>12</sub>-12 µg; Biotin-41 µg; Choline-447 mg; Folic acid-1.4 mg; Niacin-41.3 mg; Pantothenic acid-11 mg; Pyridoxine-1.1mg; Riboflavin-5.5 mg; Thiamine-1.4 mg; Iron-282 mg; Magnesium-125 mg; Manganese-275 mg; Zinc-275 mg; Copper-27.5 mg; Iodine-844 µg.

**Table 3.2. Effect of high and low fiber diets in broiler and layer chicks from 1 to 12 and 1 to 21 d of age on average daily gain (ADG), average daily feed intake (ADFI) and mortality corrected feed efficiency (FE).**

Line	Dietary fiber	1-12 d			1-21 d		
		ADG (g/bird)	ADFI (g/bird)	FE (g/kg)	ADG (g/bird)	ADFI (g/pen)	FE (g/kg)
Broiler		18.3	24.1 <sup>a</sup>	760	31.2	46.2 <sup>a</sup>	672
Layer		5.2	11.1 <sup>b</sup>	471	8.0	16.0 <sup>b</sup>	467
	High fiber	11.2	16.9	609	18.7	30.4	565
	Low fiber	12.4	17.7	621	20.0	31.8	573
..... P value .....							
Line		<0.01	<0.01	< 0.01	<0.01	<0.01	<0.01
Dietary fiber		<0.01	0.16	0.47	0.02	0.06	0.49
Line x Dietary fiber		<0.01	0.44	0.04	0.02	0.09	0.06
SEM <sup>1</sup>		0.19	0.39	12.4	0.42	0.51	8.9

<sup>a,b</sup> Values in the same column not sharing a common superscript differ significantly at  $P \leq 0.05$ .

<sup>1</sup> Pooled standard error of mean

N=11. Broiler average initial body weight = 40.0 g. Layer average initial body weight = 37.0 g

**Table 3.3. Effect of dietary fiber from DDGS and wheat bran in broiler and layer chicks fed from 1 to 21 d on nitrogen corrected Apparent Metabolizable Energy (AMEn), Ileal and apparent total tract aNDF disappearance**

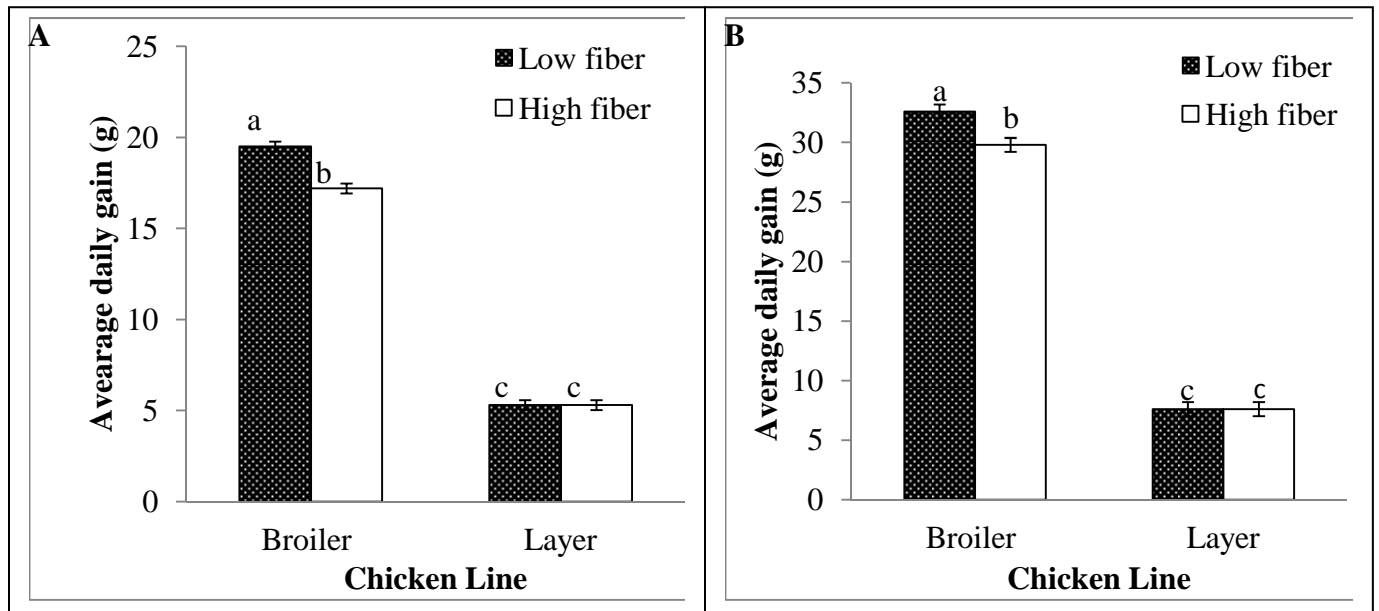
Line	Dietary fiber	AMEn (MJ/kg)	Ileal aNDF <sup>1</sup>	Excreta aNDF <sup>1</sup>
Broiler		13.42	0.21	0.19 <sup>b</sup>
Layer		13.41	0.22	0.23 <sup>a</sup>
	High fiber	13.42	0.25 <sup>a</sup>	0.23 <sup>a</sup>
	Low fiber	13.41	0.18 <sup>b</sup>	0.20 <sup>b</sup>
..... P value .....				
Line		0.96	0.39	0.01
Dietary fiber		0.99	< 0.01	0.02
Line x Dietary fiber		0.41	0.66	0.75
SEM <sup>2</sup>		0.119	0.007	0.010

<sup>a,b</sup> Values in the same column not sharing a common superscript differ significantly at  $P \leq 0.05$ .

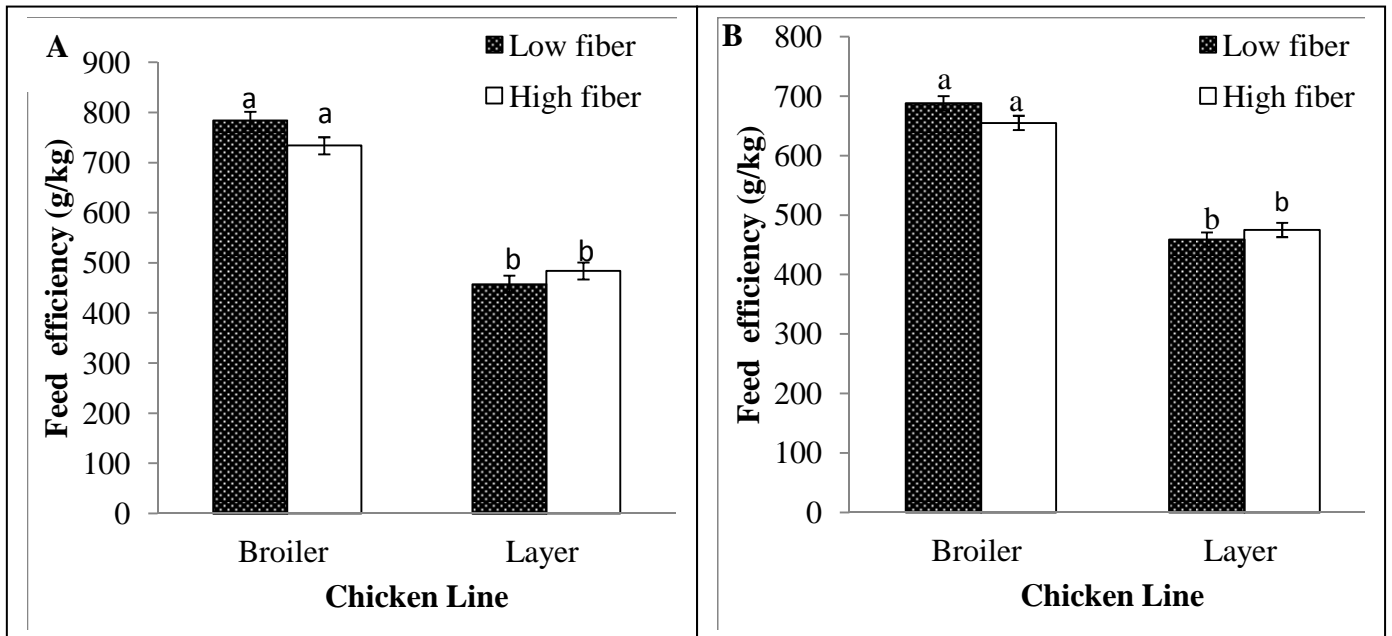
<sup>1</sup> aNDF disappearance reported in coefficients

<sup>2</sup> Pooled standard error of mean





**Figure 3.1.** Effect of low and high dietary fiber on average daily gain of broiler and layer chicks fed from 1 – 12 d and 1 – 21d of age. (A) Average daily gain from 1 – 12 d. (B) Average daily gain from 1 - 21 d. Values are reported in grams (g) as least squares means (LSM); n=44. LSM bars not sharing a common letter differ significantly,  $P \leq 0.05$ .



**Figure 3.2.** Effect of low and high dietary fiber on feed efficiency in broiler and layer chicks fed from 1-12 d and 1-21 d of age. (A) Feed efficiency from 1 – 12 d. (B) Feed efficiency from 1–21 d. Values are reported in g/kg as least squares means (LSM); n=44. LSM bars not sharing a common letters differ significantly,  $P \leq 0.05$ .

**CHAPTER 4. EFFECTS OF DIETARY FIBER ON CECAL SHORT CHAIN FATTY  
ACID CONCENTRATION AND MICROBIAL COMMUNITY OF BROILER AND  
LAYER CHICKS**

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**Abstract**

An experiment was conducted to evaluate the effects of feeding various concentrations of dietary fiber on cecal short chain fatty acid (SCFA) concentration and microbial communities of broiler and layer chicks. The lower fiber diet was based on corn-soybean meal (SBM) and the higher fiber diet was formulated using corn-SBM-dried distillers grains with solubles (DDGS) and wheat bran to contain 60.0 g/kg of both DDGS and wheat bran from 1-12 d and 80.0 g/kg of both DDGS and wheat bran from 13-21 d. Diets were formulated to meet or exceed NRC nutrient requirements. Broiler and layer chicks were randomly assigned to different treatments with 11 replicates of 8 chicks for each of the 4 treatments. The results indicated a significant interaction between bird line and dietary fiber for acetic acid (P=0.04) and total SCFA (P=0.04) concentration. There was a significantly higher concentration of acetic acid (P=0.02) and propionic acid (P<0.01) in broiler chicks compared to layer chicks. Increasing dietary fiber resulted in a significant reduction of butyric acid (P=0.03). Percent molar proportion of

individual SCFA was not different between broiler and layer chick lines. Higher dietary fiber resulted in lower percent molar butyric acid concentration ( $P=0.02$ ). The DNA of cecal microbial populations was isolated from cecal digesta samples and a variable 16S rRNA gene was amplified before digestion with both *HhaI* and *MspI* restriction enzymes and analyzed by terminal restricted length polymorphism (TRFLP). Multivariate analysis showed that cecal microbial communities varied due to diet ( $P=0.02$ ) and line ( $P=0.03$ ) with the *MspI* restriction enzyme. These results provide insights into the effect of dietary fiber on SCFA concentration and modulation of cecal microbiota in broiler and layer chicks. The increase in fermentable dietary fiber concentration in diets results in increased cecal SCFA concentrations. Cecal microbiota is altered by modification in the diet and DNA sequencing, other than TRFLP can characterize the altered bacterial colonies.

Key words: broiler, layer, short-chain fatty acid, ceca microflora,

### **Introduction**

With the increase in the price of corn and feed-grade oils and fats, the use of feed ingredients high in dietary fiber has received more interest from the poultry industry. Dietary fiber is a major component of the plant cell wall and consists of both non-starch polysaccharides (NSP) and non-carbohydrate components (Bach Knudsen, 2001). Previous experiments have reported that increasing fiber levels decreases growth rate and feed efficiency in chickens (Rick et al., 1982). However, some reports show that fiber source in poultry diets has an important role in gastrointestinal development and ultimately metabolism of energy (Jorgensen et al., 1996). Poultry are efficient at the degradation and utilization of starch as an energy source due to

efficient enzyme activity (Weurding et al., 2001), but are less efficient and with more variability in the degradation of NSP (Langhout and Schutte, 1996; Jorgensen et al., 1996). The inefficient and variable responses to dietary fiber are due to absence of digestive enzymes for fiber and the relatively short digestive tract and transit time of digesta within the chicken's gastro-intestinal tract (GIT) (Krogdahl, 1986; Iji et al., 2001)

Cecal microflora characterization started in the 1970s (Barnes et al., 1979) and recently better analysis techniques including molecular techniques have been used to address cecal microbial ecology (Zhu et al., 2002). Among the molecular techniques, the terminal restriction fragment length polymorphism (TRFLP) technique has been used to compare and contrast microflora in the duodenum, jejunum, ileum and ceca (Gong et al., 2002). Terminal restriction fragment length polymorphism examines intestinal microflora based on high-throughput, high resolution of the fingerprinting of the 16rRNA gene regions. In previous reports, the 16S ribosomal gene regions have been used to examine the bacterial diversity in the ceca. The analysis of broiler chickens retrieved 1656 bacterial nucleotide sequences from the contents of the ceca and some embedded in the cecal mucosal layer (Zhu et al., 2002).

The objective of the current experiment was to investigate the effects of increasing concentrations of dietary fiber through the addition of dried distillers grains with solubles and wheat bran on the SCFA cecal concentration, and cecal microbial ecology of broiler and layer chicks using a DNA based analytical method, terminal restricted fragment length polymorphism (TRFLP).

## **Materials and Methods**

### **Birds and experimental design**

A total of 250 male Ross 308 broiler chicks (Aviagen Group, Huntsville., AL) and 250 male Hy-line W36 (Hy-Line International, Dallas Center, IA) layer chicks were secured from separate commercial hatcheries and transported to the ISU Poultry Research and Teaching Unit. On d 1, chicks were individually weighed, sorted by weight, wing banded and assigned by line and body weight within line to battery cages to minimize differences in mean treatment bodyweight of groups at the start of the experiment. Broiler and layer chicks were assigned in different battery cages in a completely randomized design. Chicks were maintained in raised wire battery cages (432cm<sup>2</sup>/chick) with continuous light in an environmentally controlled room where all chicks had initial access to a temperature of 32 °C that was gradually decreased to 26 °C in the last week of the experiment.

Treatments were arranged as a 2 x 2 factorial with two chicken lines (broiler and layer) and two dietary fiber concentrations (lower and higher fiber). Each of the 4 treatments consisted of 11 experimental units of 8 chicks, resulting in 88 total chicks per treatment.

### **Dietary treatments**

Starter diets for both broiler and layer chicks were formulated to be isocaloric and meet or exceed National Research Council (1994) nutrient recommendations. The lower fiber diet was based on corn-soy bean meal (SBM) and the higher fiber diet was formulated based on corn-SBM-DDGS -wheat bran to contain 60.0 g/kg of both DDGS and wheat bran fed from 1-12 d

and 80.0 g/kg of both DDGS and wheat bran fed from 13-21 d (Table 4.1). Birds had *ad-libitum* access to feed and water throughout the experimental period.

### **Sample collection and DNA extraction**

On d 21, birds were euthanized by carbon dioxide asphyxiation. Both ceca were collected from one chick per each experimental unit, immediately frozen in liquid nitrogen and were later stored at -80°C until the analysis of SCFA and isolation of bacterial DNA. Nucleic acid was extracted for microbial ecology analysis. The ceca were cut open and DNA was isolated from ceca digesta samples using repeated Bead Beating plus Column (RBB+C) protocol for cell disruption as described by Yu and Morrison. (2004). DNA was quantified using 5 µl of sample in a 340 µl volume by UV 260/280 absorbance in a BioTek Synergy 2 multi-mode microplate reader (BioTek Instruments, Winooski, VT) and UV-compatible microplate.

### **SCFA concentration analysis**

The concentrations of acetic acid, propionic acid and butyric acid, were determined from the contents of one cecum per experimental unit. Frozen cecal samples were thawed at room temperature and approximately 0.45 g of cecum contents was gently squeezed into a pre-weighed 2 ml tube. 1 ml of 24% meta-phosphoric acid in 2.5 M sulfuric acid was added together with 4-methyl valeric acid as an internal standard. The acidified solution was mixed using a vortex mixer to form a homogenous solution, centrifuged at 17,000 x g for 20 min at 4°C. The supernatant were stored at -20°C until SCFA analysis was conducted. The supernatant was analyzed for SCFA using HP-FFAP column 30 m long with a 0.25 mm internal diameter, HP 6890 series Gas Chromatograph, and HP 5973 mass selective detector. There following

parameters were used; 1 µl injector volume, 12.15 psi pressure, 240°C injector temperature, 1.1 mL/min constant flow and helium carrier. The oven program used was as follows; 80°C initial temperature hold for 5 min, ramp 10°C per min to 240°C and 12 min hold at 240°C. The molar concentration of for each SCFA was calculated using 4 methyl valeric acid and the molar percentage of an individual SCFA was calculated by dividing the micro molar (µM) concentration of the individual SCFA by the µM sum of all the SCFA multiplied by 100. Individual SCFA concentrations was calculated as a percentage of the total SCFA content to determine if the increase in dietary fiber shifted the concentration of one SCFA to another.

## PCR

A variable region (341-783) of the bacterial 16S rRNA gene was amplified using 5'-fluorescein (6-FAM)-labeled 341f (5'-TCCTACGGGAGGCAGCAGT-3') primer and 5'-hexachloro-fluorescein (HEX)-labeled 783r (5'-TGGACTACCAGGTCTAATCCTGTT-3') primer (synthesized by Integrated DNA technologies, Coralville, IA). The PCR reactions were done in 0.5 mL thin-walled tubes (USA Scientific, Ocala, FL), containing 12.5 ng cecal DNA template, 5 µl of 10X *Pfx* amplification buffer (Invitrogen, Carlsbad, CA), 1.5 µl of a 10mM mixture of each of the four dideoxynucleotides triphosphates (Promega, Madison, WI), 1.5 µl of a 10 µM solution of each primer, 1 µl of 50 mM MgSO<sub>4</sub> (Invitrogen) and 0.4 µl of Platinum ® *Pfx* DNA polymerase (Invitrogen, Carlsbad, CA), made up to a final 50 µl volume with water. The thermal profile of the 16S rRNA gene amplification was as follows: denaturation at 94°C for 2 min, then 35 cycles of 94°C for 15 s, 55°C for 30 s and 68°C for 1 min, with a final extension step at 68°C for 5 min, followed by holding samples at 10°C. Negative controls, containing all components except DNA template were included in parallel. The 16S rRNA gene amplicons



were analyzed by gel electrophoresis using a 5  $\mu$ l aliquot of the product on 1% agarose gels and visualized after staining with ethidium bromide. PCR products were further purified to remove any protein using a phenol/chloroform extraction technique as follows. 50  $\mu$ l of TE buffer was added to 50  $\mu$ l PCR product and an equal volume of phenol/chloroform added to extract nucleic acid by vigorous shaking for 10-15 secs. After centrifugation (5,000 rpm for 1 min), about 90  $\mu$ l of the upper aqueous phase was transferred to a clean centrifuge tube. Exactly 9  $\mu$ l (1/10 volumes relative to total aqueous phase) sodium acetate (pH 5.5) was added, mixed briefly, and 300  $\mu$ l (ca. 3 volumes relative total aqueous absolute ethanol) added to the resultant mixture, gently mixed and stored at  $-80^{\circ}\text{C}$  for 15 min. The mixture was centrifuged (18,000 X g for 20 min) at  $4^{\circ}\text{C}$ . The supernatant was discarded and 1 x 200  $\mu$ l 75% ethanol added to wash the pellet and the spin repeated for 5 min. The Ethanol wash was discarded and the pellet air dried for 15-30 min. The dried pellet was re-dissolved in 50  $\mu$ l TE at  $55^{\circ}\text{C}$ , purified PCR products (5  $\mu$ l) were visualized by electrophoresis on 1% agarose gels with ethidium bromide staining and the remaining samples stored at  $-20^{\circ}\text{C}$  for later processing.

### **Digestion with restriction enzyme and T-RFLP analysis**

The amplified variable region DNAs were digested with either *Hha*I or *Msp*I restriction enzymes. The reactions set up in 0.5 mL thin-walled tubes containing 9  $\mu$ l of purified PCR sample, 2  $\mu$ l of 10X reaction buffer 4 (New England Biolabs, Ipswich, MA), and 1  $\mu$ l of 20 U/ $\mu$ l of either *Hha*I or *Msp*I in 20  $\mu$ l final volumes made up with water. BSA (NEB; 100 $\mu$ g/ $\mu$ l) was added in the *Hha*I reaction as required by the manufacturer. The digests were performed at  $37^{\circ}\text{C}$  for 2 hours and samples were later stored at  $-20^{\circ}\text{C}$  for later analysis. Fluorescent labeled terminal restricted fragments (T-RFs; 1.5  $\mu$ l) were separated using an automatic sequence analyzer, ABI

3730 (Applied Biosystems) and the T-RFs sizes in the range of 35-500 bp were determined using internal size standard (GeneScan-500 ROX; Applied Biosystems). T-RFLP electropherograms were analyzed with GelQuest 3.1.7 software (SequentiX, Germany).

### **Identification of operational taxonomical units (OTUs)**

The amplified variable region DNAs were digested with either *HhaI* or *MspI* restriction enzymes. The reactions set up in 0.5 mL thin-walled tubes containing 9  $\mu$ l of purified PCR sample, 2  $\mu$ l of 10X reaction buffer 4 (New England Biolabs, Ipswich, MA), and 1  $\mu$ l of 20 U/ $\mu$ l of either *HhaI* or *MspI* in 20  $\mu$ l final volumes made up with water. BSA (NEB; 100 $\mu$ g/ $\mu$ l) was added in the *HhaI* reaction as required by the manufacturer. The digests were performed at 37°C for 2 hours and samples were later stored at -20°C for later analysis. Fluorescent labeled terminal restricted fragments (T-RFs; 1.5  $\mu$ l) were separated using an automatic sequence analyzer, ABI 3730 (Applied Biosystems) and the T-RFs sizes in the range of 35-500 bp were determined using internal size standard (GeneScan-500 ROX; Applied Biosystems). T-RFLP electropherograms were analyzed with GelQuest 3.1.7 software (SequentiX, Germany).

### **Statistical analyses**

The SCFA concentration data were analyzed using GLM procedure (SAS 9.3, 2012) in a two way ANOVA with the battery cage as an experimental unit. Tukey's Honestly Significant Difference test was used to find difference among treatment means and the significance was considered at  $P \leq 0.05$ . The analysis of the microbial community was done separately for each restriction enzyme, *HhaI* and *MspI*, in both forward and reverse direction. Because a few OTU's dominated the microbial assemblage, peak heights were quarter root transformed, as

recommended by Clark and Green (1988). The Bray-Curtis measure of distance (Bray and Curtis, 1957) was calculated between each pair of samples. Relationships between samples were visualized using non-Metric Multidimensional Scaling (Gotelli and Ellison, 2013). The nMDS places all the samples in a two or three-dimensional plot so that the pairwise Bray-Curtis distances are maximally related to the distances between points on the plot. Hence, a pair of cecal microbial assemblages with similar composition will tend to be plotted near each other on the plot.

The differences in microbial assemblage composition were evaluated using a two-way analysis of variance on species composition data (perMANOVA, Anderson 2001). The analyses of the microbial community composition were done using the `vegdist()`, `metaMDS()` and `adonis()` functions in the `vegan` library version 2.0-7 (Oksanen et al., 2013) in R (R Core Team 2013).

## Results

The results for cecal SCFA content are shown in Table 4.2. Acetate concentration was highest, followed by butyrate and propionate. There was increased cecal acetic acid ( $P=0.02$ ) and propionic acid ( $P<0.01$ ) concentrations in broiler chicks in comparison to layer chicks. Increasing dietary fiber resulted in a significant ( $P=0.03$ ) decrease in butyric acid, but did not have an effect on other SCFA concentrations. There were no interactions ( $P>0.05$ ) between bird line and dietary fiber for both propionic and acetic acids, but significant interactions were observed in acetic acid ( $P=0.04$ ) and total SCFA ( $P=0.04$ ), as the increase in dietary fiber resulted in significant decrease of SCFA concentration in layer chicks in comparison to broiler chicks (Figure 4.1).

There were no interactions between chicken line and dietary fiber treatment for all the SCFA components when individual SCFA were computed as a percentage of the total SCFA. The percent proportion of butyric acid was higher for lower dietary fiber compared to high dietary fiber (22.66 vs  $17.31 \pm 1.62$  %, respectively;  $P=0.02$ ). There were no significant differences in percent proportions of acetic and propionic acids regardless of dietary fiber concentration or chicken line.

Multivariate statistical analysis showed that the composition of the microbial community was significantly different in the ceca among the dietary treatments when *MspI* restriction enzyme was used. The differences were due to diet ( $P = 0.02$ ) and line ( $P = 0.03$ ), with no evidence of an interaction. No significant differences in microbial populations were observed with *HhaI* restriction enzyme for forward and reverse primer dyes (Table 4.3).

The differences in cecal bacterial community associated with diet and line using the *MspI* restriction enzyme are shown in Figure 4.2 and Figure 4.3 respectively. These relationships are represented in three dimensional space. The stress value of the three-dimensional ordination is reasonably low (less than 0.10), implying that it is a good representation of the overall cecal microbial community. The more similarity that exists between samples, the closer the points on the NMDS plot, assuming the plot is a good representation of the distance matrix.

## Discussion

In chapter 3, results showed significant differences in performance and aNDF disappearance between broiler and layer chicks. This report further investigates whether the differences in performance can be attributed to SCFA concentration or microbial ecology between the two chicken lines fed various concentrations of dietary fiber. As expected, there was

increased acetic acid concentration compared to propionic and butyric acid regardless of chicken line. This is consistent with previous reports which show that acetic acid production starts early and was present at 3 days of age in chickens while propionic acid and butyric acid were detected after 12 days of age (Van der Wielen et al., 2000; Lan et al., 2005). In the current experiment, we observed differences in concentrations of the SCFA between broiler and layer chicks, with exception of butyric acid. Previous reports have shown that there is relatively longer intestinal length in layer chicks compared to broiler chicks (Kaminiska, 1979) which could contribute to a difference in SCFA concentration between broiler and layer chicks. However, the difference in intestine length is not thought to be a major contributing factor in the current experiment because the two chick lines are bred ignoring differences in the intestinal lengths. In addition, modern broiler chickens have been reported to have high rates of intestinal development (Lumpkins et al., 2010). The variation in rates of intestinal development between broilers and layer chicks could also provide a possible explanation for the differences in SCFA concentration between the two lines as broiler chicks may ferment more dietary fiber to support the rapid growth rate of intestinal development.

The percentage of butyric acid was significantly lowered when dietary fiber ingredients were increased in the diet, unlike other SCFA that were not different between higher and lower fiber diets. In the current experiment, the ingredients used were high in insoluble fiber that is mainly a nutrient diluent and does not directly affect digestion (Angkanaporn et al., 1994; Edwards, 1995). Since the feed ingredients used in the current experiment contain high insoluble fiber levels, the results of total SCFA concentration between high and low fiber treatments are not surprising as insoluble fiber is not highly fermented in poultry. All the indigestible components of dietary fiber in the diet such crude fiber will have insignificant fermentation and

may not effect SCFA concentration chicks ceca (Angkanaporn et al., 1994). In contrast to previous reports with insoluble fiber, the relative concentration of acetic acid decreased as dietary fiber ingredients were increased in broiler chick diets while butyric acid significantly increased with high dietary fiber (Danayrolles et al., 2007). In the current experiment, DDGS and wheat bran were utilized as high fiber ingredients in a corn-SBM basal diet. The ingredients used (i.e corn, SBM and DDGS) are known to contain high proportions of insoluble fiber, with exception of wheat bran which contains slightly high amount of soluble fiber. However, the highest amount of wheat bran used was only 80 g/kg and would not cause a significant impact on fermentation. The results might be different with higher soluble fiber ingredients due to increases in microbial proliferation that would cause more SCFA acid generation as dietary fiber increases in the diet.

Short chain fatty acids have been shown to inhibit pathogenic bacteria (Russell, 1992) and also have toxic effects on some *Enterobacteriaceae* (McHan and Shotts, 1993; Van der Weielen et al., 2000). But they do not have negative effect on beneficial gastrointestinal tract bacteria such as *Lactobacillus* (Van der Weielen et al., 2000).

In this experiment, the overall cecal bacterial community structure was analyzed using a molecular, culture independent, fingerprinting technique. The terminated restricted fragment length polymorphism (T-RFLP) is a rapid analysis method that is robust and reproducible and is important in comparing microbial communities in several biological systems (Moeseneder et al., 1999; Dunbar et al., 2001; Egert et al., 2004). The method has a high resolution and can detect differences in microbial species that are less abundant compared to other microbial profiling analysis techniques such as the denaturing gradient gel electrophoresis (Moeseneder et al., 1999). However, T-RFLP like all other PCR-based technologies has some limitations. There may be

biased representation of microbial communities due to primer choice (Zhu et al., 2002), concentration of DNA template (Chandler et al., 1997) or the number of PCR cycles that are used (Suzuki and Giovannoni, 1996) and does not reveal the specific microbes present. These conditions may have affected our results.

Diet and line contributed to the difference in composition of cecal microbial communities with the *MspI* restriction enzyme. As expected, increasing dietary fiber resulted in significant differences in cecal microbial communities. In agreement with previous reports, diet has been shown to modify the overall microbial community (Apajalahti et al., 1998; Lan et al., 2005). This response is again consistent with previous research which indicated that source of feed and local feed modifications can significantly influence intestinal microbial communities, whereas birds that were fed on identical feed regimes had closely aligned microbial profiles (Apajalahti et al., 2001; Hume et al., 2003). The current experiment showed a difference in microbial communities between broiler and layer lines. In agreement with previous reports, difference in genetic lines of chickens with varying growth rates of intestinal development have previously resulted in a difference in bacterial populations (Lumpkins et al., 2010).

In conclusion, the current experiment was conducted to determine the effect of increasing dietary fiber on cecal SCFA concentration and microbial ecology. It appears that cecal microbiota fermented diet ingredients to acetate, propionate and butyrate as the major products. Based on NMDS, cecal microbiota can be altered by changes in the dietary fiber and different chicken lines may be inhabited by different microbial organisms. The results of this experiment provide insights in understanding the effects of dietary fiber on cecal SCFA concentration and microbiota of broiler and layer chicks. The increase in fermentable dietary fiber concentration in diets results in increased cecal SCFA concentrations. Cecal microbiota is altered by modification

in the diet, and broiler and layer chicks are inhabited by different microbes which could partly explain why the two chicken lines had differences in performance as reported in chapter 3. The characterization of bacteria by DNA sequencing would provide more detail about the microbial colonies in the chick ceca.

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### Figures and Tables

**Table 4.1. Ingredient composition and nutrient content of broiler starter diets fed from 1-21d of age.**

Ingredient Composition (g/kg)	Low fiber	High fiber	High fiber
	1-21d	1-12d	13-21d
Corn	609.9	505.6	470.8
Soybean Meal 48	307.6	271.8	259.9
Dried Distillers Grains with Solubles	0.0	60.0	80.0
Wheat Bran	0.0	60.0	80.0
Meat/bone Meal	20.0	20.0	20.0
Animal Vegetable blend	24.5	45.6	52.6
Salt	2.9	2.6	2.5
DL Methionine	1.7	1.7	1.7
Limestone	10.1	10.8	11.1
Dicalcium Phosphate	13.5	12.1	11.6
Choline Chloride 60	1.0	1.0	1.0
Vitamin and Mineral Premix <sup>a</sup>	6.25	6.25	6.25
Titanium Dioxide	2.5	2.5	2.5
<b>Calculated Composition (g/kg)</b>			
Crude Protein	210	210	210
Metabolizable Energy (MJ/Kg)	12.98	12.98	12.98
Calcium	10.0	10.0	10.0
Non-Phytate Phosphorus	4.5	4.5	4.5
Fat	53.4	76.7	84.5
Digestible Methionine + Cysteine	8.5	8.5	8.5
Digestible Lysine	11.2	10.7	10.5
Digestible Threonine	8.1	7.9	7.9
<b>Analyzed Composition (g/kg)</b>			
Crude Protein	203	208	210
Neutral Detergent Fiber	100.2	140.2	149.7
Acid Detergent Fiber	24.5	37.5	42.5

<sup>a</sup> Provided per kg of Diet: Selenium-250 µg; Vitamin A-8,250 IU; Vitamin D<sub>3</sub>-2,750 IU; Vitamin A-17.9 IU; Menadione 1.1 mg; Vitamin B<sub>12</sub>-12 µg; Biotin-41 µg; Choline-447 mg; Folic acid-1.4 mg; Niacin-41.3 mg; Pantothenic acid-11 mg; Pyridoxine-1.1mg; Riboflavin-5.5 mg; Thiamine-1.4 mg; Iron-282 mg; Magnesium-125 mg; Manganese-275 mg; Zinc-275 mg; Copper-27.5 mg; Iodine-844 µg.

**Table 4.2. Effect of increasing dietary fiber on short chain fatty acid (SCFA) concentration in broiler and layer chicks at 21 d of age.**

Line	Dietary fiber	Acetic acid	Propionic acid	Butyric acid	Total SCFA
..... $\mu\text{mol/g}$ .....					
Broiler		48.03 <sup>a</sup>	7.97 <sup>a</sup>	13.99	70.95 <sup>a</sup>
Layer		41.57 <sup>b</sup>	5.32 <sup>b</sup>	12.53	60.16 <sup>b</sup>
	High fiber	46.05	6.60	11.20 <sup>b</sup>	64.55
	Low fiber	43.54	6.69	15.34 <sup>a</sup>	66.56
..... P value .....					
Line		0.02	<0.01	0.43	<0.01
Dietary fiber		0.35	0.89	0.03	0.54
Line x Dietary fiber		0.04	0.91	0.53	0.04
SEM <sup>1</sup>		1.862	0.456	1.305	2.326
..... % Proportion .....					
Broiler		68.00	11.25	19.38	
Layer		69.00	8.88	20.83	
	High fiber	71.32	10.30	17.31 <sup>b</sup>	
	Low fiber	65.92	9.93	22.66 <sup>a</sup>	
..... P value .....					
Line		0.60	0.62	0.67	
Dietary fiber		0.06	0.21	0.02	
Line x Dietary fiber		0.97	0.16	0.71	
SEM <sup>1</sup>		2.072	2.452	1.624	

<sup>a,b</sup> Values in the same column not sharing a common superscript differ significantly at  $P \leq 0.05$ .

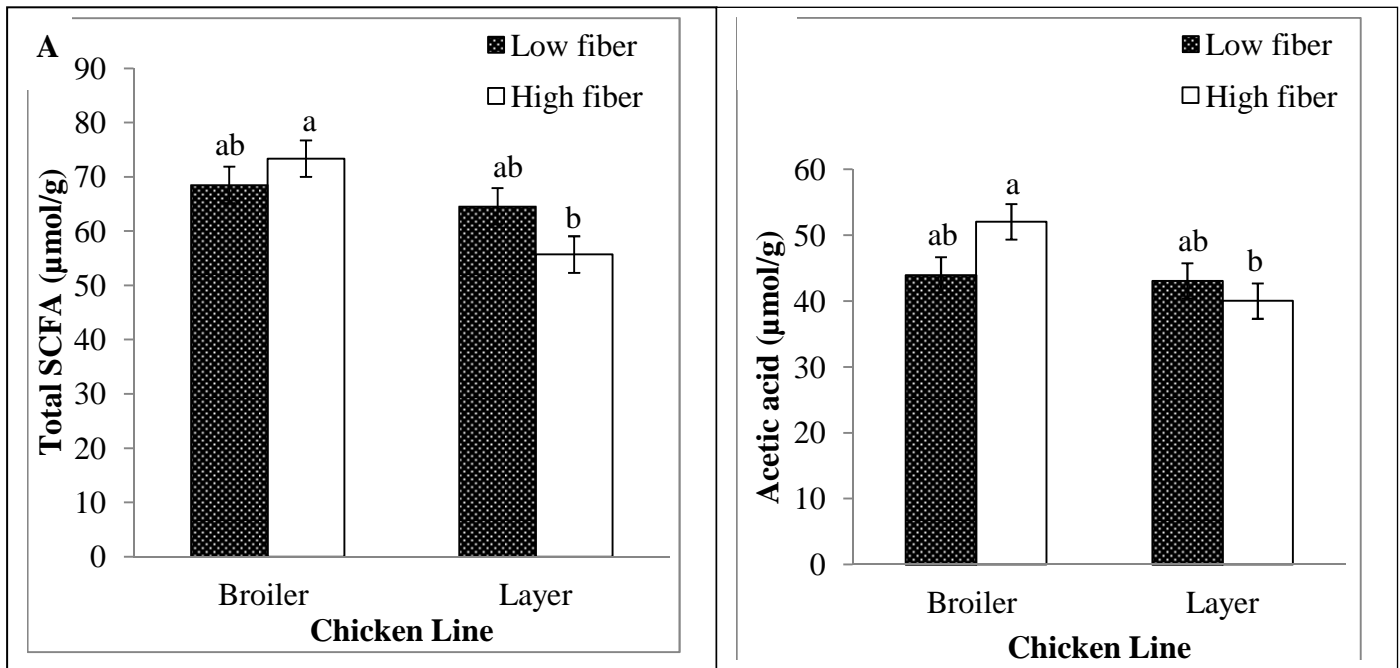
<sup>1</sup> Pooled standard error of mean

**Table 4.3. Two-way PerMANOVA of cecal microbial communities associated with diet and line for the two primers and two restriction enzymes<sup>a</sup>**

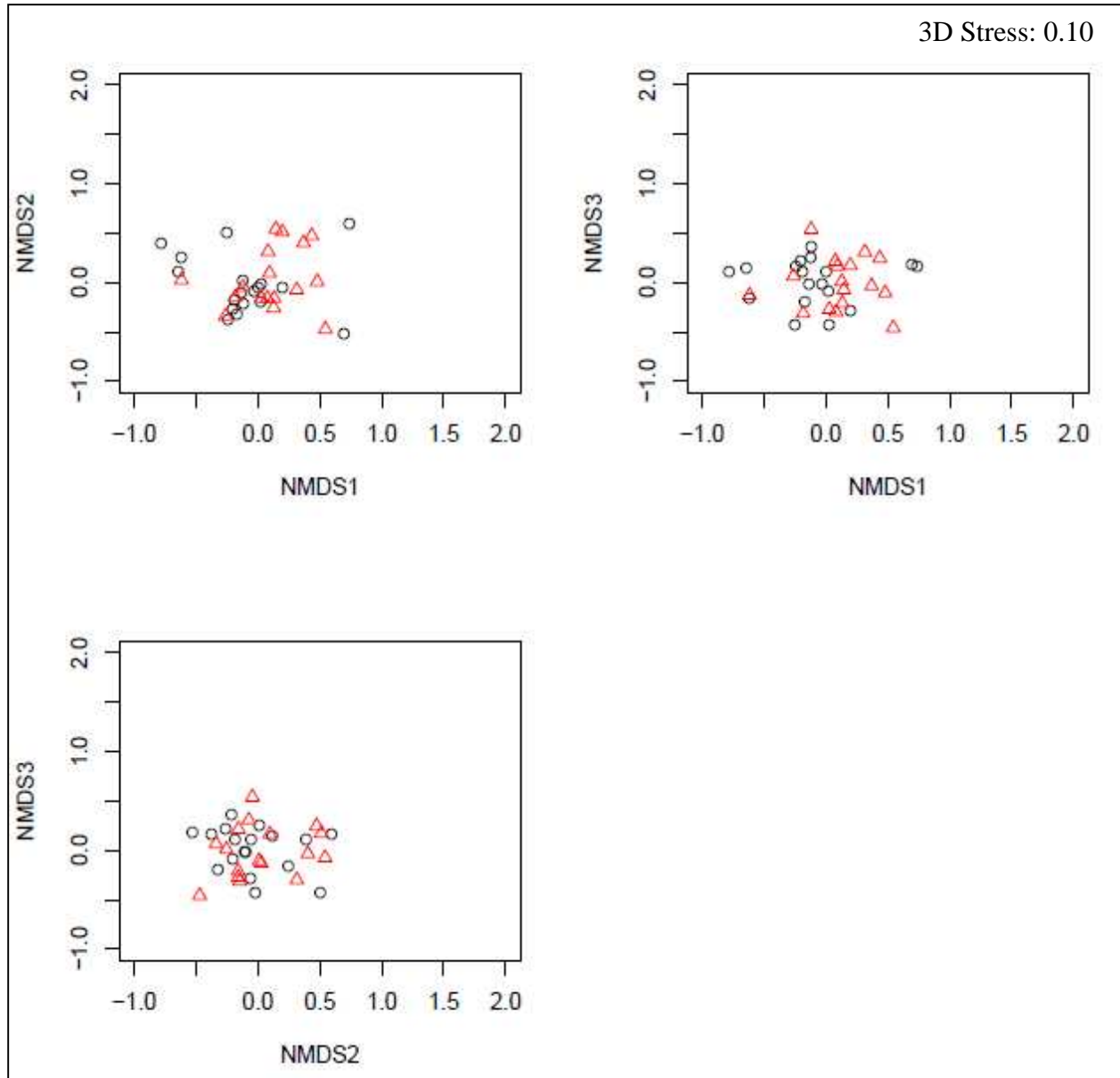
Parameter	P values			
	Hforward	Hreverse	Mforward	Mreverse
Diet	0.640	0.646	0.023	0.263
Line	0.425	0.458	0.268	0.028
Diet X Line	0.129	0.110	0.111	0.241

<sup>a</sup>The p-value for each of the factors with forward (blue) and reverse (green) primers and each restriction enzyme, *HhaI* (H) and *MspI* (M)

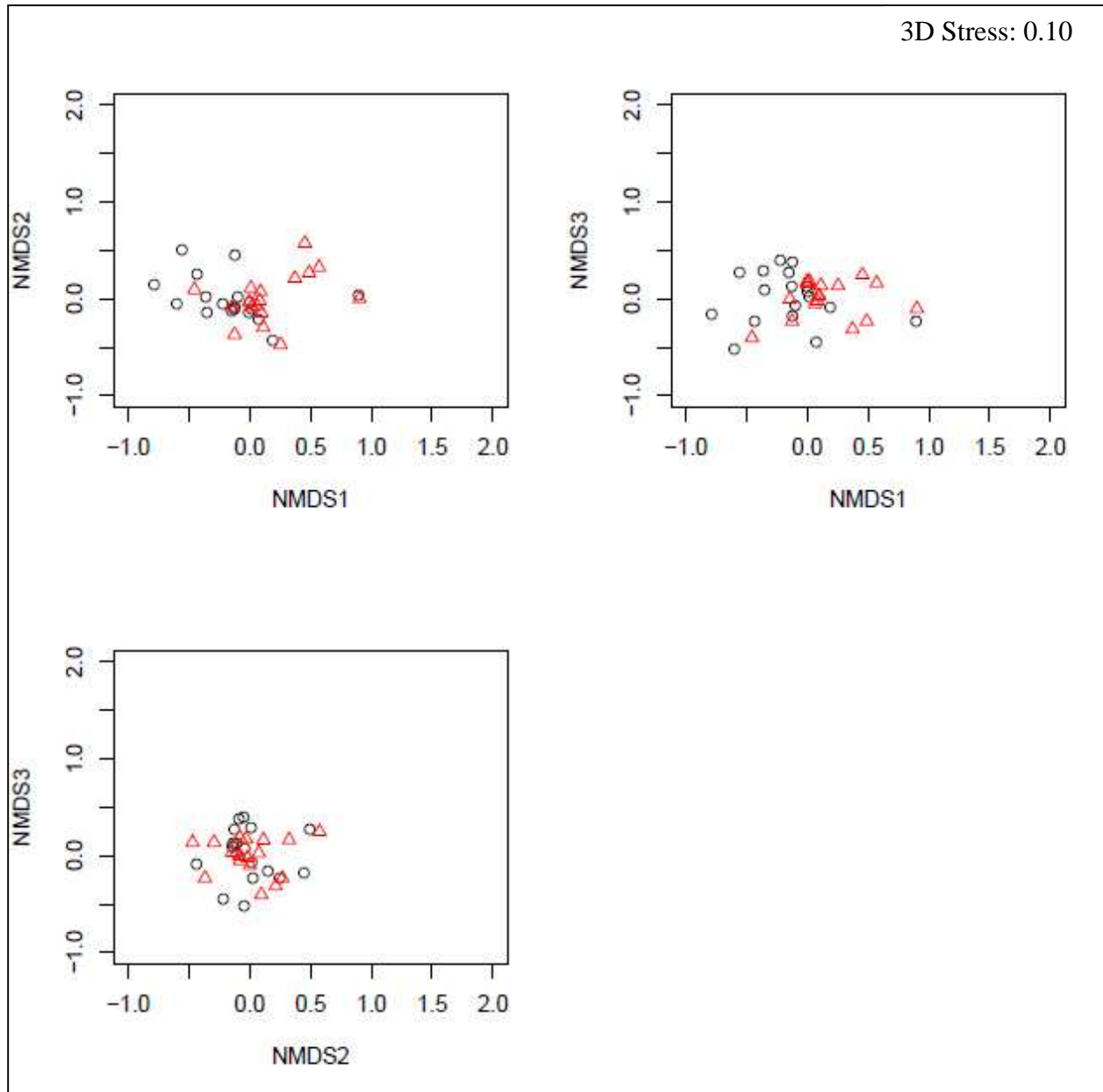




**Figure 4.1.** Effect of increasing dietary fiber on acetic acid and total short chain fatty acid (SCFA) concentration in broiler and layer chicks at 21 d of age. (A) Total chain fatty production. (B) Acetic acid production. Values are reported as least squares means (LSM); n=44. LSM bars not sharing common letters differ significantly,  $p \leq 0.05$ .



**Figure 4.2.** Pairwise plots of the 3-dimensional nonmetric multidimensional scaling (nMDS) ordination of cecal microbial communities from MspI blue (Mblue) dye of higher (red open triangles) and lower (black open circles) fiber diets showing the overall microbial community pattern



**Figure 4.3.** Pairwise plots of the 3-dimensional nonmetric multidimensional scaling (nMDS) ordination of ceal microbial communities from MspI green (Mgreen) dye of broiler (black open circles) and layer (red open triangles) chick lines showing the overall microbial community pattern

## CHAPTER 5. GENERAL CONCLUSIONS

The hypothesis for the thesis was that increasing dietary fiber by the use of DDGS and wheat bran would lead to no change in performance allowing for the feeding of lower cost alternatives to corn and soybean meal in poultry diets. This would be accomplished by altering energy utilization and cecal microbiota populations in both broiler and layer chicks. The ability of high dietary fiber ingredients to maintain performance and energy utilization would validate commercial producers' use of maximum inclusion of high dietary fiber feed ingredient in chick starter diets so as to achieve optimal performance using lower cost feed ingredients. Therefore the objective of the thesis was to evaluate the effects of feeding high dietary fiber from corn dried distillers grains with solubles (DDGS) and wheat bran on broiler and layer chicks' performance, metabolizable energy, aNDF disappearance, short chain fatty acid (SCFA) concentration and cecal microbial ecology.

Feeding dietary fiber impacts performance as increased fermentation of fermentable fiber such as NDF in the ceca would lead to increased SCFA concentration which in turn contributes to cecal microbiota proliferation, resulting in further fermentation. Although the amount of energy generated from the SCFA in the chicken ceca may be low, it could have significant impact on the performance of broiler or layer chicks. The effect on performance will also depend on the type of fiber in the feed ingredients as the fermentative ability of cecal microbiota correlates with the amount and type of dietary fiber components. Hence, fermentation of dietary fiber to SCFA will result into proliferation of cecal microbes.. The proliferation causes further SCFA production and ultimately contributes to the energy requirements of the birds.

The experimental high fiber diet formulated with 60.0 and 80.0 g/kg of both DDGS and wheat bran for the first and second feeding periods, respectively, in broiler starter diets clearly affected broiler chick performance, but did not have negative effects on layer chicks. Average daily gain for broiler chicks decreased significantly ( $P \leq 0.05$ ) as dietary fiber was increased over the two periods of feeding. Feed intake levels were not altered in either broiler or layer chicks by the inclusion of the two high fiber feed ingredients into the dietary formulation. Fiber analysis revealed that layer chicks had significantly higher total tract neutral detergent fiber (aNDF) disappearance in comparison to broiler chicks. Although there were differences in aNDF disappearance between the two chicken lines, apparent metabolizable energy was not affected. The results of the experiment lead to a conclusion that layer chicks can utilize feed ingredients richer in dietary fiber better compared to broiler chicks and that 80.0 g/kg of both DDGS and wheat bran is acceptable for starter diets in layer chicks, with no detrimental effects on performance. Therefore one recommendation from this research is that high fiber ingredients can be included at higher rates in layer starter diets in comparison to broiler starter diets. Additional research on diet and fiber transit time might better elucidate the mechanism that allows layer chicks to better tolerate high dietary fiber concentration compared to broiler chicks.

Several experiments have been conducted in broilers and layer hens, and up to 50.0 g/kg and 100.0 g/kg DDGS have been recommended in broiler starter and finisher diets, respectively. The current experiment showed that layer chicks utilized high fiber diets formulated with DDGS and wheat bran better in comparison to broiler chicks possibly due to the increased aNDF disappearance. In addition, a separate experiment could be conducted to determine both ileal and faecal starch and non-starch polysaccharides disappearance. Because chickens do not secrete digestive enzymes to digest dietary fiber, total faecal disappearance will be greater than ileal

disappearance and it will be assumed that that very little of the NDF and acid detergent fiber (ADF) fractions will be altered before reaching the ceca. An experiment which also involves the determination of starch digestion rate between broiler and layer chicks will address any differences in starch digestion rate between the lines.

The final part of the thesis investigated the effects of increasing dietary fiber on cecal SCFA concentration and microbial population. Increasing dietary fiber resulted in significantly higher ( $P \leq 0.05$ ) SCFA concentration in broiler chicks in comparison to layer chicks. However, no changes in percent proportion of SCFA were detected between the chicken lines. Cecal microbial analysis revealed that modification of diet through use of high fiber feed ingredients altered microbial population. The cecal microbial population also appeared different as a result of lines difference, indicating that broiler and layer chicks were inhabited by different microbiota. In healthy chickens, bacteria like *Enterococcus spp*, *Lactobacillus spp*, and *Enterobacteriaceae spp* predominate the ceca during the first days of life, while *Eubacterium spp* and *Bacteriodes spp* establish themselves after about two subsequent weeks of life. Increasing dietary fiber will most likely alter those particular bacterial species as the current experiment involved d 1 old chicks that were kept under experimentation for 21 d of age. Alteration of beneficial bacteria could alter SCFA concentration in the chicks' ceca resulting in the difference noted between the two lines of chickens.

Terminal restricted length polymorphism (TRFLP) is a robust analytical method of studying only gross differences among gastrointestinal tract microbial (GIT) populations and hence further research is recommended to fully understand the particular microbial species that are altered. The TRFLP analytical method gives an insight of the microbial community by presenting concentration of the bacteria present but only selected identified bacterial fragment

peaks can be used. Other analytical methods such as the denaturing gradient gel electrophoresis have been used to estimate bacterial diversity in the chicken GIT, but they do not provide full knowledge of the lines or species. Hence, to fully characterize and identify the bacterial lines from any section of the GIT such as the ceca, total genomic DNA should be isolated from the cecal fecal samples and then sequenced. Cecal samples could be collected at different time periods (4, 7, 14, and 21 d) to further investigate whether cecal microbiota changes with time.

In conclusion, higher fiber ingredients can be an important feed ingredient in commercial chick starter diets so as to reduce feed cost without compromising chicken performance. Performance results of the experiment are in agreement with previous the literature reports for broiler chicks but there are fewer reports on chicks and this is the first report to compare the two. High fiber feed ingredients are suitable substitutes for higher supplementation rates in layer chick starter diets in comparison to broiler chick starter diets.