EFFECT OF DIETARY FIBRE LEVELS AND PARTICLE SIZE ON BROILER PERFORMANCE, GUT MORPHOGENESIS AND IMMUNE STATUS

BY

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DECLARATION

I, EUNICE AGYEI HENNEH, declare that this thesis submitted for the award of the degree of Master in Philosophy (Animal Science) is entirely my own conducted research. Apart from references to other works, which have been duly cited, this thesis has neither been submitted or being submitted simultaneously for a degree elsewhere.

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ABSTRACT

The study investigated the effect of increasing dietary fibre levels and varying maize particle size on nutrient digestibility, growth performance, gut morphogenesis and immune status of broilers. Three hundred Cobb 500 broiler chicks were randomly allocated to six dietary treatments in a 2 X 3 factorial arrangement (two maize particle sizes and three levels of wheat bran inclusion). Each treatment was replicated five times with ten broilers per replicate and the trial lasted for 49 days. Treatments 1 (T1), 2 (T2; control diet) and 3 (T3) contained fine maize particles with treatments 4 (T4), 5 (T5) and 6 (T6) containing coarse maize particles. Birds on T1 and T4, T2 and T5, and T3 and T6 were fed 6, 8 and 10% wheat bran respectively from day one to 21. Wheat bran inclusion from day 22 to 42 increased to 13, 15 and 17% for birds on T1 and T4, T2 and T5, and T3 and T6 respectively. There were no significant differences ($p \geq 0.05$) in nutrient digestibility among treatments. Overall, average daily feed intake increased for broilers on T4 (107.15g) with no dietary treatment effect being observed on average daily gain and final body weight. However, broilers fed coarse maize particles were the most efficient in converting feed to gain. The relative empty gizzard weight of broilers fed coarse maize particles was significantly higher ($p \leq 0.05$) than that of broilers fed fine maize particles. No significant differences ($p \geq 0.05$) were observed in relative carcass weights, caecal short-chain fatty acid concentrations, haematological indices and serum glucose content. Averagely, concentration of acetic acid in the caecum (8147.14 µg/g) was the highest compared with the other short-chain fatty acids. Results of this study indicate that birds can tolerate diets containing up to 17% (finisher phase) WB with fine or coarse maize particles without any adverse effect on growth, gut health and immune status. Feed manufacturers can include up to 17% WB (finisher phase) with fine or coarse particles in broiler diets in order to cut down feed cost with no adverse effect on broiler performance and gut health.

DEDICATION

I dedicate this work to the blessed memory of my gifted mentor, Dr. Thomas N. N. Nortey, Senior lecturer, Department of Animal Science, School of Agriculture, College of Basic and Applied Sciences, University of Ghana for sowing in me seeds of academic and lifetime excellence. With God's grace, I will surely make these seeds blossom. God richly bless you and your family.

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LIST OF ABBREVIATIONS

AA	Acetic Acid
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
AFI	Alternative Feed Ingredients
ANF	Anti-Nutritional Factor
BA	Butyric Acid
BW	Body Weight
CD	Crypt Depth
CF	Crude Fibre
CP	Crude Protein
DDGS	Dried Distillers Grains with Solubles
DF	Dietary Fibre
DM	Dry Matter
EE	Ether Extract
FOS	Fructo-Oligosaccharides
GIT	Gastro-Intestinal Tract
GOS	Galacto-oligosaccharides
GSD	Geometric Standard Diameter
HCL	Hydrochloric Acid
HCT	Haematocrit
HGB	Haemoglobin
HPLC	High-Performance Liquid Chromatography-Ultraviolet Detection
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Cell Volume
MOS	Mannan-Oligosaccharides
NCP	Non-Cellulosic Polysaccharides
NFE	Nitrogen Free Extracts
NSP	Non-Starch Polysaccharides
OM	Organic Matter
PA	Propionic Acid

PCV	Packed Cell Volume
RBC	Red Blood Cell
RS	Resistant Starch
SCFA	Short-Chain Fatty Acids
SDF	Soluble Dietary Fibre
TNSP	Total Non-Starch Polysaccharides
TOS	Total Oligosaccharides
VFA	Volatile Fatty Acids
VH	Villi Height
WB	Wheat Bran
WBC	White Blood Cell

CHAPTER ONE

INTRODUCTION

Poultry meat and their associated products are the leading protein choice in human nutrition internationally (Mottet and Tempo, 2017). Consequently, in most developing countries, the poultry sub-sector is one of the fastest growing sectors in the agricultural industry (Adeyonu *et al*., 2021). This rapid growth is attributed to factors such as urbanization, continuous rise in global population and increase in purchasing power (Borda-Molina *et al.*, 2018). For instance, about 130 million tons of chicken was projected to be produced globally in 2020 to match the demands of the growing world population (FA0, 2020). Again, according to Alexandratos and Bruinsma (2012), the production of poultry meat will increase by 121% by 2050 in order to prevent food scarcity. Additionally, Mottet and Tempio (2017) stated that, the comparative ease in the day-to-day management of poultry also makes it a preferred enterprise by smallholder farmers.

Researchers are constantly searching for intervening measures to increase production efficiency while maximizing profit (Yegani and Korver, 2008). These intervening measures such as increased broiler growth rates and feed efficiency are essential as they can potentially offset some of the 60–70% feed cost (Cooper and Songer, 2009). However, these advances made in growth rates and feed efficiency of broilers have made maintaining their health status very demanding (Cooper *et al.*, 2013). This is due to the possible increase in the incidence of diseases and infections, which can lead to the loss of profit. Several studies have indicated the maintenance of the gut (gastrointestinal tract, GIT) ecosystem as an effective way of enhancing broiler health and optimal performance (Thursby and Juge, 2017; Jha *et al.*, 2019). The gut is responsible for digestion and absorption of nutrients, pathogen destruction and immune system development (Dittoe *et al.*, 2018). Traditionally, the inclusion of antibiotics in broiler diets helped maintain the gut ecosystem

(Huyghebaert *et al.*, 2011). However, incidence of microbial resistance and antibiotic residues in chicken have been associated with the long-term use of antibiotics in animals (Mathew *et al.*, 2007). The inclusion of feed ingredients which have the ability to create a favourable environment for the growth of beneficial bacteria while maintaining gut integrity and health has been recommended as an alternative nutritional strategy to the use of antibiotics (Rinttilä and Apajalahti, 2013; Borda-Molina *et al.*, 2018). This implies that, the objective of feed formulation must be geared towards enabling broilers achieve their optimum growth performance while boosting gut health (Ghaffari *et al.*, 2007).

Currently, the concept of alternative feed ingredients (AFIs) inclusion in broiler feed has been largely considered as a way to reduce broiler production cost, boost gut health while maximizing optimum growth and profit (Nortey *et al.*, 2013). This is because these AFIs are readily available, less costly and are not consumed by humans. Agro-industrial/feed processing by-products such as wheat bran, cassava root meal, cocoa pod husks, corn cobs and sorghum barley brewer's spent grain are examples of AFIs (Nortey *et al.*, 2013; Nortey *et al.*, 2015). The use of agro-industrial/feed processing ingredients partially in feed manufacture for broilers is widely practiced by Ghanaian smallholder and rural broiler farmers in order to reduce feed cost. Also, feed manufacturing companies are gradually embracing the use of AFIs (groundnut cake, palm kernel cake and fish meal) as replacements for major broiler feed ingredients (soybean meal, wheat and maize (5m editor, 2011). However, these AFIs are high in dietary fibre (DF). This limits their inclusion in broiler diets due to the absence of endogenous enzymes in broilers (Jha *et al.*, 2019). Traditionally, all broiler diets contain an amount of DF due to the grains and legume-based feed ingredients used in broiler diets (Mateos *et al.*, 2012). As such, commercial feed mills produce diets with about three percent crude fibre content (Mateos *et al.*, 2012).

Dietary fibre is the indigestible portion in the cell walls of plant carbohydrates such as cereals, legumes and agro-industrial/feed processing by-products (Tiwari and Jha, 2017). Recent studies have shown the positive effects dietary fibre (DF) has on promoting optimum growth performance and gut health (Chawla and Patil, 2010; Jha and Berrocoso, 2016; Tiwari and Jha, 2017). In the area of gut health, microbial fermentation of dietary fibre (such as wheat bran) promotes high antioxidant activities, selective growth of beneficial bacteria (*Bifidobacteria* and *Lactobacillus spp.*) and the production of short-chain fatty acids (SCFAs) such as butyrate (Ahsan *et al.*, 2016; Iseri, 2017). Butyrate inhibits the occurrence of gut disorders such as diarrhoea and coccidiosis, increases villi height, villi height to crypt depth ratio and promotes overall broiler growth performance (Bedford and Gong, 2018). On the other hand, dietary fibre is described as an anti-nutritive factor (ANF) due to the negative effects it exerts nutrient utilization and voluntary feed intake (Jha *et al.*, 2019) in monogastrics. However, these effects are largely influenced by fibre type, feed form and physiochemical properties (Jha and Berrocoso, 2016).

According to Adibmoradi *et al.* (2016) and Zaefarian *et al.* (2016), manipulation of the ingredient particle size can help improve upon these negative effects of dietary fibre. Optimum growth performance has been associated with the inclusion of structural components such as coarse particles or whole grains of maize in monogastric diets (Xu *et al.*, 2015a, Kheravii *et al.*, 2018). Jiménez-Moreno *et al.* (2013) showed that, the development of digestive organs such as the gizzard, crop and proventriculus and overall gut development was enhanced by the inclusion of coarse feed particles in broiler diets. The development of these organs enhances nutrient digestibility through an increase in enzymatic activities and reverse contractions (Svihus, 2014). This results in an increase in feed efficiency and growth. There is the need to explore the

relationship between dietary fibre (wheat bran), particle size and their overall effect on gut health, nutrition and feed cost in broiler production.

1.1. Hypothesis

Feeding broilers diets with arabinoxylan-type fibre (wheat bran) and coarse maize particle sizes will improve growth performance, gut morphogenesis and immune status in broilers.

1.2. Objectives

The main objective of this study was to explore the effect of dietary fibre levels (using wheat bran (WB) as a dietary fibre source) and maize particle sizes on broiler growth performance, gut and immune health status.

Specific objectives were to:

1. Evaluate the main and interactive effects dietary fibre levels (using wheat bran (WB) as a dietary fibre source) and maize particle sizes had on broiler:
    a. Nutrient digestibility
    b. Growth performance
    c. Carcass characteristics
    d. Caecal butyric acid concentration
    e. Caecal short-chain fatty acid profile
    f. Caecal microbial population and pH
    g. Gut morphology
    h. Haematological indices
    i. Serum glucose content
    j. Immune status
2. Determine the cost effectiveness of such diets and implications for feed manufacturers

CHAPTER TWO

LITERATURE REVIEW

2.1. The Ghanaian poultry industry

About 80% the Ghanaian poultry industry is primarily made up of medium (120, 000 birds – 50, 000 birds) and small scale (below 10, 000 birds) businesses with 20% being made up of privately owned large scale (over 50, 000 birds) commercial poultry farms (Anon, 2019). Normally, chickens (*Gallus gallus domesticus*) are reared for their meat, feathers, eggs and aesthetic value (USDA, 2017). In Ghana, chicken is the most consumed poultry meat followed by that of guinea fowls, ducks, turkeys and ostrich (USDA, 2017). Indigenous chicken production, serves as an emergency source of funds and food for most people living in the rural areas (Kryger *et al.*, 2010). Patterns observed in the growing population and the emergence of new hotels, rest stops, eating joints and restaurants has led to a rise in the demand for chicken. (5m editor, 2011). However, the domestic production of chicken is declining due to the high demand for imported frozen chicken (Woolverton and Frimpong 2013). Reduced prices and pre-cut nature of imported frozen chicken makes them the preferred choice of local consumers. Annually, Ghana spends about GH₵ 2,150 million in the importation of chicken and this supplies about 50% of the local demand (Appiakorang Jnr, 2019; Arhinful, 2020).

In addition to the preference for imported frozen chicken, broiler production in Ghana is affected by a lot of consistent setbacks. High prices of feed ingredients have been a persistent factor affecting feed cost in broiler production in Ghana (Alabi *et al.*, 2017). This is primarily because feed accounts for 60-70% of overall production (USDA, 2017). Globally, maize, soybean, millet and wheat are the conventional feed ingredients used in the preparation of broiler diets (Lindberg,

2014). These raw materials are also in high demand for human consumption resulting in a continuous competition between animals and humans for these cereals and grains (Donkoh *et al.*, 2003). In Ghana, maize, wheat bran and soybean meal form about 60, 6, and 20 percent respectively of broiler diets (Andam *et al.*, 2017). Due to this, 30% of locally produced maize is used in the production of poultry (broiler) feed (FAO, 2014).

2.2. The Impact of Feed Formulation on Broiler Growth and Feed Cost

Feed ingredients used in broiler diets are variable based on factors such as breed, age, sex, purpose and strain of birds. Globally, maize, wheat, sorghum and millet serve as energy sources in broiler diets (Onu and Aniebo, 2011). In Ghana, locally available protein-based ingredients used in broiler diets include: fish meal, cotton-seed meal, kernel cake, copra cake, groundnut cake meal and soybean cake (USDA, 2017). Dietary manipulation of these feed ingredients must be geared towards making animal production more efficient and profitable (Makkar, 2016). Currently, various management and feeding strategies are being adapted by nutritionists to make this possible. Feeding strategies such as: the inclusion of treated unconventional feed resources in diets for monogastrics, phase feeding and the formulation of feed according to the breeders' specifications have been employed (Makkar*l.*, 2016). These feeding strategies aim at promoting optimum nutrient utilization to enhance animal performance. However, the ability of monogastrics to utilize feed is largely dependent on factors such as: (1) how well diets are formulated and (2) the nutritional composition of their feed (Adeola *et al.*, 2016).

The inclusion of dietary fibre (DF) is limited in broiler diets due to its negative effect on bioavailability of nutrients (Walugembe *et al*., 2015). Generally, all broiler diets contain an amount of dietary fibre due to the grains and legume-based feed ingredients used in the manufacture of broiler feed. According to Mateos *et al.* (2012), feed manufacturing companies include up to 3%

crude fibre in broiler diets. This is due to the absence of endogenous enzymes suitable for the digestion and utilization of fibre.

2.3. Dietary Fibre

Indigestible carbohydrates can be referred to as dietary fibre (Mudgil and Barask., 2013). Dietary fibre is basically the non-starch polysaccharides (NSPs) and lignin components present in plant cell walls (Lindberg, 2014). However, resistant starch and oligosaccharides are also termed as dietary fibre because of their similar physiological characteristics as lignin and NSPs (Perry and Ying, 2016) (Figure 2.1). Dietary fibre was originally referred to as roughage, bulk, or ballast before 1965 and measured as 'crude fibre' (Chawla and Patil, 2010). Scientists such as McCance and Lawrence later modified this definition to describe roughage as the indigestible part of plant-based foods obtained after a process of solvent, dilute acid and dilute alkali extraction is carried out (Dai and Chau, 2017).

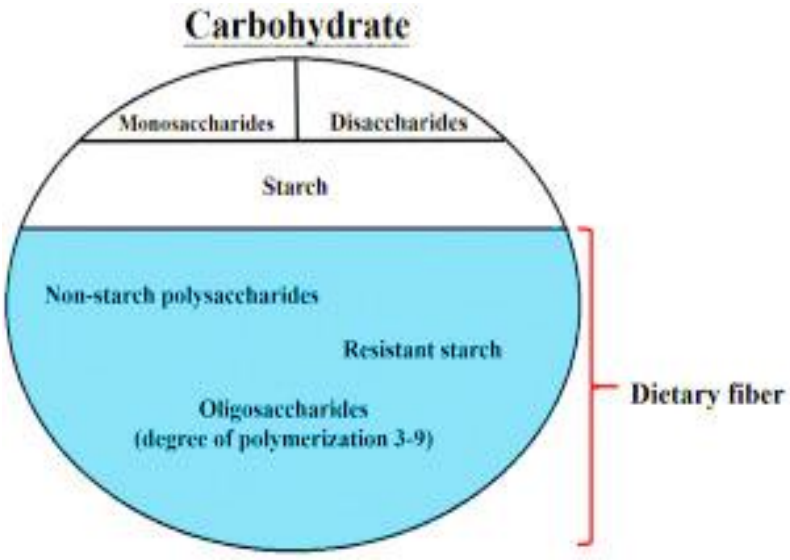

Figure 2. 1: Components of carbohydrates (Dai and Chau, 2017)

However, the term 'dietary fibre' was introduced by a British physician, Eben Hipsley in 1953 where he defined it as the non-digestible constituents of the plant cell wall which is unavailable for digestion (Hipsley, 1953; Chawla and Patil, 2010; Phillips and Cui, 2011). The Codex Alimentarius Commission on Nutrition and Foods for Special Dietary Uses (CCNFSDU) accepted an official definition of DF in 2009 (Codex Alimentarius, 2009). Dietary fibre was officially defined as "carbohydrate polymers with 10 or more monomeric units, which are not hydrolyzed by endogenous enzymes in the small intestine of humans, and which are either edible carbohydrate polymers naturally occurring in the food, or carbohydrate polymers which have been obtained from raw food materials by physical, enzymatic or chemical means or synthetic carbohydrate polymers." The commission on the other hand, indicated that based on scientific evidence, qualified national authorities may approve descriptions 'b' and 'c' (Codex Alimentarius Commission, 2009). However, points 'b' and 'c' are subject to approval from the qualified national authorities based on scientific evidence that proves the physiological effect of dietary fibre on health. (Codex Alimentarius Commission, 2009).

2.3.1. Types of dietary fibre

The components of DF can be grouped under total non- starch polysaccharides (TNSPs), oligosaccharides, analogous carbohydrates (such as resistant starch), associated NSPs substances and lignin (Dhingra *et al.*, 2012) (Figure 2.2).

2.3.1a. Oligosaccharides

These are digestion-resistant carbohydrate polymers with 3-9 monosaccharide units (Capuano, 2017). Examples of oligosaccharides include fructo-oligosaccharides (FOS), inulin, polydextrose, xylosaccharides and resistant maltodextrins and galacto-oligosaccharides (Hamaker and Tunchul,

2014; McCleary, 2014). They are absent in the plant cell walls hence were not initially classified as dietary fibre until 2009. Fructo-oligosaccharides (FOS) and inulin are collectively known as fructans (Mensink *et al.*, 2015). They are usually found in fruits and vegetables such as bananas, onions, garlic, wheat, asparagus and artichokes (Links, 2018).

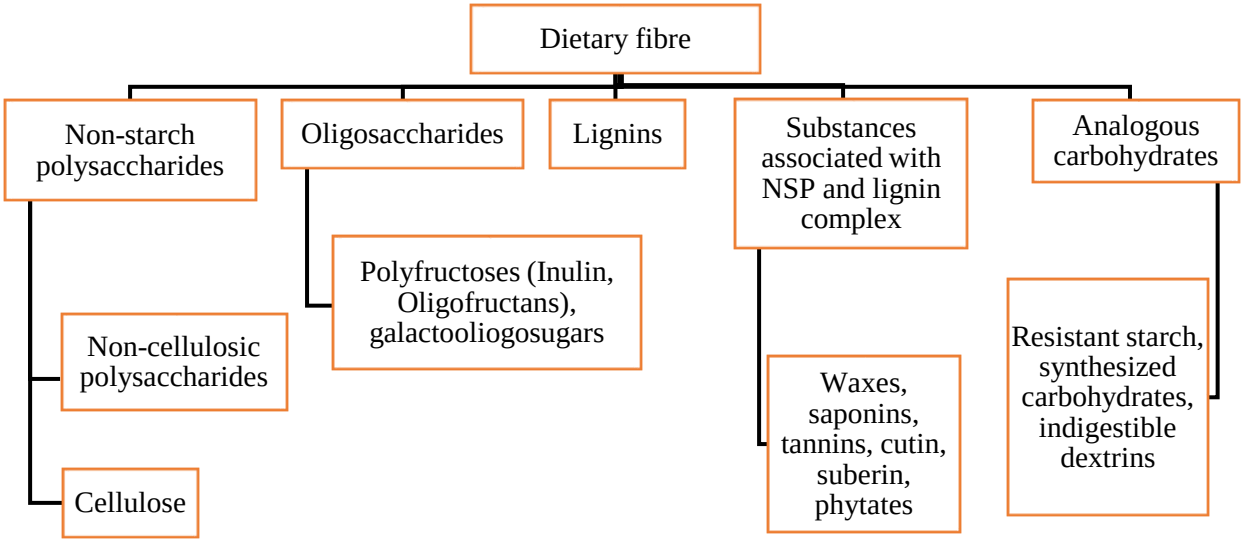

Figure 2. 2: Components of dietary fibre (Dhingra *et al*., 2021)

Another common oligosaccharide is galacto-oligosaccharides (GOS). They are made up of polymers of galactose and a terminal glucose with 2-10 degrees of polymerization (Holscher, 2017). Some natural sources of GOS include leguminous seeds and plants such as soybeans, peas, cowpea, lentils and lupines (Kao *et al.*, 2016). They can also be produced from lactose (Rodriguez-Colinas *et al.*, 2012).

2.3.1b. Analogous carbohydrates - Resistant starch (RS)

Total plant starch is classified by its digestive rates into: quickly digestible, slowly digestible and resistant starch (Dai and Chau, 2017). Resistant starch describes the indigestible part of starch, which remains undigested 120 minutes after ingestion (Fuentes-Zaragoza *et al.*, 2011) as

illustrated in Figure 4. Resistant starch is made up of amylose and amylopectin granules which form linear molecules bonded by α-1, 4-D-glucan linkages (Haralampu, 2000). Whole grains, seeds and barley are sources of RS.

2.3.1c. Lignin

It is a complex and multiple-branched polymer formed through dehydrogenative polymerization of phenylpropane units into p-hydroxyphenyl, guaiacyl and syringyl polymers (Wang *et al.*, 2019). Lignin is not a polysaccharide but it is considered a component of dietary fibre due to its constant association with cell wall polysaccharides such as cellulose and hemicellulose (Zhao *et al.*, 2020). During plant growth, lignin binds directly or indirectly to cellulose or hemicellulose through sugar residues and ferulic acid (Davin *et al.*, 2008). Lignin serves as a protective barrier for plant cell walls due to its rigidity (Rinaldi, 2017).

2.3.1d. Non-Starch Polysaccharides (NSP)

Non-starch polysaccharides (NSP) are complex non-digestible carbohydrates that make up about 90% of primary and secondary plant cell walls and are bonded together by α or β-glycosidic linkages (Knudsen, 2014). Due to this, they function as structural polysaccharides and normally occur together with other compounds such as proteins (Albersheim *et al.*, 2011). Cellulose, pectins and hemicellulose are examples of NSPs, which occur in large amounts in plant cell walls. Others such as fructans, glucomannans and galactomannans are known as plant storage polysaccharides and occur in smaller quantities (Navarro *et al.*, 2019). Most cereals such as wheat, maize and sorghum contain NSPs. Figure 2.3 shows NSPs classification.

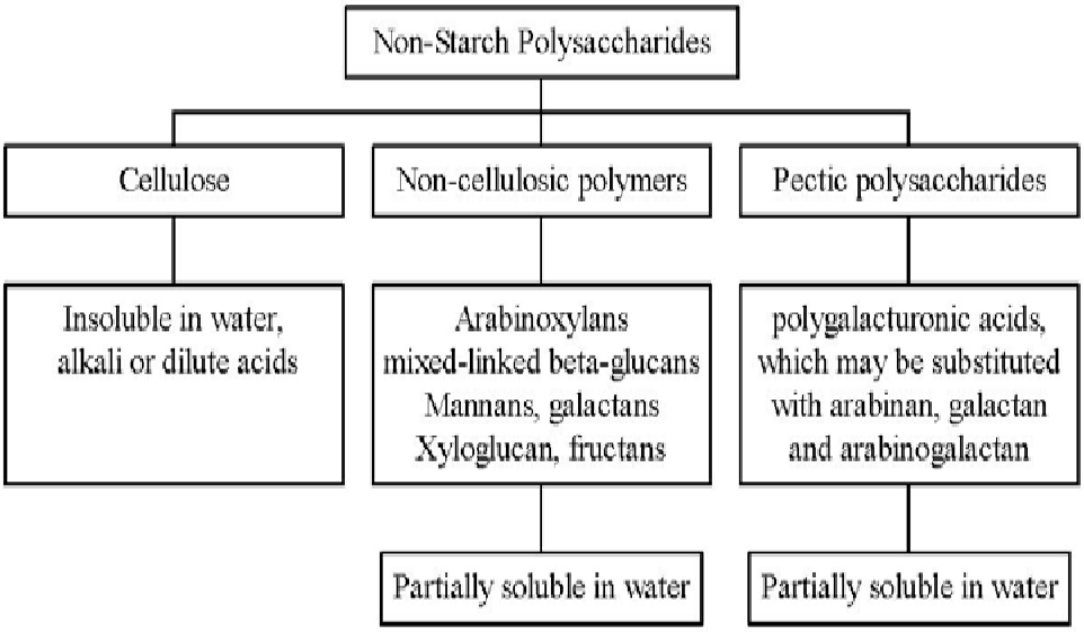

Figure 2. 3: Classification of non–starch polysaccharides (Choct, 2015)

2.3.1d.1. Cellulose

Cellulose is the most abundant NSPs found in plants cell walls with small quantities in every plant tissue such as the endosperm and cotyledon (Knudsen, 2014). It is made up of linear chains linked together by β-1–4 bonds. Usually, cellulose molecules are arranged in a defined manner within microfibrils (Lavanya *et al.*, 2011; Moon *et al.*, 2011).

2.3.1d.2. Non-cellulosic NSPs

Non-cellulosic NSPs are a group of structural polymers with pyranoside sugars held together by 1-4 linkages (Sinha *et al.*, 2011). These NSPs differ from one other based on the degree of branching and their uronic acid content (Căpriţă, *et al.*, 2010). Examples include mannans, mixed-linked β-glucans, xyloglucan and arabinoxylans (Moreira and Filho, 2008). Table 2.1 shows some examples of non-cellulosic NSPs and their properties.

2.3.1d.3. Pectins

Pectins have an α-1-4 glycosidic backbone chain linked to D-galacturonic acid units (Wefers *et al.*, 2014). Their gel–like nature gives them the ability to act as intercellular cement and are the second most abundant polysaccharides found in fruits and vegetables (Zaragoza *et al.*, 2010). Pectins can be grouped into arabinogalactans (legumes and rapeseeds), arabinans (cereal products) and galactans (citrus) based on structure and, or degree of substitution (Wefers *et al.*, 2014).

Table 2. 1: Examples of non-cellulosic NSPs, their properties and food sources

Polysaccharide	Monomeric Residue	Description	Food Sources
Arabinoxylans	β-D-xylopyranosyl α-L-arabinofuranosyl	a. β-(1 → 4)-linked xylose units replaced at the O-2 or O-3 position with arabinose b. They differ based on the ratio of arabinose: xylose c. Ratio of arabinose-xylose influences their viscosity	Wheat, rye, barley, oat, rice, sorghum
Mixed-linked β-glucans	Glucose	a. β-(1 → 3) and β-(1 → 4) linkages present b. They are a characteristic of the order of Poales such as grasses c. Interact with cellulose microfibrils at the subaleurone and endospermic cell wall	Oat and barley
Galactomannans	Galactose, Mannans	a. Serve as storage polysaccharides in the endosperm of leguminous seeds b. Ratio of galactose: mannan influences their viscosity and solubility c. Viscous nature of some galactomannans promotes slower gastric emptying hence increasing satiety d. Retains water in seeds hence promoting effective seed germination e. Replacement for fat in may food products since it is noted for the unique fat-rich mouth feel	Locust bean, arabic, tara and guar gum

2.3.2. Classification of Dietary fibre

Each dietary fibre type has its own distinctive physical, chemical, structural and physiochemical properties (Daou and Zhang, 2014). Hence, their inclusion in the diets of animals causes different physiological effects in the body. Some ways by which dietary fibre is classified include:

1. Origin or source: plants, native or synthetic sources
2. Site of digestion: stomach, colon and small intestines.
3. Products of fermentation: acetic, propionic, valeric, fumaric, lactic acids
4. Applications: food, pharmaceutical industries (Stephen *et al.*, 2017)

The physiochemical properties of fibre results in beneficial physiological effects. Ways by which dietary fibre can be classified based on their physiochemical properties are as follows:

5. Structure: linear or nonlinear molecular polysaccharides
6. Solubility in a buffer at a defined pH: water or alkaline/acids
7. Viscosity in water or in the digestive system
8. Fermentability pattern: rate of fermentation and short-chain fatty acids' profile
9. Bulking effect (Chawla and Patil, 2010)

2.3.2a. Classification based on solubility and viscosity of dietary fibre

Solubility of dietary fibre refers to its ability to dissolve in water, a buffer or an enzyme solution prepared to suit the aqueous enzyme environment of the digestive system (Elleuch *et al.*, 2011; El Khoury *et al.*, 2012). The solubility of dietary fibre depends on factors such as chemical (random or orderly arrangement) configuration, physical structures and interactions with other dietary fibre types (such as lignocellulosic complex), the presence of a substitution group (COOH or SO_2), temperature and ionic strength (Choct, 2015). Dietary fibre types with orderly

arranged molecules plus less branching is more stable in the solid form than in a solution (Guillon and Champ, 2000). Highly branched dietary fibres (gum acacia) and ionic groups have a high solubility level (Dhingra *et al.*, 2012).

The viscosity of fibre refers to its resistance to flow (Guillon and Champ, 2000). It is determined by the ratio of shear stress (C): shear rate (c), where an increased shear rate results in an increase/decrease solubility (Elleuch *et al.*, 2011). The type and concentration of water-soluble fibre influences viscosity (Theuwissen and Mensink, 2008).

2.3.2a.1. Soluble Dietary fibres (SDF)

Soluble DFs include pectins, galactomannans gums, mucilages, oligosaccharides (including fructooligosaccharides; FOS), β- glucans (oat and barley grains), alginate, and psyllium (Maziarz, 2013). Also, they are gummy in water and are known to ferment well in an aqueous enzyme solution (Esposito *et al.*, 2005). Some water-soluble DFs are viscous in nature while some are not (Choct, 2015). Water-soluble dietary fibres with low-molecular weights are highly soluble in water and have low viscosity (Dhingra *et al.*, 2012). Such dietary fibres also have short polymer chains, which are highly branched at irregular intervals. Water-soluble dietary fibres play a major role in maintaining the stability of serum lipid concentration (Surampudi *et al.*, 2016). High viscous water-soluble dietary fibres easily dissolves in water but have a slower rate of movement (Dikeman and Fahey, 2006). They form gels when in water which thickens the digesta content making movement very slow in the gastro-intestinal tract. This results in a delay in gastric emptying increasing total transit time in the gastro-intestinal tract. Low nutrient digestion and absorption is associated with viscous soluble dietary fibres (Brennan, 2005). Arabinoxylans and mixed linked

beta-glucans such as starchy endosperm and aleurone layer of wheat, rye and barley are examples of viscous water-soluble dietary fibres (Stephen *et al.*, 2017).

2.3.2a.2. Insoluble fibres (IDF)

Insoluble fibres refer to indigestible carbohydrates which do not dissolve in water or an aqueous enzymatic solution due to the hydrogen bonds that exist between them (Dai and Chau, 2017). Figure 2.4 shows the different ways by which IDF can be classified (Papanikou, 2016). Cellulose, some hemicelluloses, resistant starch and lignin are examples of insoluble dietary fibre (Williams *et al.*, 2017). They have a high water absorbing quality, which promotes a good bulking effect. The addition of wheat bran, whole grains and vegetables to a diet increases the rate of passage in the gastro-intestinal tract (Kheravii *et al.*, 2018). This goes a long way to maintain the normal motility of the gastro-intestinal tract (Ho *et al.*, 2012).

2.3.2b. Classification based on Fermentability

Fibre fermentability refers to the ability of the beneficial gut microbiota to break down fibre into essential products in the gut (Leech, 2017). Examples include beta-glucans, oligofructose, inulin, guar gums and pectins. Fermentable fibres promote growth of beneficial microbiota through the production of short-chain fatty acids. (Parnell and Reimar, 2012). Some insoluble fibres are fermented to some extent as shown in figure 2.4. This is possible based on how porous and available the surface area of these insoluble fibres are at a moment. The porosity and availability of dietary fibre depends on the chemical structure and source of fibre (Capuano, 2017). The hydrogen bonds between the polymers of insoluble fibres (hemicellulose, cellulose and lignin) make it increasingly difficult for the microbial degradation (Williams *et al.*, 2017).

2.3.3. The Role of Dietary Fibre in Monogastric Nutrition

Low voluntary intake, reduced nutrient digestibility and utilization as well as overall poor growth performance have been associated with the inclusion of DF sources in monogastric diets (Nortey *et al.*, 2015). However, most feed ingredients such as cereals and legumes contain a very significant amount of dietary fibre (Table 2.2). In addition, poultry often peck at their litter materials (example wood shavings) which contain some amount of fibre (Choct, 2015). Recent studies have highlighted the positive effects dietary fibre has on monogastrics and nutritive ways by which the undesirable effects of dietary fibre can be mitigated (González-Alvarado *et al.*, 2010; Lindberg, 2014; Choct, 2015; Kheravii *et al.*, 2018).

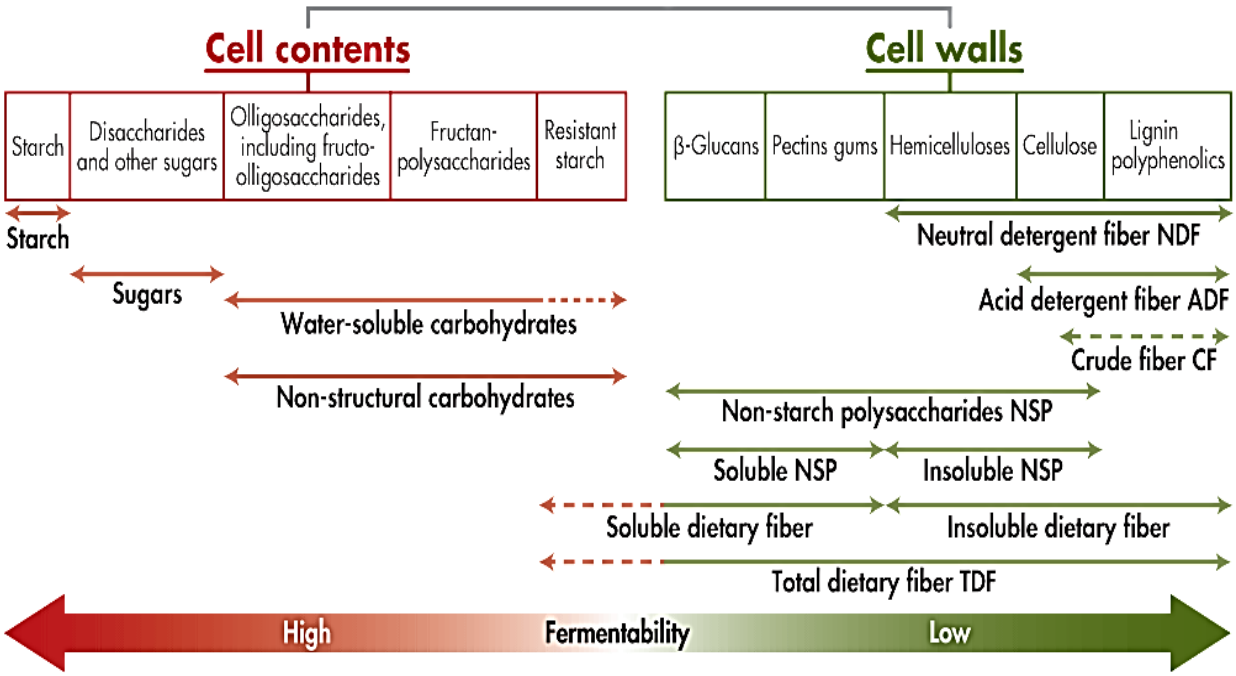

Figure 2. 4: Classification of dietary fibre based on fermentability (Papanikou, 2016)

Dietary fibre is known to enhance intestinal mucosa integrity, gut morphology, physiochemical properties of digesta and overall gut health (Canibe and Bach Knudsen, 2002; Molist *et al.*, 2012; Liu *et al.*, 2012).

Also, dietary fibre reduces the expression of some immune indicators which may be attributed to the presence of stressors and inflammation in the gut (Teng and Kim, 2018; Tiwari *et al.*, 2018). As such, inclusion of moderate levels of dietary fibre in monogastric diets is highly recommended in recent times. Strategies such as particle size manipulation and supplementation of exogenous enzymes have been used in many studies to reduce the negative effects associated with DF intake in monogastrics (Anadón *et al.*, 2019). The effect of DF on the physiology of monogastrics depends largely on the physical, chemical and physico-chemical properties of the fibre used, the level of inclusion in the diet among other reasons (Holscher, 2017).

Table 2. 2: Proximate nutrient composition of some common cereal grains and co-products (g/kg DM)

	Wheat[1]	Corn[1]	Sorghum[1]	Wheat bran[2]	Barley[2,3,4]	DDGS[1]
Starch	618	620	690	222	587	86
Cellulose	13	17	15	72	39	58
NCP						
Soluble	19	25	4	29	56	34
Insoluble	62	38	47	273	88	158
NSP						
Arabinoxylan	81	17	17	238	12	61
β-glucan	8	6	1	24	43	63
Mannose	2	2	1	5	2	9
Galactose	3	8	3	9	2	14
Uronic acids	4	8	4	15	2	16
Total NSP	95	81	66	374	167	192
Lignin	18	8	16	75	35	32
Dietary Fibre	112	89	83	449	202	322

DM = dry matter, NCP = non-cellulosic polysaccharides; NSP = non-starch polysaccharides; DDGS = distilers dried grains with solubles; [1]Jaworski *et al.* (2015); [2]Knudsen (1997); [3]According Holtekjølen *et al.* (2006); [4]According to Jha *et al.* (2011)

2.3.3a. Effect of Dietary Fibre on Voluntary Feed Intake

The level, nature and type of dietary fibre included in monogastric diets affect voluntary feed intake of monogastrics (Ratanpaul *et al.*, 2019). Early gut fill and satiety could be reasons for low voluntary feed intake (Ndou *et al.*, 2013). Viscous and soluble fibres may lead to low voluntary feed intake by increasing the period of chewing and saliva production (Howarth *et al.*, 2001). In order to break down bulky fibres, monogastrics will have to chew ingested feed for a relatively longer period of time. The prolonged stay of the ingested feed in the mouth increases the amount of saliva produced. This can result in early satiety which may affect the level of energy intake.

When feed formulated has low energy values, an early satiety will cause a decrease in energy intake below the required level (Burton-Freeman, 2000). However, a well-balanced diet with a fibrous component may be beneficial to layers in promoting general performance and production (Gurbuz *et al*, 2011; Martínez *et al.*, 2015). In a study conducted in barrows using sugar beet pulp, a lower voluntary feed intake was recorded when the inclusion of sugar beet pulp was higher than 35.0% (Zhang *et al.*, 2013). Also, the movement of bulky and viscous fibres along the walls of the stomach may cause an increase in the secretion of gastric juices (Benelam, 2009). This may lead to a distension of the stomach causing a feeling of satiety through afferent vagal signals sent to the nervous system (Burton-Freeman, 2000).

2.3.3b. Effect of Dietary Fibre on Nutrient Digestibility

Even though dietary fibre is not digested by endogenous enzymes, their presence in broiler diets can negatively or positively influence digestion and nutrient utilization (Tiwari *et al.*, 2018). However, this is subject to factors such as age, breed, level of inclusion, type and nature of fibre (Jha and Berrocoso, 2015).

The type and nature of dietary fibre may affect digesta viscosity, transit time and faecal bulk (Zhang *et al.*, 2013). For instance, soluble dietary fibre has a high-water holding capacity which delays gastric emptying and reduces nutrient absorption (Serena *et al.*, 2008). On the other hand, insoluble fibre may bind to organic compounds, increase gut transit time resulting in a higher portion of digesta moving to the hindgut (Montagne *et al.*, 2003). Dietary fibre can also form complexes with organic compounds, thus preventing their digestion and absorption. Also, the movement of some types of dietary fibre such as rice hulls may cause an abrasive effect on the intestinal wall leading to losses of endogenous cells and lumen nutrients (Mateos *et al.*, 2012). This may reduce the rate of absorption as the number of endogenous cells and pores which make up the villi is reduced. With respect to age, nutrient digestibility in mature animals may be higher than that in young animals (Lindeberg, 2014). This may be due to the fact that, the gastro-intestinal tract (GIT) of matured animals may be well adapted to the fibrous nature of the feed (Le Goff *et al.*, 2002). As such, the rate of digestion and fermentation (in the hindgut) will be higher in the mature animals compared to the younger animals. According to Jha *et al.* (2019), the level of fibre inclusion in a diet is inversely related to the level of digestion and nutrient utilization.

However, recent studies have indicated that, the presence of structural components such as dietary fibre can enhance nutrient digestibility in broilers (Kimiaeitalab *et al.*, 2017). This can be attributed to their positive effects on development of digestive organs such as the gizzard, crop and proventriculus (Jiménez-Moreno *et al.*, 2013). This results in an increase in enzymatic activities (secretion of cholecystokinin and pepsin), secretion of HCL and reverse contractions which are key indicators of enhanced nutrient digestibility (Svihus, 2014). Also, gut motility is improved which encourages better mixing of digesta with digestive juices (González-Alvarado *et al.*, 2007). In a study carried out by Jiménez-Moreno *et al*. (2013), coarse fibrous diets fed to

broilers positively affected the secretion and efficacy of digestive enzymes. This was attributed to a longer retention time in the gizzard, which led to an effective breakdown of the structural components. Bacterial fermentation in the crop was enhanced as well as protein digestibility (Afsharmanesh and Pourreza, 2005; Classen *et al.*, 2016).

Knowledge about the suitable inclusion rate, type of fibre, age and other dietary components gives the nutritionist the opportunity to formulate diets that can mitigate the negative effects of dietary fibre and enhance their beneficial effects on nutrient digestibility in broilers.

2.3.4. The Gut Microbiota and Dietary Fibre Fermentability in Poultry

The microbiota plays various roles in the gastro-intestinal tract and are often referred to as the 'forgotten organ' (O'Hara and Shanahan, 2006). The microbiota of the gut refers to commensal and pathogenic bacteria (Montagne *et al.*, 2003). An optimum intestinal microbiota has a higher ratio of commensal bacteria to pathogenic bacteria or at most a balance (Thursby and Juge, 2017). Commensal bacteria include species belonging to: *Lactobacillus*, *Streptococcus*, *Bifidobacteria*, *Bacillus* and *Saccharomyces* (Kabir, 2009). They have the ability to act and protect their host by preventing the colonization and invasion of pathogenic bacteria (*Listeria spp., Salmonella spp., C. perfringens and Campylobacter jejuni)* (Mor-Mur and Yuste, 2010; Khan *et al.*, 2019). Pathogenic bacteria are infectious microorganisms, which cause diseases (Montagne *et al.*, 2003). Gut microbes secretes enzymes, which acts on the fibre substrates. Cellulolytic bacteria secrete cellulase to act on cellulose (Karmakar and Ray, 2011) while amylolytic bacteria secrete amylase to act on starch (Gopinath *et al.*, 2017).

Fibre fermentability refers to the ability of the beneficial microbes to break down indigestible fibre sources to produce short chain fatty acids and other metabolites (Jha and Berrocoso, 2015). In the absence of fibre-degrading endogenous enzymes, dietary fibre is degraded and fermented by the

gut microbial population. Even though fibre fermentation may occur along the gut, it largely occurs in the caeca where there is a dense population of microbes (Kheravii *et al.*, 2018). The principles of bacterial fermentation have been widely studied than those of other microbes. Studies suggest two different groups of bacteria exist in the gut based on their mode of operation: free-living bacteria and substrate-dependent bacteria (Vermeulen *et al.*, 2018). During fermentation, substrate-dependent bacteria form a colony around fibrous particles by adhering to their surfaces in order to ferment them (Hamaker and Tuncil, 2014). Strains of bacteria such as *Lactobacillus spp., Enterococcus spp. and Streptococcus spp*. have been identified in the upper gut of broilers with *Lactobacillus spp.* forming about 80-90% of the microbial population (Rinttilä and Apajalahti, 2013). The dominance of *Lactobacillus spp*. may be due to their ability to exist in a highly acidic environment such as the crop. Gong *et al.* (2008) reported *Lactobacillus aviaries* and *Lactobacillus salivarius* as the dominant bacteria species found in the upper gut of broilers. In the caeca of chickens, bacteria from the orders, *Clostridiales*, *Bacteroidales* and *Lactobacillales*, have being identified (Torok *et al*, 2011). Furthermore, it was observed that these bacteria disappeared after sometime due to reasons such as: (1) high rate of bacterial washout, (2) high pH and (3) rapid flow of digesta (Torok *et al*, 2011).

Fermentation of arabinoxylans, pectin and soluble β-glucans usually occurs in the proximal colon and caecum with that of cellulose occurring at the distal part of the colon (Quinn, 2017). This may be an indication that dietary fibre fermentation is site-specific which could be due to the presence of particular strains of bacteria. Bacterial fermentation in the lower gut in very efficient as undigested feed residues from the upper gut are used as substrates (Williams *et al.*, 2017). Hence, there is relatively little competition between the host and lower gut microbes for the same feed resources. As such, feeding monogastrics with fibrous diets will rather provide suitable substrates

for the metabolic activities of lower gut microbiota while providing the host animal with end products of fermentation such as SCFAs (Den Besten *et al.*, 2013). In the absence of complex indigestible polysaccharides, the lower gut bacteria ferment indigestible protein sources in order to obtain energy. This leads to the production of toxins and carcinogens (protein fermentation) and phenols, indoles and ammonia (amino acid fermentation). The presence of these can be harmful to the health of the monogastric animal. Hence, addition of dietary fibre to monogastric diet is essential to maintain efficient metabolic processes and gut health.

2.3.4a. End products of Dietary Fibre Fermentation

End products of bacteria fermentation vary based on the source and physiochemical properties of the dietary fibre (Jha and Berrocoso, 2016). However, short chain fatty acids (SCFAs) or volatile fatty acids (VFAs), gases such as hydrogen (H_2), carbon dioxide (CO_2) and methane (CH_4) are generally produced as end-products of fibre fermentation (Jha and Berrocoso, 2015). Figure 2.5 shows the process of dietary fibre fermentation and the end products produced. The most common SCFAs produced are: acetate, succinate, propionate, butyrate and lactate. Acetate contributes about 60% of the total SCFAs produced with butyrate and propionate making about 15 and 25% (Jha *et al.*, 2019). Growing pigs and gestating sows derive 15% and 30% of their energy requirements from SCFAs (Bedford and Gong, 2018).

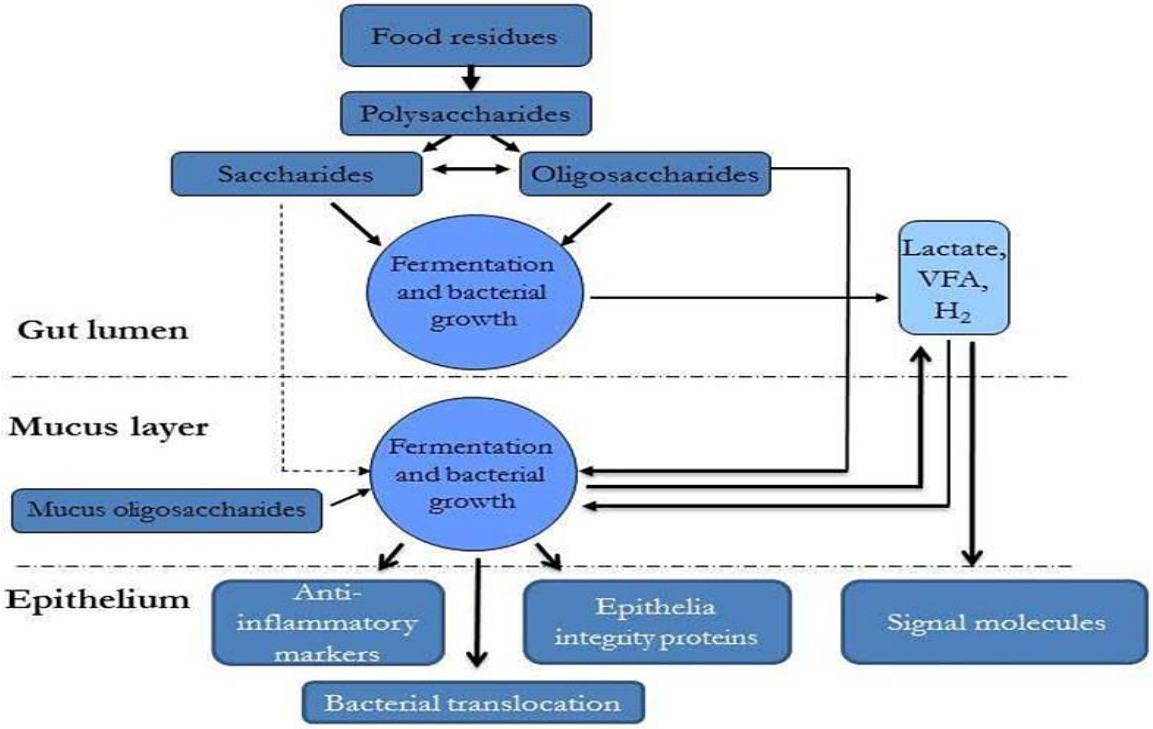

Figure 2. 5: Dietary fibre fermentation end products (Lindberg, 2014)

2.3.5. Dietary Fibre as a Potential Prebiotic in Poultry Nutrition

The incidence of enteric diseases is of a great worry to farmers due to their associated high mortality rate, low productivity and contaminated poultry products (Hajati and Rezaei, 2010; Ayeh-Kumi *et al.*, 2016). Research findings over the years have conclusively pointed to the crucial role gut microbiome play in promoting growth and maintaining optimal health in poultry (Montagne *et al.*, 2003; Jha *et al.*, 2019; Yegani and Korver, 2008; Yadav and Jha, 2019). Composition of broiler diets has been established as a primary source by which gut microbiome can be modulated (Yadav and Jha, 2019). Consequently, the inclusion of prebiotics in poultry diets is increasingly becoming popular due to their beneficial effect on gut microbiome (Ricke *et al.*, 2020). Recent findings have proven their role as growth and gut health promoters making prebiotics a potential substitute for antibiotic use in poultry nutrition (Solis-Cruz *et al.*, 2019).

Gibson and Roberfroid originally defined prebiotic as a "non-digestible feed ingredient that beneficially affects the host by selectively stimulating the growth and or activity of some beneficial bacteria in the colon, and thus improves host health" (Gibson *et al.*, 1995; Ganguly, 2015). However, in 2010, Gibson and his colleagues redefined prebiotics as "a selectively fermented ingredient that allows specific changes, both in the composition and or activity of the gastrointestinal microbiota that confers benefits" (Gibson *et al.*, 2010). Prebiotics must be fermentable by intestinal microbiota, resistant to gastrointestinal absorption, gastric acidity and hydrolysis by endogenous enzymes (Gibson *et al.*, 2004). One critical characteristic of a prebiotic is its ability to enhance the selective growth and metabolism of indigenous intestinal microbiota. Prebiotics do not introduce novel forms of bacteria but rather serve as a substrate for the already existing ones. The inclusion of non-digestible carbohydrates in poultry diets have reportedly enriched the counts and diversity of gut microbiome such as *Lactobacilli* and *Bifidobacteria* (Gibson and Roberfroid, 1995; Singh *et al.*, 2017). Currently, the possible effects of prebiotics on other beneficial microbes such as butyrate-producing microbes have been reported even though the exact mode of action is yet to be fully understood (Thomas *et al.*, 2015).

Most prebiotics are plant-based (Slawinska *et al.*, 2019). They are primarily dietary fibres; predominantly oligosaccharides of galactose, fructose or mannose but favourable effects of certain proteins, lipids and peptides on gut microbiome (Hajati and Rezaei, 2010) have been reported. Examples of specific prebiotics include: stachyose, fructooligosaccharide (FOS), transgalactooligosaccharide (TOS), inulin, RS, glucooligosaccharide, xylooligosaccharide, isomaltooligosaccharide, lactulose, soybean oligosaccharide, polydextrose, lactosucrose and NSPs such as β-glucans (Propulla, 2008; Pourabedin and Zhao, 2015; Hutkins, *et al.*, 2016; Ricke, 2018).

Natural plant sources such as tomato, garlic, leek, chicory and honey also exert prebiotic effects (Ganguly, 2013).

Basically, dietary fibre as a prebiotic is indigestible by gut endogenous enzymes as only about 20 endogenous enzymes glucosidases have been discovered to digest some starch polysaccharides (Holscher, 2017). Hence most dietary fibre remains undigested till it reaches the caeca where it undergoes possible microbial fermentation (Józefiak *et al.*, 2004). Gut bacteria (predominantly *Bacteriodes*) through the symbiotic relationship that exist between the microbiota and the host mostly carry out microbial fermentation (Holscher, 2017). When dietary fibre is consumed by a host, it functions as biological agent, which serves as a substrate for microbial growth and metabolism. The host in turn benefits from the fermentative end products such as SCFAs. The gut microbes are able to ferment DF due to their genetic nature that allows them to produce carbohydrate-active enzyme (CAZymes) such as glycoside hydrolases, glycosyltransferases, polysaccharide lyases, and carbohydrate esterases (Flint *et al.*, 2012). These enzymes are activated based on the substrate binding site available on the undigested DF. The substrate-binding site available is influenced by the properties of DF such as chemical structure. Physiochemical properties such as the extent of fermentability are influenced by how soluble or viscous the fibre type is (Elleuch *et al.*, 2011; McRorie and Fahey, 2013). Hemicellulose, cellulose and lignin are insoluble, less viscous hence less fermentable as compared to soluble fibre. Due to their less viscous nature, gut transit time is quicker therefore lowering the extent of microbial fermentation. In their work, Abazari *et al.* (2016) observed an increase in the counts of *Lactobacillus spp.* when broilers were fed rice husks. On the other hand, oligosaccharides, fructans, resistant starch, pectins and β-glucans are highly viscous and very soluble, hence, are easily fermentable. Feeding birds infected with *E. tenella* soy soluble carbohydrates increased the counts of lactic acid bacteria

leading to their survival (Lan *et al.*, 2005). In addition, fibre levels in a particular diet significantly influences the growth and diversification of the microbial community (Mateos *et al.*, 2012).

A lot of theories about how dietary fibre work as prebiotics have been reported, even so, these mechanisms are not exclusive (Figure 2.7). Generally, the production of SCFAs triggers a series of actions such as a low pH, which in turn enhances the rate of digestion and nutrient utilization. Specific mode of actions employed by gut microbes include:

a. Bacteria antagonism: Beneficial bacteria inhibit the growth of pathogenic bacteria through competition for limiting nutrients and production of toxins (such as curvacin. nisin, and bifidocin) (Ricke, 2003; Solis-Cruz *et al.*, 2019). As a result, the gut microbiota population shifts more towards the non-pathogenic bacteria. For instance, Fukuda *et al.*, (2011) established a primary relationship was established between the survival of mice infected with *enterohaemorrhagic E. coli* 0157: H7 and the increase in counts of *Bifidobacterium* species (Fukuda *et al.*, 2011). In addition, acid-intolerant pathogens such as *Salmonella*, *E. coli, and C. perfringens* survive at a pH range of 5.4-7.2. However, the production of SCFAs by beneficial microbes can lower the pH to about 5 or even below, which indirectly inhibits the growth of pathogenic bacteria. Butyric, acetic and propionic acids directly destroy pathogenic bacteria by attacking their cell structures (cell wall and cytoplasmic membrane) and inhibiting their cellular functions related to cell synthesis and development (Ricke, 2003; Fernández-Rubio *et al.*, 2009).
b. Stimulation of immune system: The increase in beneficial bacteria, prevention of pathogen adherence to intestinal mucosa and increase in immunoglobulin protection are some immunological effects linked with oligosaccharides (Seifert and Watzl, 2007). Dietary supplementation of fructooligosaccharide-inulin reduced the counts of viable *Salmonella*

enteritidis and decreased inflammasome activation (Babu *et al.*, 2012; Pourabedin and Zhao, 2015).

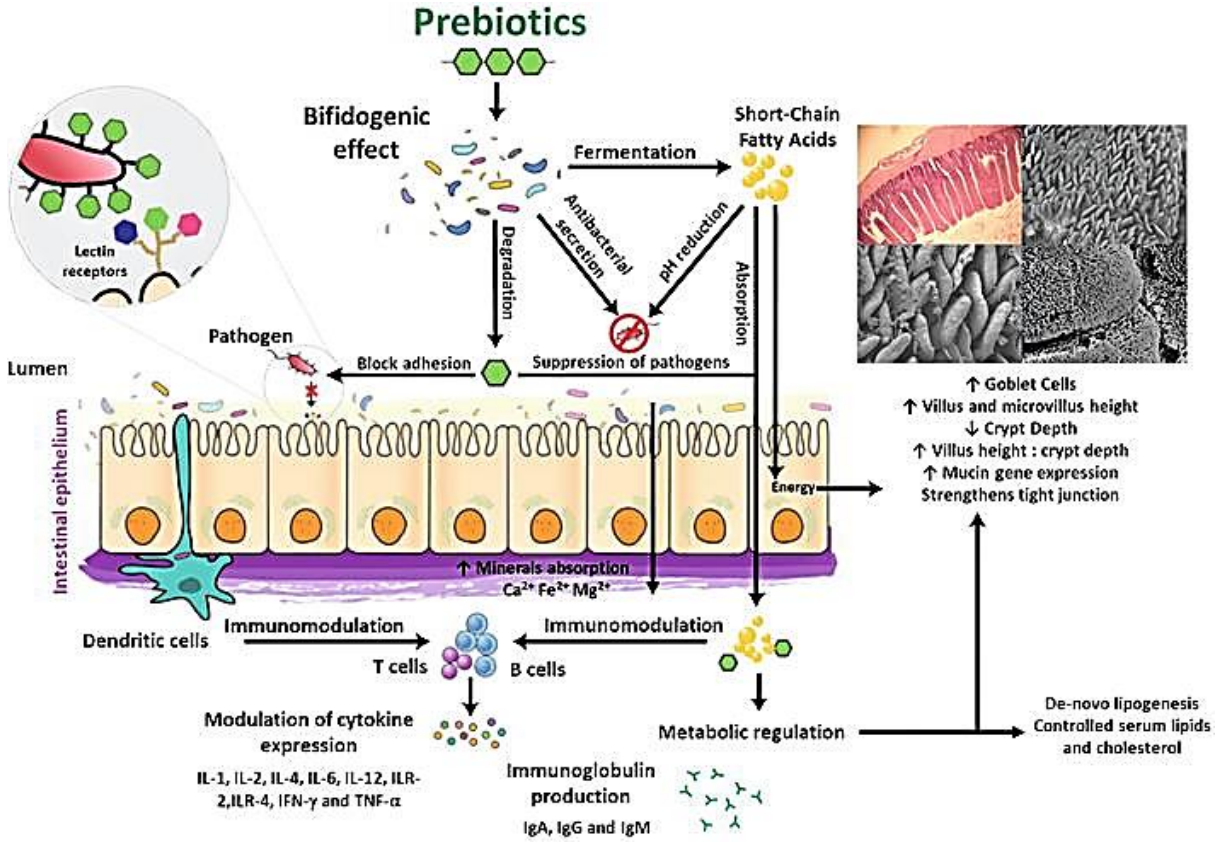

Figure 2. 6: Mechanisms by which dietary fibre & SCFAs work as a prebiotic (Pourabedin and Zhao, 2015)

Also, initial stages of *Clostridium perfringens* infection was contained by feeding broilers mannan-oligosaccharides. The mannan-oligosaccharides helped induce a T-helper type-1 cell-associated control pathway, which is responsible for producing proteins that promote the production of antibodies and phagocytosis. According to Buzás *et al.* (2006), an agonist relationship exists between dietary fibre and toll-like receptors which aides in the control of pathogens and their activities.

c. Improved intestinal morphological structure: Increased villi height, reduced crypt depth and villi height to crypt depth ratio (VH: CD) in different parts of broiler gut (Chen, 2003; Rehman *et al.*, 2007) have been associated with the presence of DF in broiler diets. The increase in villi height and VH: CD improves gut development, which enhances nutrient digestion and absorption (Jha *et al.*, 2019). In studies conducted by Loddi (2003) and Pelicano *et al*. (21005), longer villi height was observed when birds were fed MOS. In addition, Johansson *et al.* (2008) indicated a relationship between the dietary supplementation of prebiotics and multiplication of intestinal goblets cells; goblet cells are needed for the production of mucins. Mucins attach themselves to pathogens while preventing their attachment to intestinal mucosa.

2.3.5a. Effect of Dietary Fibre on the Production of Short Chain Fatty Acids (SCFAs)

Also, short-chain fatty acids (mostly butyric, acetic, propionic, valeric) are produced along the gut through the microbial fermentation of indigestible fractions of a diet (Holscher *et al*, 2015). Short-chain fatty acids are organic compounds with a carboxyl functional group having the chemical structure, R-COOH (Haq *et al.*, 2017). Unlike pathogenic bacteria, beneficial bacteria are able to produce SCFAs (Jamroz *et al.*, 2002). They serve as a source of energy for gut microbiota with about 90-99% used or at times absorbed back into the gut (Ruppin *et al.*, 1980). Acetic acid positively affects the blood-brain barrier and serves as a source of energy for muscle tissue in the liver (Perry *et al.*, 2016). Propionic acid is usually used as a substrate for gluconeogenesis (De Vadder *et al.*, 2014). Butyric acid is a preferred source of energy for metabolic activities of colonocytes and enterocytes. Again, an increase in butyric acid levels enhances gut mucosal health of monogastrics by inhibiting the occurrence of GIT disorders (Bedford and Gong, 2018). Feeding coarser particles or supplementation of a butyrate product improved body weight gain and feed

conversion ratio (FCR) of broilers significantly (Qaisrani *et al.*, 2015). To add, butyric acid is also known to reduce hindgut protein fermentation because it suppresses protein-fermenting microbiota, especially the gram-negative population in broilers (Gunal *et al.*, 2006). The reduction in the colonization of harmful bacteria in the digestive tract of broilers was attributed to the beneficial effect of SCFAs (Zhang *et al.*, 2011 and Chamba *et al.*, 2014). As stated earlier, the production of SCFAs lowers gut pH. This creates a concentration gradient enabling the diffusion of SCFAs into the cell walls of pathogenic bacteria to inhibit cellular synthesis and development. Generally, SCFAs: a) stimulate nutrient digestibility through the enhancement of proteolytic activities; b) boost beneficial bacteria growth through their bacteriostatic and bactericidal effects on harmful bacteria; c) promote digestive enzymatic processes; d) encourage gut health and e) lower intestinal pH (Mansoub *et al.*, 2011; Papatsiros *et al.*, 2013).

Dietary composition is a major determinant of the efficiency and quantity of SCFAs produced in the gut (Papatsiros *et al.*, 2013). Dietary fibre is a key component that largely influences the production of SCFAs due to their indigestible but fermentable nature. The production of SCFAs is one of the intermediate metabolic pathways by which gut microbiota utilizes dietary fibre as an energy source in animals (Hsieh *et al.*, 2015). Due to their heterogeneous nature, they serve as a means by which a broad spectrum of SCFAs is produced. The varied dietary fibre compositions in plant-based diets was reported to have influenced the production of SCFAs in broilers (Tsukahara and Ushida, 2000). Additionally, the type of dietary fibre determines the composition of SCFAs produced (Gibson *et al.*, 2010). Inclusion of dried distillers' grains with solubles and wheat bran in broiler diets resulted in an increase in production of acetic, butyric and propionic acids in a decreasing order (Walugembe *et al.*, 2015). Furthermore, fructans are noted for their direct effect on the production of acetate and indirect production of butyrate through microbial cross-feeding

(Holscher *et al.*, 2015). Fermentable dietary fibres such as arabinoxylan-oligosaccharides, galacto-oligosaccharides and inulin-type fructans largely influence the production of SCFAs. This may be due to the effect of increasing the counts of saccharolytic bacterial population (Gibson *et al.*, 2010).

Again, the level of DF included in a diet can influence the profile and concentration of SCFAs produced (Prasad and Bondy, 2019). This may be due to the linear increase in the proliferation of beneficial bacteria and their fermentative processes as the level of DF increases (Kheravii *et al.*, 2018). However, in testing for the effect of DF levels on the production of SCFAs, Bogusławska-Tryk *et al.* (2015) observed an increase in levels of SCFAs in the caecum and ileum when lignocellulose was fed at 0.5% as compared to 0.25 and 1% inclusion rates. Likewise, Walugembe *et al.* (2015) observed a reduction in butyric acid levels in broilers fed diets containing up to 80 g/kg each of WB and dried distillers' grains with solubles (DDGS) as compared to those fed 0% fibre. The lack of increase in SCFAs despite the increasing levels of DF in the two studies mentioned above was attributed to the effect of the fibre type used, insoluble fibre, on gut transit time. Insoluble fibre is known to reduce gut transit time, which may have reduced the extent of fermentation. Total SCFAs and butyric acid levels were elevated when birds were fed diets with 3% WB in a study conducted by Shang *et al.* (2020). The inconsistencies observed from these reports can also be attributed to the differences in breed, age or stage of gastro-intestinal development of birds (Kheravii *et al.*, 2018). Again, consistency in the daily intake of a fibre type may affect SCFAs production (Den Besten *et al.*, 2013). Work done by Heo *et al.* (2014) showed that fermentation of raw potato starch (resistant starch) was possible in pigs when dietary treatments were fed consistently for 21 days. This may be due to a progressive change in the microbial profile in response to the type of fibre fed over the period of time. Hence, changing an

insoluble fibre-based monogastric diet abruptly to a soluble fibre-based diet might decrease the amount of SCFAs produced due to a change in microbial profile.

2.4. Particle Size

Particle size refers to the fineness or coarseness of an ingredient or feed (Amerah *et al.*, 2007a). It is expressed as geometric mean diameter (Nir *et al.*, 1994a). Any variation in particle size distribution is known as geometric standard deviation (GSD; S_{gw}) which helps to establish a range of variation and determines uniformity (Amerah *et al.*, 2007a). Addo *et al.* (2012) reported that a smaller geometric standard deviation indicates a high uniformity. Particle size distribution of feed or feed ingredient can be determined by dry or wet sieving (Ruhnke *et al.*, 2015; Kalivoda *et al.*, 2017). Usually, dry sieve analysis is usually carried out on feed samples while wet sieve analysis can be performed on feed samples, excreta or digesta (Ruhnke *et al.*, 2015).

2.4.1. Importance of Dietary Particle Size in Poultry Nutrition

Birds and layers consume diets based on their taste, visual, tactile and olfactory stimuli (Picard *et al.*, 2002). According to Gentle (1979), birds use their mechanoreceptors (found on their beaks) to differentiate between particle sizes. For instance, chicks are attracted to and prefer larger and brightly coloured feed particles (Schiffman, 1968; Oppong-Sekyere *et al.*, 2012). However, their preferences tend to change within the course of their growth (Nir *et al.*, 1989) and according to Gentle (1979), this may be influenced by the growth in size of a bird's gape. These characteristics of a bird's physiology makes the knowledge about particle size in poultry diets essential in achieving the desired growth performance and productivity.

In previous years, little attention has been given to the role particle size plays in feed manufacturing and its subsequent effect on the optimal growth and productivity of poultry. However, based on

the physiological needs of the monogastric animal, the optimal ingredient particle size will enhance efficient nutrient utilization and productivity (Nir and Ptichi, 2001). This may be because of the influence of particle size on the surface area available for enzymatic action (Addo *et al.*, 2012). For example, researchers have established a link between larger particles in mash diets and higher rate of amylase activity and bile acid concentration (Svihus., 2011). This may be due to the longer grinding and transit time, which encourages better digestion. Yegani and Korver (2008) reported the need to provide monogastrics diets with uniformly distributed particles in order to optimize performance. Betscher *et al.* (2010) attributed the improved morphological structure, gut health and broiler performance to the positive effect of diet structure and a well-distributed feed. Again, dietary particle size affects the development of digestive (Zaefarian *et al.*, 2016) organs and an optimal performance of the GIT is crucial in a bird's performance (Mateos *et al.*, 2012). According to Nir and Ptichi (2001), dietary particle size of mash diets influences the extent of grind by the gizzard, hence directly affects gizzard weights of birds. Excessive milling and thermal treatments to produce pelleted or highly processed feed may limit the role particle size plays in animal production (Ruhnke *et al.*, 2015).

2.4.1a. Factors that Influence Dietary Particle Size in Poultry Nutrition

Feed and ingredient processing are important in broiler nutrition as the final product can improve or destroy the digestive physiology and health of the animal (Yegani and Korver, 2008). Generally, the desired target size of feed, normally influenced by age of the animal, determines the conditions for the grinding processes (Oppong-Sekyere *et al.*, 2012). The age of the animal is considered in relation to the stage of their GIT development. Traditionally, grains fed to day-old chicks up to three weeks are reduced to fine particles while those for birds above three weeks are ground to a coarse texture (Mateos *et al.*, 2012). Also, the type of mill, screen size and speed (of hammers or

rollers) influence the particle size of feed (Schofield, 2005; Amerah *et al.,* 2007a). Hammer mills produce more regular-sized particles, which are influenced by screen size and hammer speed (Koch, 1996). Roller mills, on the other hand, produce a higher proportion of medium to coarse particles with a less amount of fines (Nir and Ptichi, 2001). This is influenced by the type of corrugation on these rollers. Characteristics of grains such as endosperm hardness, moisture content, type of grain, maturity and size determine grinding conditions (Amerah *et al.,* 2007a; Kiarie and Mills, 2019). Regular and finer particles are identified with the milling of softer endosperms (Carré *et al.,* 2005). A better broiler growth performance is linked to harder endosperms when manufacturing mash diets as they give coarser and irregular particles (Pirgozliev *et al.,* 2003). Again, cost increases as the extent of grinding increases and this tends to influence grinding conditions to a larger extent (Wondra *et al.,* 1995).

2.4.1b. Effect of Grinding on Feed Utilisation in Poultry Nutrition

Grinding of some feed ingredients is an inevitable step in the feed manufacturing process (Oppong-Sekyere *et al.*, 2012). Usually, commercial protein-based ingredients (soybean meal, meat meal and canola cake) are pre-processed and are available at an already determined particle size (Amerah *et al.*, 2007a). However, the extent of particle size reduction of carbohydrate-based ingredients such as cereals and legumes differ per feed mill as they come as raw grains. Hence, they have to be ground at individual feed mills to achieve a desired size (Oppong-Sekyere *et al.*, 2012). Reducing the size of feed ingredients lessens segregation of nutrients during handling and makes mixing easier (Pacheco *et al.*, 2018). Particle size reduction is a two-step process that involves the splitting of the outer coat to expose the endosperm of a seed (Amerah *et al.*, 2007a; Oppong-Sekyere *et al.*, 2012). It is usually done by using hammer or roller mills. According to Anguita *et al.* (2006), grinding conditions are specific to grain sizes, maturity, crystallinity and

brittleness. Over the years, feed millers have preferred fine grinding in order to increase surface area for the efficient work of digestive enzymes. In pigs, potential negative effects associated with fine grinding include development of ulcers in the oesophageal region and increased susceptibility to the colonization of harmful bacteria (Nir *et al.*, 1994b). However, the inclusion of structural components such as coarse or whole grains in monogastric diets have received much attention (Ferket and Gernat, 2006). This is due to the effects on intestinal morphology, microbiota profile and better peristalsis movement (Amerah *et al.*, 2007b). The impact of coarse or fine grinding is affected by the feed form; mash, crumbled or pelleted diets. The optimum particle size of monogastric diets based on maize or sorghum ranges from 600-900µm (Nir *et al.*, 1994a; Amerah *et al.*, 2007a).

2.4.2. Effect of Dietary Particle Size on Growth Performance

There are inconsistencies in the reports of studies carried out on the influence of feed particle size on broiler growth performance (Amerah *et al.*, 2007a; Jacobs *et al.*, 2010; Chewning *et al.*, 2012; Oppong-Sekyere *et al.*, 2012). The response of broilers to feed particle size is influenced by rearing systems, type of grain, age of bird, phase at which a particle size is fed, feed form and the stage of GIT development (Jacobs *et al.*, 2010; Kheravii *et al.*, 2018).

Broiler diets must be formulated to improve GIT development right from day one in order to achieve an optimal growth performance (Rubio *et al.*, 2020). Previous studies have indicated that feeding broilers larger particles stimulates a better development of digestive organs such as crop, proventriculus and gizzard (Naderinejad *et al.*, 2016; Kiarie and Mills, 2019). Consequently, this enhances an efficient breakdown and absorption of nutrients. Similarly, Nir *et al.* (1995) reported that feeding larger particle sizes to broilers stimulates better development of the gizzard which enhances physical and chemical activities; and this can potentially improve nutrient utilization. In

addition, optimal nutrient digestion occurs from one to about day 14 in broilers. Hence, strategies geared towards improving weight gain potential for a good market weight must be initiated during this period (Batal and Parsons, 2002). Rubio *et al.* (2020) observed no improvement in feed conversion ratio when broilers were fed coarser (1,664µm) corn particles during the finisher phase (day 28 to day 42). This was attributed to poor development of the GIT during the starter phase (day 1 to day 27) such that the birds were unable to effectively utilize the coarser particles during the finisher phase. They further indicated that particle size of corn (average of 650µm) fed to the birds from day one to 28 may have not been structurally adequate to prepare the GIT for the corn particle size fed from day 28 to 42 (1,664µm). On the other hand, Xu *et al.* (2015a) demonstrated a decrease in growth performance when birds were fed coarse particles (1642µm) from day one. Feed additives such as premixes are finer, hence can be rejected during feed intake by birds due to their selective preference for larger feed particles. Hence, Xu *et al.* (2015a) attributed the decline in growth performance to the consumption of less balanced nutrients. Also, the impact of dietary particle size on growth performance can be influenced by the type of rearing system used (Rubio *et al.*, 2020). In rearing systems such as deep litter system, structural components such as litter (wood shavings) are used as bedding materials in cages. According to Xu *et al.* (2017), the ingestion of wood shavings by broilers reared in a deep-litter system elicits similar effects as the consumption of large feed particles; that is mechanical stimulation of the gizzard. On the other hand, this is not so in birds reared in a battery cage system due to the absence of a bedding material (wood shavings). As such, dietary particle size manipulation must be done considering the presence or absence of other structural components in the environs of broilers to avoid negatively affecting growth performance. Figure 2.8 shows how particle size of broiler diets triggers a series of biological effects. Due to the complex nature of dietary fibre and coarse feed particles, the

secretion of digestive enzymes increases in order to break down the complex structure of dietary fibre or coarse feed particles. This creates a favourable acidic environment for microbial fermentation and seeding of beneficial bacteria. Consequently, nutrient utilization and absorption is increased with short chain fatty acids increasing as well.

2.4.3. Effect of Dietary Particle Size on Microbial Profile

Primarily, the mode of action by which particle size changes gut microbial profile is primarily based on alterations in gizzard activity, gut pH and digesta transit time (Zaefarian *et al.*, 2016).

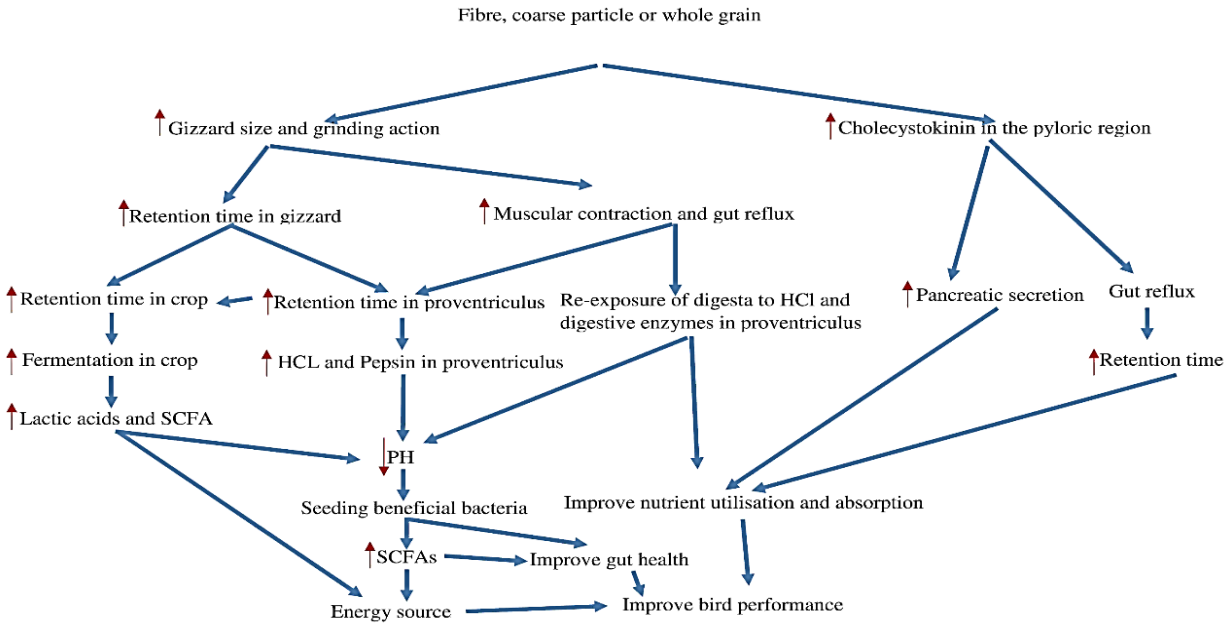

Figure 2. 7: Effect of particle size on nutrient digestion, growth and gut health in poultry (Kheravii *et al*., 2018)

As stated earlier, providing broilers coarse feed particles enhances the mechanical activity of the gizzard, which triggers the release of digestive enzymes resulting in an acidic environment (Engberg *et al.*, 2002). Coupled with the increased digesta transit time, the survival rate of acid-intolerant pathogenic bacteria (such as *E. coli* and *S. enterica serovar Typhimurium*) present in feed will be reduced (Svihus, 2014).

Also, the acidic environment creates an opportunity for an increase in early seeding of beneficial bacteria such as *Lactobacilli*, *Streptococci* and *Coliform spp*. in upper gut (Fuller, 2001). An early abundance of non-pathogenic bacteria stimulates the release of compounds such flavonoids (Sulek *et al.*, 2014). Flavonoids are critical in maintaining gut integrity and in the stimulation of immune responses (Sulek *et al.*, 2014). Inclusion of coarsely ground maize (>1000µm) in broiler diets elevated the counts of *Lactobacillus spp*. and *Bifidobacteria spp*. but reduced the numbers of *Campylobacter spp*., *E. coli* and *Bacteroides spp*. (Jacobs *et al.*, 2010; Singh *et al.*, 2014).

A fast gut transit time is associated with feeding finer or smaller particles, which reduces nutrient digestion and utilization due to the shorter digesta retention time (Amerah *et al.*, 2007b; Kheravii *et al.*, 2018). As a result, a greater proportion of digesta accumulates in the distal gut, which can be harmful to the host depending on the nature of diet (Kiarie and Mills, 2019). If a soluble diet is fed, a lower amount of energy will be made available to the host. This is because the microbes will use some of the energy generated for their own metabolic activities (Lan *et al.*, 2005). Hindgut protein fermentation leads to the production of toxic compounds such as phenols, amines, ammonia and thiols (Timbermont *et al.*, 2011). These compounds create a suitable gut environment for the growth of pathogenic bacteria such as *Clostridium spp*. (Timbermont *et al.*, 2011).

2.4.4. Effect of Dietary Particle Size on the Production of SCFAs

Recent reports indicate a possible effect of dietary particle size on SCFAs production. According to Kheravii *et al.* (2018), the inclusion of large fibrous particles in broiler diets, extends digesta passage rate in the foregut while lowering the pH in the crop. This initiates early microbial fermentation of DF in the foregut which improves gut health. Bogusławska-Tryk *et al.* (2015) reported early proliferation of *Lactobacillus spp*. in the crop of broilers when lignocellulose was

added to their diets. The early reduction of pH in the crop is essential to the inhibition of pathogenic bacteria in the gut (Classen *et al.*, 2016). According to Van Immerseel *et al.* (2006), SCFAs remain longer in un-dissociated forms in a low environmental pH, which enhances their antimicrobial properties.

2.4.4a. Impact of Short-Chain Fatty Acids on Nutrient Utilization and Digestibility

Nutrient digestion and utilization occur in the gut, hence, a proper functioning gut is essential for optimal growth performance of broilers (Jha *et al.*, 2019). The gut is the ultimate hub of a host's immunity; hence if the gut is healthy, available nutrients and metabolites will be geared towards growth and development (Montagne *et al.*, 2003). A positive link between the levels of SCFAs in the distal gut and broiler growth performance has been established (Singh *et al.,* 2012; Park *et al.,* 2013). This may due to the positive influence of SCFAs on the development of microscopic structures in the small intestines, digestive enzyme secretions, maintenance of intestinal epithelial integrity, defense systems and reduction in pathogenic virulence (Guilloteau *et al.,* 2010). Kiarie *et al.* (2014) concluded that the higher levels of acetic and butyric acids in birds fed wheat-based diets may have elicited a higher growth performance as compared to birds fed corn-based diets. Additionally, via the peptide YY pathway, SCFAs are able to influence nutrient digestion and utilization (Park *et al.,* 2013). The peptide YY is a hormone synthesized and secreted in the distal ileum and colon in response to the presence of SCFAs in the intestinal lumen. The release of the hormone causes ileal brake, where appetite and feed intake are reduced, leading to a delayed gastric emptying (Cuche *et al.,* 2000). The extended digesta retention time can promote a better digestion and absorption of nutrients in the small intestines (Cuche *et al.*, 2000). The effect of the peptide YY hormone on nutrient digestibility and utilization has been demonstrated in rats (Cherbut *et al.*, 1998) and piglets (Cuche *et al.*, 2000). The ability of SCFAs to regulate intestinal flow goes a long

way to promote efficient nutrient digestion and utilization (Pan and Yu, 2014). Inclusion of fibrous feed ingredients in broiler diets away from the traditional dietary composition of easily digestible components results in the generation of 'extra' energy from SCFAs which can improve feed conversion ratio (Lan *et al.,* 2005). Due to their acidic nature, the presence of SCFAs in the gut increases the rate of protein synthesis by triggering the release of enzymes such as pepsin (Muramatsu *et al.,* 1988).

2.4.4b. Impact of Short-Chain Fatty Acids on Gut Morphology

Enterocytes and colonocytes are examples of cells that make up the epithelium covering the small intestines and colon respectively. Colonocytes are instrumental in the absorption of water, sodium, and chloride from undigested feed materials (Hamer *et al.,* 2010). Many factors are identified with healthy guts but the morphology of the small intestines structures (villi and crypts) are baseline indicators (Wang and Peng, 2008). The villus is noted for its role in digestion and absorption of nutrients; hence a longer villus is ideal as it increases the luminal absorptive area of the small intestines (Laudadio *et al.,* 2012). On the other hand, the crypt, which serves as the site for growth and multiplication of enterocytes, must be shallow (Oliveira *et al.,* 2008). A shallow crypt reflects a healthy set of villi, indicating a lower need for the synthesis of new enterocytes for villi growth. Therefore, energy can be used for the development of other structures (Miles *et al.,* 2006). Previous studies have proven the positive effects of SCFAs, especially butyric acid, on the development of gut structure and its functioning (Guerra-Ordaz *et al.,* 2013; Khatibjoo *et al.,* 2018) (Figure 2.8). Butyric acid stimulates the occurrence of metabolic activities such as cellular differentiation in the epithelium, which may result in an increase in intestinal tissue weight (Fukunaga *et al.,* 2003; Rinttilä and Apajalahti, 2013). Kien *et al.* (2007) observed a linear increase in villi height (VH), crypt depth (CD) and villi to crypt depth ratio (VH: CD) of piglets as butyrate levels rose with age.

This was attributed to the anti-peristaltic movement of butyrate from the caecum into small intestines, which influenced cell proliferation.

Butyric acid also preserves gut integrity by protecting tight junction proteins to avoid the 'leaky gut' situation (Sun and O'Riordan, 2013). On the other hand, acetic acid is responsible for effective blood flow in the colon and reduces apoptosis (Scheppach, 1994). It also promotes efficient ileal motility and mucin production (Liu *et al.*, 2017).

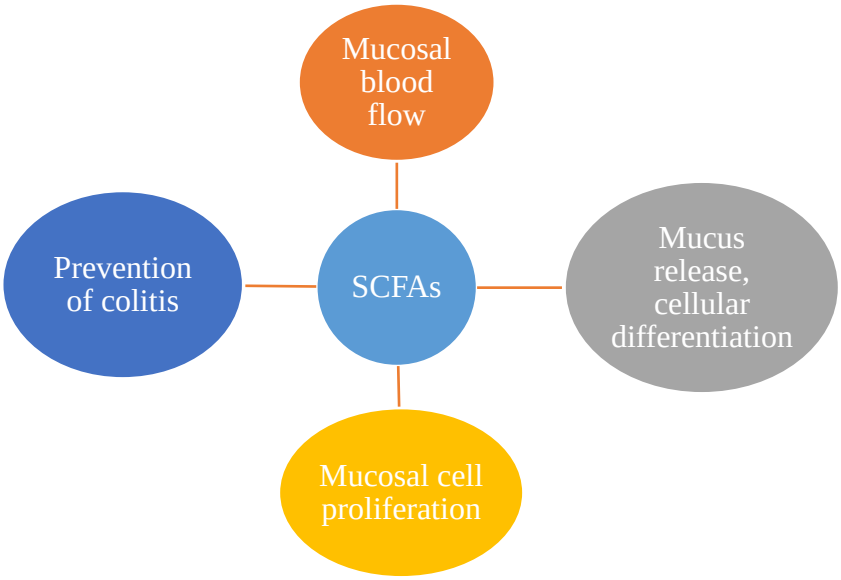

Figure 2. 8: Effect of SCFAs on gut morphology (Scheppach, 1994)

Some researchers believe that the influence of SCFAs and the microbial community on gut structure and function are not exclusive (Liao *et al.*, 2020). This may be due to the spatial heterogeneity of microbial communities along the gut (Gong *et al.*, 2007). According to Choi *et al.* (2014), the composition of the microbial communities at every point along the gut may pronounce a distinct effect on gut morphology structure. This interaction is significant as farmers

can regulate and promote good intestinal development in broilers through dietary manipulation (Liao *et al.*, 2020).

2.5. Wheat bran as a dietary fibre source

Wheat bran is an insoluble (arabinoxylan and cellulose) DF with about <5% of soluble components (β-glucans) (Onipe *et al.*, 2015). It is an agro-industrial by-product obtained after the processing of wheat grains (*Triticum aestivum* L.) into flour (Vermeulen *et al.*, 2018). Based on the variety of wheat and the milling process, about 10-19% of WB can be obtained from the kernel while the production of wheat offal can yield about 50% WB (Ash, 1992). Annually, about 150 million tonnes of WB are gathered as by-products globally (Wanzenböck *et al.*, 2018). Wheat bran is highly rich in minerals (iron, zinc and phosphorus), vitamins (thiamin and riboflavin) and bioactive components (ferulic acid, flavonoids, and carotenoids) (Andersson *et al.*, 2014; Onipe *et al.*, 2015). The chemical composition of WB is shown in Table 2.3. Even though it has a higher oil content than the whole grain, it has a lower energy value (Chalamacharla *et al.*, 2018). This necessitates the inclusion of oil to WB-based diets to supplement the dietary energy levels.

2.5a. Influence of Particle Size and Wheat Bran on Butyric Acid Production

In a study conducted by Kjølbæk *et al.* (2020), the degradation of arabinoxylan-ferulic acid linkages in WB resulted in the production of butyric acid as the dominant SCFA in the colon. Courtin *et al.* (2008) and Akhtar *et al.* (2012) reported positive influences of WB-derived arabinoxylans on gut microbial composition, anti-inflammatory effects and intestinal barrier function in broilers.

Table 2. 3: Chemical composition of wheat bran

Parameter	Composition
Dry matter (% as fed)	83.6-90.3
Crude fibre (% DM)	6.3-14.7
Crude protein (% DM)	14.1-20.5
Ash (% DM)	4.0-7.3
Ether extract (% DM)	2.1-5.7
Neutral detergent fibre (% DM)	32.4-56.5
Acid-detergent fibre (% DM)	8.4-17.6
Lignin (% DM)	1.9-5.3
Starch (Polarimetry) (% DM)	11.1-35.4
Gross energy (MJ/kg DM)	18.0-19.9

Heuzé *et al.* (2015)

Additionally, an improvement in growth performance and phosphorus utilization in broilers was attributed to the effect of WB phytate (Cavalcanti and Behnke, 2004). On the other hand, the phytate effect of WB can be limited when WB-based diets are pelleted. This can be attributed to the negative effect of heat treatment on phytate (Cavalcanti and Behnke, 2004). The microbial fermentation of WB is very beneficial to the host due to its positive effects on microbial cross-feeding (Deroover *et al.*, 2017). As mentioned earlier, WB is a very good source of butyrate due to its arabinoxylan component (Kjølbæk *et al.*, 2020). However, in the absence of indigenous butyrate-producing microorganisms, primary degraders such as *Bifidobacteria* and *Lactobacillus spp*. feed on WB to produce acetic and lactic acids respectively (De Maesschalck, *et al.*, 2015). However, in the presence of *Faecalibacterium* and *Roseburia*, acetic and lactic acids can then be converted into butyric acid (Moens *et al.*, 2016). Hence, a broad spectrum of SCFAs is produced. Vermeulen *et al.* (2018) identified the effect of WB on cross-feeding and the growth of beneficial bacteria in broilers.

Post-weaning diarrhoea, a common cause of mortalities in newly weaned piglets is caused by *E. coli* and *Salmonella* five to ten days after weaning (Lu *et al.*, 2018). Molist *et al.* (2012) fed *E. coli*

K88-challenged piglets with coarsely ground WB. A reduction in the severity of diarrhoea, an increase in butyric acid and a decrease in *E. coli* K88 adhesion were observed. The results obtained may be as a result of the reduction effect of butyric acid on the counts of *Salmonella* and *Enterobacteria* in the gut (Bedford and Gong, 2018). In poultry, the occurrence of *Salmonellosis* causes great economic losses as well as severe infections in humans (Antunes *et al.*, 2016). *Salmonellosis* is caused *by Salmonella enterica, serovars Enteritidis* or *Typhimurium* (Dar *et al.*, 2017). Evidence from studies conducted have proven the antimicrobial role WB plays in the reduction of the incidence of *Salmonellosis*. *Salmonella Typhimurium* post-challenge and pH were reduced when whole wheat was supplemented in broiler diets (Jha *et al.*, 2019). The inclusion of arabinoxylo-oligosaccharides (a derivative of partial hydrolysis of WB) in the diets of broilers significantly increased the number of *Bifidobacteria* in the caeca (Eeckhaut *et al.*, 2008). According to Belenguer *et al.* (2006), *Bifidobacteria* uses arabinoxylans as substrates for the production of butyric acid. Due to their lignified and insoluble nature, the inclusion of large or moderate particles of WB in monogastric diets is important to promote the growth of beneficial bacteria (Zaefarian *et al.*, 2016). Large or moderate particle sizes of WB will increase retention time and enhance fermentation (Mateos *et al.*, 2012).

2.6. Nutritional immune modulation as an alternative to antibiotic usage in broiler production

Nutritional immune modulation refers to the manipulation of the development, maintenance and response of the immune system via nutrients and non-nutritive components of feed (Korver, 2012; Swaggerty *et al.,* 2019). It is a management tool that exploits how the over or undersupply, balance or imbalance of nutrients affects immune response. For instance, the oversupply of selenium in broiler diets leads to toxicity, which compromises the immune system (Peng *et al.,* 2011). The use

of a single immune measurement as a marker for accessing nutritional effectiveness on immunity has been reported to be less effective (Koutsos and Klasing, 2014).

As a result, recent studies are indicating the need to consider supplementing broiler diets with components that enhance the performance of gut microbial community and mucosal immune system. Enhancing gut health is a sure way of promoting the overall wellbeing of the immune system. The concept of gut health encompasses a diet which provides sufficient nutrients, mucosa that maintains gut integrity, and a microbial community that maintains a balanced, healthy environment (Montagne *et al.*, 2003). Also, the guts of pigs and poultry contain about 70% of the body's immune cells (Celi *et al.*, 2019), hence the need to promote gut health. An interactive relationship between the immune system and the gut microbiota has been reported (Oakley and Kogut, 2016).

Prebiotics such as dietary fibre have been considered in this regard due to their effect on leucocytes (Koutsos and Klasing, 2014). For instance, mannanoligosaccharides (MOS) have the ability to elevate immune signals in birds (Teng and Kim, 2018). These immune influences may be strongly correlated to the beneficial influence of DF on the gut microbiome and mucosal surface (Sunkara *et al.*, 2011). Again, the inclusion of β-glucan in the diets of pullets prevented the colonization oby *Salmonella enteritidis* in the visceral organs (Chen *et al.*, 2008). The inclusion of butyrate in chick diets improved mucosal modulation, which led to a better growth performance and a lower colonization of *Salmonella* (Van Immerseel *et al.*, 2004). The weights of the spleen, thymus and Bursa of Fabricus were increased in birds fed sodium-butyrate supplemented diets (Sikandar *et al.*, 2017). The thymus medulla and germinal centre area increased indicating a possible influence on the systemic immune system of the birds. Similarly, Eshak *et al.* (2016) observed an increase in bursa weights when broilers were treated with sodium butyrate. An effective interaction between

the immune system of monogastrics and the microflora of the gut maintains homeostasis and reduces the potential occurrence of diseases (Chassaing *et al.*, 2014).

2.7. Haematological response of broilers to wheat bran as a dietary fibre source in broiler diets

The haematological status of a bird is an indication of its physiological, clinical and nutritional health condition (Olabanji *et al.*, 2007). Largely, diets, drugs or environmental effects may influence the health status of the bird (Adamu *et al.*, 2006). The haematological status refers to the numbers and morphology of blood cellular components and their calculated indices (Merck Manual, 2012). In feeding trials, haematological assessment reflects how test diets affect overall broiler performance, positively or adversely (Olabanji *et al.*, 2007). When haematological values fall within the normal reference range, they indicate the test diet is nutritionally adequate to support all the required metabolism of the bird (Togun *et al.*, 2007). Low or high values demonstrate either extremely high or inadequate amounts of some dietary components (Bawala *et al.*, 2007). In a study by Idan, (2019), increasing levels of WB up to 12% did not have any adverse effect on the haematological indices of broilers. Furthermore, Martínez *et al.* (2015) reported no significant change in the levels of haemoglobin and haematocrit when up to 200g/kg WB was included in the diet of pullets. Also, the inclusion of 5% and 10% of neem kernel cake to broilers did not affect haematological indices apart from red blood cells (Frimpong, 2013).

2.7.1. Blood haematological indices and what they imply

The red blood cells (RBCs) are haemoglobin carriers and are involved in the transport of oxygen and carbon dioxide in the body (Waugh and Grant, 2001). Dietary protein levels, iron, copper, vitamin B2, B6, B12 and folic acid directly affect red blood cells (Nyaulingo, 2013). Low count of RBCs indicates a reduced amount of oxygen transport to the tissues as well as low transport of

carbon dioxide to the lungs for exhalation (Isaac *et al.,* 2013). Blood haemoglobin is the protein pigment in RBCs that contains iron (Onyishi *et al.,* 2017). It is significant in the diagnosis of anaemia and poor heart, liver or marrow function (Olagunju *et al.,* 2013). Packed cell volume (PCV) or haematocrit (Ht/Hct) is the percentage of RBCs in the blood (Nyaulingo, 2013). It is related mainly to the transport of oxygen in the blood. A high Hct indicates an abnormal production of RBCs, dehydration or a reduced plasma volume (Chineke *et al.,* 2006). A high or normal Hb and Hct is associated with a better feed conversion efficiency in broilers (Mitruka and Rawnsley, 1977). The mean corpuscular volume (MCV) denotes the average volume of individual RBCs (Onyishi *et al.*, 2017) A MCV value above the normal reference range implies a microcytic condition due to a larger shape of the RBCs. On the other hand, a lower MCV suggests the RBCs are smaller in shape (Jain, 1993). This parameter can be influenced by the environmental conditions or genotype of birds (Onyishi *et al.,* 2017). Mean corpuscular haemoglobin (MCH) refers to the average mass of haemoglobin found in a red blood cell (Nyaulingo, 2013). It is directly affected by the Hb count of the blood (Onyishi *et al.*, 2017). Mean corpuscular haemoglobin concentration (MCHC) also indicates the levels of haemoglobin in an amount of packed red blood cells (Aster, 2004). Blood platelets are responsible for blood clotting. A low platelet concentration suggests a large amount of blood will be lost in case of injury (Merck Manual, 2012).

The white blood cells (WBCs) are responsible for fighting infections through phagocytosis and the production of antibodies (Soetan *et al.,* 2013). They are sustained by amino acids and they indicate how well the bird responds to stressful conditions (Lumeij, 1997). An increase in WBC levels suggests the occurrence of inflammation, trauma or toxicity while a decrease reflects swelling or a chronic infection (Hidanah *et al.,* 2018). White blood cells can be further categorized

into differentials, which add up to 100 – eosinophils, basophils, heterophils, lymphocytes and monocytes. Eosinophils are responsible for dealing with parasitic invasion and their effects (Irizaary-Rovira, 2004). Basophils respond to allergic reactions by secreting histamine and control antigen invasion (Vleck *et al.,* 2000). Heterophils have antibacterial abilities and elevated levels of heterophils reflect the occurrence of leukaemia, parasitic or bacterial infections (Mitchell and Johns, 2008). However, in extreme cases of bacterial infections or haematopoietic cell diseases, heterophil counts are low (Latimer and Bienzle, 2000). Lymphocytes produce antibodies to fight chronic antigenic stimulations (Hidanah *et al.*, 2018). The heterophil to lymphocyte (H: L) ratio is basically used as a stress indicator (Cotter, 2015). A higher percentage of monocytes in the blood indicates a large number of dead cells. Monocytes normally differentiate into macrophages and are responsible for phagocytosis, tissue repair, secretion of microbicidal factors, pro-inflammatory and anti-inflammatory cytokines (Abbas *et al.*, 2014; Bayona *et al.*, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Experimental Site

The trial was conducted at the Livestock and Poultry Research Centre (LIPREC), School of Agriculture, College of Basic and Applied Sciences, University of Ghana, Legon. LIPREC is located in the Greater Accra Region of Ghana, specifically on latitude $05^0$40'N and longitude $00^0$16'W. It is about 8 km off the Legon-Aburi road from the 'Ritz Junction' at Madina and within the Coastal Savannah zone. LIPREC has a natural rangeland with mainly fire resistant shrubs, trees and tussock plants on it (Osei-Amponsah, 2010). Averagely, it has a temperature of 26.9°C but fluctuates between 24.8°C to 28.3°C in August and February respectively (Osei-Amponsah *et al.*, 2014). The research centre experiences a bimodal rainfall pattern comprising of major and minor seasons. The major season occurs between mid-March to July with the minor season occurring between mid-August to November. LIPREC has an annual rainfall range of 1280-1709 mm with a mean rainfall of 785 mm (Osei-Amponsah, 2014). At 1500 h, relative humidity is between 58 to 83.7% but is slightly lower midmornings.

3.2. Particle Size Analysis

Particle size analysis was carried out at the Nutrition laboratory, Animal Science Department, School of Agriculture, University of Ghana. Samples of maize ground with a hammer mill fitted with 6 and 8 mm screens (Plate 3.1) were obtained and used for the analysis.

A: 6 mm screen B: 8 mm screen

Plate 3. 1: Screens used in the milling of maize

A Ro-tap sieve shaker (Model RX-29, W. S. Tyler Industrial Group, Mentor, OH) (Plate 3.2) which operates simultaneously by a mechanical and circular motion with a stack of sieves (13 in this case) that separates feed particles based on their sizes was used (Kalivoda *et al.*, 2017).

Plate 3. 2: A Rotap machine with 7 sieves

The protocol for standard particle size analysis, ANSI/ASAE S319.4 as described by Kalivoda *et al.* (2017) was followed.

Each sieve was weighed individually with sieve agitators (rubber balls and bristle sieve cleaners) to obtain a tare weight. Sieve agitators ensured the equal flow of the maize particles through the sieve openings (Table 3.1). Approximately 100g (± 5) of sample was weighed, mixed with 0.5g of dispersion agent (Model SSA-58, Gilson Company, Inc., Lewis Center, OH) and poured on the top sieve. The dispersion agent ensured uniform dispersion and prevented the maize particles from binding onto sieve screen surfaces. The sieve stack was then placed inside the Ro-tap machine and run for 15 minutes.

Table 3. 1: Sieve and sieve agitator (s) arrangement

U.S. sieve number	Sieve opening (µm)	Sieve agitator (s)
6	3,360	None
8	2,380	None
12	1,680	3 rubber balls
16	1,190	3 rubber balls
20	841	3 rubber balls
30	595	1 rubber ball; 1 bristle sieve cleaner
40	420	1 rubber ball; 1 bristle sieve cleaner
50	297	1 rubber ball; 1 bristle sieve cleaner
70	210	1 rubber ball; 1 bristle sieve cleaner
100	149	1 bristle sieve cleaner
140	105	1 bristle sieve cleaner
200	74	1 bristle sieve cleaner
270	53	1 bristle sieve cleaner
Pan	-	None

(Kalivoda *et al.*, 2017)

At the end of the run, the sieve stack was removed and each sieve weighed together with their sieve agitator (s). The weight obtained represented the residual sample, which was used to calculate the geometric mean diameter (d_{gw}) and the geometric standard deviation (S_{gw}). Formulas as stated by Kalivoda *et al.*, (2016) were used in these calculations.

3.3. Maize Particle Size Distribution

The particle size distribution for maize samples ground using 6mm and 8mm screens are shown respectively in Figures 1 and 2.

The particle size of maize ground using a 6mm screen averaged 725µm with a geometric standard deviation (GSD) of 3.18µm. The highest proportions of the particle size distribution were retained on the 1700µm (17.23%) and 1800µm (18.80%) sieves.

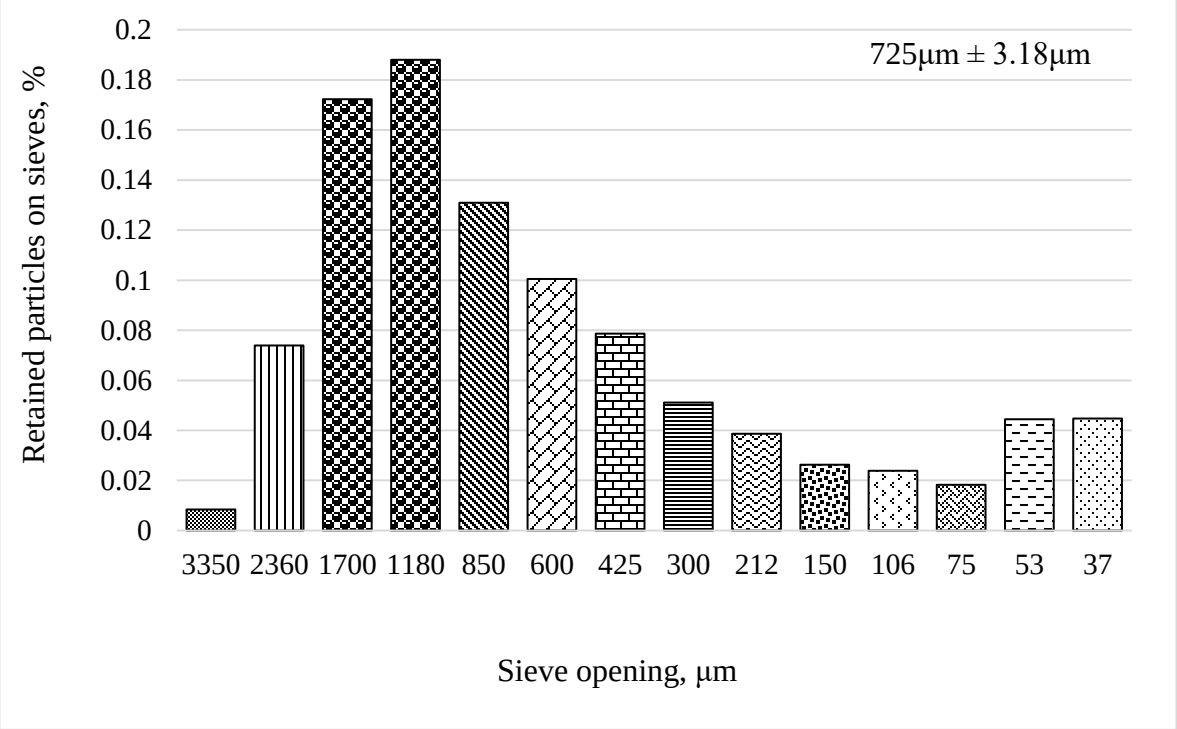

Figure 3. 1: Particle size distribution of maize ground using a 6mm screen

For maize ground using an 8mm screen, most particles were collected on the 2360µm (20.58%) and 1700µm (17.43%) sieves.

The geometric mean average was 1178µm with a geometric standard deviation (GSD) of 3.07µm.

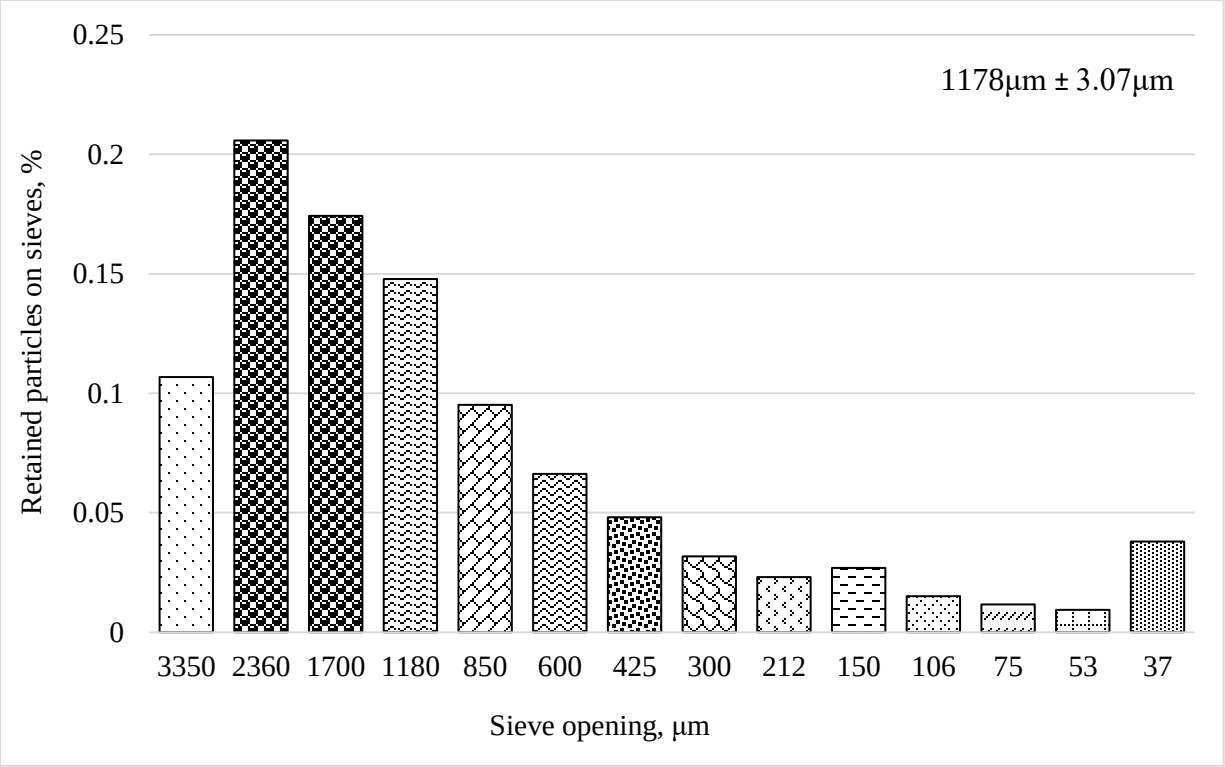

Figure 3. 2: Particle size distribution of maize ground using an 8mm screen

These maize particle sizes ranges were used in the formulation of the experimental diets.

3.4. Experimental Diets

In this trial, wheat bran was used as the dietary fibre source and its inclusion increased among treatments and per phase (Table 3.2).

During the brooding period (one week), a common broiler starter was fed to all birds, which contained 8% wheat bran (WB) with fine maize particles. Subsequently, six dietary treatments were fed in a two-phase feeding regime comprising of a starter (three weeks) and finisher (three weeks) phase (Tables 3.2). To create the experimental treatments concerning the two different maize particle sizes, treatment 1 (T1), treatment 2 (T2) and treatment 3 (T3) were processed to contain fine maize particles whereas treatment 4 (T4), treatment 5 (T5) and treatment 6 (T6) contained coarse maize particles. These particle sizes were maintained for both phases.

During the starter phase, T1, T2 and T3 were formulated to contain 6, 8 and 10%WB. The WB inclusion rate for treatments 4, 5 and 6 followed the same inclusion rate as T1, T2 and T3. With the finisher phase, T1 and T4 contained 13% WB with T2 and T5 containing 15% WB. Treatments 3 and 6 were formulated to contain 17% WB.

Table 3. 2: Experimental diets

Treatment	Particle size (µm)	Level of wheat bran (%)	
		Starter	Finisher
1	Fine	6	13
2	Fine	8	15
3	Fine	10	17
4	Coarse	6	13
5	Coarse	8	15
6	Coarse	10	17

All diets were formulated to meet the recommended broiler nutrient requirements according to Cobb 500 specifications (Cobb 500, 2018). In addition, the dietary treatments were in a mash form and formulated to be isonitrogenous and isocaloric. The composition, calculated nutrient contents and analysed chemical composition of dietary treatments are shown in Table 3.3 and 3.4.

3.5. Experimental Birds, Experimental Design and Housing

A total of three-hundred-day-old mixed sex broiler chicks were obtained from a prominent commercial hatchery.

Wheat Table 3. 3: Percentage composition of the dietary treatments fed during the starter phase (day one to day 21)

Ingredients (%)	Treatment					
	T1	T2[1]	T3	T4	T5	T6
	6%WB +Fine	8%WB +Fine	10%WB +Fine	6%WB +Coarse	8%WB +Coarse	10%WB +Coarse
Wheat bran	6.00	8.00	10.00	6.00	8.00	10.00
Maize	55.50	52.60	49.70	55.50	52.60	49.70
Soybean meal	36.00	35.90	35.80	36.00	35.90	35.80
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Oyster shell	0.55	0.55	0.55	0.55	0.55	0.55
L-Lysine	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Dicalcium phosphate	0.20	0.20	0.20	0.20	0.20	0.20
Toxin binder	0.20	0.20	0.20	0.20	0.20	0.20
Broiler premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Vegetable oil	0.50	1.50	2.50	0.50	1.50	2.50
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analyses						
ME[3] (MJ/kg)	3218.7	3218.3	3217.9	3218.7	3218.3	3217.9
Crude protein (%)	23.22	23.22	23.23	23.22	23.22	23.23
Analysed composition						
Dry matter (%)	86.66	87.79	86.72	87.02	86.17	86.71
CP (% of DM)[4]	26.63	26.69	27.15	26.73	27.05	27.10
CF (% of DM)[5]	3.14	3.19	3.44	3.17	3.50	3.75
EE (% of DM)[6]	4.51	4.46	4.21	4.37	4.49	4.49
Ash (% of DM)[7]	4.10	4.16	2.27	3.61	2.12	3.74

[1]Control diet
[2]Vitamin premix supplied the following kilogram per diet: vit A, 10,000 IU; vit D3, 2000 IU; vit E, 10 IU; vit K, 3 mg; riboflavin, 4.4 mg; cobalamin, 0.05 mg; pantothenic acid, 8 mg; niacin, 16.5 mg; choline, 175 mg; folic acid, 0.5 mg; Mg, 2.3 mg; Fe, 30.5 mg; Zn, 50 mg; Co, 0.27 mg.
[3]Metabolizable energy

These were assigned to six treatment diets. There were five replications, ten birds per replicate and fifty birds per treatment. The experiment was set up in a 2 x 3 factorial arrangement to access the interactive effects between maize particle sizes (fine/coarse) and levels of fibre (wheat bran) inclusion [(starter: 6, 8 and 10%; finisher: 13, 15 and 17%)] in broilers. The birds were completely randomized into thirty wire mesh-sided concrete floor pens (ten birds per pen) with short walls

measuring about 2ft walls. The dimensions for each pen were 1m x 1m, hence, each pen had a stocking density of one bird per $0.1m^2$.

Table 3. 4: Percentage composition dietary treatments fed during the finisher phase (day 22 to day 42)

Ingredients (%)	Treatment (kg)					
	T1	T2[1]	T3	T4	T5	T6
	13%WB +Fine	15%WB +Fine	17%WB +Fine	13%WB +Coarse	15%WB +Coarse	17%WB +Coarse
Wheat bran	13.00	15.00	17.00	13.00	15.00	17.00
Maize	59.00	56.10	53.20	59.00	56.10	53.20
Soybean meal	25.00	24.90	24.80	25.00	24.90	24.80
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Oyster shell	0.55	0.55	0.55	0.55	0.55	0.55
L-Lysine	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Dicalcium phosphate	0.20	0.20	0.20	0.20	0.20	0.20
Toxin binder	0.20	0.20	0.20	0.20	0.20	0.20
Broiler premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Vegetable oil	1.00	2.00	3.00	1.00	2.00	3.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analyses						
ME[3] (MJ/kg)	3123	3122	3122	3123	3122	3122
Crude protein (%)	19.35	19.36	19.36	19.35	19.36	19.36
Analyzed composition						
Dry matter (%)	87.22	86.42	86.96	87.65	86.85	87.56
CP (% of DM)[4]	23.43	22.19	22.89	23.63	22.24	23.71
CF (% of DM)[5]	4.20	4.50	4.78	4.48	4.54	4.80
EE (% of DM)[6]	4.17	4.44	4.24	3.18	4.02	4.28
Ash (% of DM)[7]	5.12	3.60	4.42	4.78	4.54	4.55

[1]Control diet
[2]Vitamin premix supplied the following kilogram per diet: vit A, 10,000 IU; vit D3, 2000 IU; vit E, 10 IU; vit K, 3 mg; riboflavin, 4.4 mg; cobalamin, 0.05 mg; pantothenic acid, 8 mg; niacin, 16.5 mg; choline, 175 mg; folic acid, 0.5 mg; Mg, 2.3 mg; Fe, 30.5 mg; Zn, 50 mg; Co, 0.27 mg.
[3]Metabolizable energy

3.6. Management of Experimental Birds

Day-old broiler chicks (Cobb 500; initial average body weight, 43.86g) were brooded for one week, which served as an acclimatization period (Plate 3.3). Antibiotics and vitamins were

administered through water for two days and a common starter diet was fed for the brooding period. Coal-heated clay pots served as a source of heat for the chicks during the brooding period. Individual chick weights at d7 (between 151 – 207g; average weight, 180g) were used as a baseline to aid in the allocation of the birds to randomized pens.

A: Set-up for brooding period

B: Birds at brooding period

Plate 3. 3: Brooding period

At the start of the experiment, each pen was filled with new wood shavings. These shavings were changed periodically in order to maintain good hygiene. A plastic feeding tube and waterer were placed in each pen. Birds had *ad-libitum* access to clean water and feed throughout the experiment. The birds in each replicate (experimental unit) were collectively fed pre-determined amounts of feed. The feeders were shaken at least once a day. Sunlight and electric lamps served as sources of light. The temperature of the house was maintained at 32 ^{o}C to 33^{o}C, 29^{o}C and 27^{o}C from day

1 to day 7, day 8 to day 14 and day 15 to day 42 respectively. Standard vaccinations and all recommended prophylactic measures were duly followed throughout the entire period. Birds were vaccinated such Newcastle and Gumboro with medications such as coccidiostat being provided.

3.7. Data Collection

3.7.1. Growth Trial

The growth trial lasted 42 days. Initial and weekly body weights (BW) (d0, 7, 14, 21, 28, 35 and 42) of birds per pen (10 birds) were taken and used to calculate average daily gain (ADG) using formula A.

A.

$$\text{ADG (g)} = \frac{\text{Total body weight gained}}{\text{Number of days} \times \text{Number of birds in a pen}}$$

Weekly amounts of feed given and feed refused were recorded and used in the computation of feed disappearance and average daily feed intake (ADFI) using formula B.

B.

$$\text{ADFI (g)} = \frac{\text{Total amount of feed offered} - \text{residual feed}}{\text{Number of days} \times \text{Number of birds in a pen}}$$

Feed conversion ratio was calculated by dividing ADFI by ADG of broilers (c). Mortalities were recorded daily and used to adjust feed conversion ratio.

C.

$$\text{FCE} = \frac{\text{ADFI}}{\text{ADG}}$$

Finally, percentage mortality (P.E.) was calculated using formula D.

D.

$$\text{P. E. (\%)} = \left\{\frac{\text{Total number of dead birds}}{\text{Total number of experimental birds}}\right\} \text{x}100$$

3.7.2. Carcass Characteristics Analysis and Caecal pH Measurement

At the end of the experiment (day 43), one bird of average body weight in each pen (replicate) was randomly selected (five birds per treatment) for carcass traits and caecal pH measurement.

On day 43 the live weight of each bird was taken and recorded as the final weight. Birds were slaughtered by the severance of the carotid arteries and allowed to bleed out completely, then defeathered. Defeathered weights of the birds were taken after which organs of interest were harvested and weighed. Weights of organs such as the gizzard, crop, intestines, kidney, liver, thymus and spleen were taken. After the removal of the viscera, the weights of the carcass, breast, thigh and wings were also recorded.

The caeca of each bird were excised and their contents pooled into a container. The pH of the caecal content was measured using a Milwaukee SM101 pH meter.

3.7.3. Blood Sampling

On day 43, five birds (one per replicate) of average weight from each treatment were selected for the determination of haematological profile and glucose levels. Two mls of blood was drawn from

wing vein using a sterile needle into Ethylene diamine tetra acetic acid (EDTA) vacutainer tubes. Blood samples were placed on ice and transported to the laboratory for blood analysis.

3.7.3.1. Determination of Haematological Indices

The URIT-5250Vet Haematology Analyzer was used in determining/calculating the following blood parameters: red blood cells (RBC), haemoglobin (HGB), haematocrit (HcT), mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC).

3.7.3.2. Differential White Cell Count

Relative percentages of the various white blood cells (basophils, eosinophils, lymphocytes, monocytes and heterophils) present in the blood samples were determined manually. The protocol of Evatt *et al.* (1992) was used. A thin smear of a homogenous blood sample was made on a clean slide and waved to air dry. One volume of Leishman stain (for 1-2 minutes) and two volumes of buffered water (5-7 minutes) were applied on the smear consecutively and allowed to stand. The slide was then washed in excess buffer and allowed to stand for 2 minutes for effective differentiation. A drop of oil was used to mark a portion on the slide and the differential counting in 100 white blood cells was done using an oil immersion objective lens (40X) of a light microscope.

3.7.3.3. Serum Glucose Determination

Drops of blood from each bird were used to determine their glucose level using a Gold-Accu Blood Glucose Monitoring system (Changsha Sinocare Inc., China).

3.7.4. Gut morphological analysis

After blood collection, birds were exsanguinated and allowed to bleed thoroughly from the jugular veins. Abdominal cavities were opened and their small intestines removed. Portions of the jejunum and ileum were taken for the analysis of intestinal morphological changes.

Tissues from the jejunum (extends from pancreo-billiary duct to Meckel's diverticulum) and ileum (extends from the Meckel's diverticulum to the ileo-caecal valve) were taken from each bird. Samples were flushed, injected with 10% neutral formalin and immediately placed in containers filled with appropriate amount of fixative. This fixation step was necessary to preserve and protect the integrity of the tissues. Further intestinal morphometric examination was then carried out on the samples. The protocol of Hair bin Bejo (1990) was followed. The jejunal and ileal samples were sliced with a microbiome blade to display their mucosal surface. The formalin was then washed off by placing sliced samples under running water for about 30 minutes. The formalin-free samples were then dehydrated by immersing them in a series of ethanol solutions with increasing concentrations (70, 80, 90 and 100%) at an interval of 15 minutes each. Ileal and jejunal tissues were then immersed in 100% ethanol at intervals of 30 and 45 minutes apart. Tissue samples were further soaked in xylene, an intermediate solvent, at 20, 20 and 45 minutes apart, which enabled infiltration of molten paraffin wax at 60°C. The waxed samples were kept overnight and allowed to cool to solidification at 20°C. Using an 'embedding centre', the intestinal samples were blocked and sectioned at 6 µm thickness, at an angle of 5^{o} using a microbiome. Sections were fixed on glass slides, heated on water (40^{o}C) to dryness and deparaffinized. They were then stained with haematoxylin and eosin and mounted on histological slides with cover slips. Using a binocular light microscope with a ToupView camera adaptor, the slides were examined. The villi height and crypt depth were then read using a binocular light microscope with a ToupView camera adaptor. The villi

height to crypt depth ratio was also calculated. Values from three well-oriented villi and crypts of each bird were measured in micrometres and the average taken.

3.7.5. Analysis of Caecal Short-Chain Fatty Acids Concentrations

Again, after the blood sampling, the caecal contents of the birds were pooled together per replicate and used for the analysis of short-chain fatty acid concentrations. The short-chain fatty acids (SCFAs) that were analysed included acetic, propionic and butyric acids. Caecal contents of the individual birds were gently squeezed into well labelled Eppendorf tubes and immediately placed on ice for transportation to the laboratory Samples were stored at 4^0C. Stock solutions of acetic, propionic and butyric acid were prepared at a concentration of 1000 mg/ml in 0.4% hydrochloric acid solution (HCL). Serial dilution was carried out for each standard to obtain five concentrations (100, 50, 25, 12.5 and 6.25 µg/ml) separately prior to HPLC analysis. Accurately, 0.5g of the caecal content were weighed into a tube and thoroughly mixed with 5mL of 0.4% HCL. The mixture was initially centrifuged at 4000rpm for 30 minutes and at 10,000rpm for 15 minutes. After the separation, the supernatant was loaded into an HPLC vial for analysis.

Short-chain fatty acids concentrations were determined in caecal samples using the principle of high-performance liquid chromatography-ultraviolet detection (HPLC). The protocols of Reuter (2015) and Kim (2016) were followed. The HPLC analysis was carried out using an Agilent 1100 system (Santa Clara, CA, USA), composed of degasser, quaternary pump, auto sampler, diode array detector (DAD), and HP ChemStation Software. Chromatographic separation was carried out on an ODS C18 (250 x 4.6 mm id., 5 µm particle size) analytical column maintained at 30^0C, 20 µL injection volume and the wavelength monitored at 254nm. The reverse phase principle of HPLC was applied in the chromatographic separation. The mobile phase consisted of A: 10mM KH_2PO_4, which was adjusted to a pH of 2.4 using phosphoric acid and B: Acetonitrile. Table 3.5

shows the gradient time program used for the analysis. With the retention time and peak area generated, standard curves for acetic, propionic and butyric acid were obtained which served as a baseline for the determination of SCFAs levels in the caecal samples expressed in µg/g.

Table 3. 5: Gradient time program for HPLC analysis

Time (minutes)	Flow rate (mL/min)	%A[1]	%B[2]
0.0	1	80	20
10.0	1	40	60
12.5	1	40	60
12.6	1	80	20
15.0	1	80	20

[1]Moble phase A: 10-mM KH_2PO_4
[2]Mobile phase B: Acetonitrile

3.7.6. Microbial Population

The counts of *Lactobacillus spp.* and *E. coli* in caecal samples were analysed. Approximately 1g of the caeca contents were emptied in well-labelled sterile containers and stored at 4^0C pending further analysis. The modified protocol of Hu and Guo (2007) was followed.

From each sample, 0.1g was weighed into sterile containers and diluted in a 1:10 ratio with saline solution (sodium chloride). Further serial dilutions ($10^{-2} – 10^{-9}$) were carried out on the resulting homogenate using saline solution as a diluent. One hundred microliters of each homogenate per sample was plated on sterilized agar plates. *Lactobacillus spp.* were cultured on de man, Rogosa, Sharpe (M.R.S.) agar (CM0361, Oxoid LTD., Basingstoke, Hampshire, England) with the following components: peptone 10.0; 'Lab-Lemco' powder 8.0; yeast powder 4.0; Glucose 20.0; Sorbitan mono-oleate 1ml; Di-potassium hydrogen phosphate 2.0; Sodium acetate $2H_2O$ 5.0; Tri-ammonium citrate 2.0; Magnesium sulphate $7H_2O$ 0.2; Manganese sulphate $4H_2O$ 0.05 and agar 10.0. *E. coli* growth was measured on Eosin methylene blue (E.M.B.) agar (M317-500G, HiMedia

Laboratories Pvt. Ltd., India) made up of: peptic digesta of animal tissue 10.00; Dipotassium phosphate 2.00; lactose 5.00; sucrose 5.00; eosin –Y 0.40; methylene blue 0.065 and agar 13.50. *Lactobacillus spp*. agar plates were incubated anaerobically at 35^0C while *E. coli* agar plates were incubated at 44^0C. Countable colonies were recorded and expressed as log10 cfu/g.

3.7.7. Apparent Nutrient Digestibility Trial

Three birds (replicates) from each treatment (18 birds in total) were selected and used for a one-week digestibility trial. Each bird was placed in a clean and well- labelled metallic battery cage equipped with a nipple drinker and a feeding trough. An adaptation period of 4 days was allowed, followed by a withdrawal of feed for 12 hours. On the 5^{th}, 6^{th} and 7^{th} days, birds were given 250g (average daily feed intake) of their respective diets while water was provided *ad-libitum*. For three consecutive days, total excreta collection was done after every 24-hour period (0800 h – 0800 h) and the excreta immediately frozen at -20^0C for further analysis. Daily refusals were recorded and used to compute daily feed intake. At the end of the 7^{th} day, daily excreta samples of each replicate were thawed, pooled, and weighed. Samples were then dried at 55^0C in an air-flow type oven for five days followed by chemical analysis. The total tract apparent nutrient digestibility was calculated using the digestibility formula by Adeola (2000) as:

$$\text{Digestibility, \%} = 100 \text{ X} \left[\frac{\text{Component intake} - \text{Component faeces}}{\text{Component intake}} \right]$$

3.8. Economics of Production

A feed cost analysis was carried out on all treatments based on the cost of individual feed ingredients (Table 3.6). Parameters such as cost of feed per kilogram (GH₵), total cost of feed consumed (GH₵), and total cost of feed per kilogram weight gain (GH₵), were calculated. Feed

costs per kg for each of the experimental diets were calculated based on the prevailing prices of the ingredients at the time of the experiment.

Table 3. 6: Cost of feed ingredients on a per kilogram basis

Ingredient	Weight per bag (kg)	Cost per bag (GH¢)	Cost per kilogram (GH¢)
Wheat bran	25	16.5	0.66
Maize	50	85	1.7
Soybean meal	50	140	2.8
Dicalcium phosphate	25	95	3.8
Oyster shell grit	50	25	0.5
Salt	50	40	0.8
Lysine	25	240	9.6
Methionine	25	500	20
Broiler premix	25	200	8
Toxin binder	25	190	7.6
Oil	24	165	6.88

3.9. Chemical Analysis

Representative feed and excreta samples were finely ground with a Retsch mill (Model ZM200, Retsch GmbH, Retsch-Allee 1-5, Haan Germany) fitted with a 0.5 mm sieve. Each sample was subjected to proximate analysis according to the Association of Official Analytical Chemist (AOAC, 1984) procedures to determine the dry matter (DM), crude protein (CP), ether extract (EE), crude fibre (CF) and acid-insoluble ash contents. The nitrogen free extract (NFE) was calculated using the formula:

$$NFE = DM\% - (CP\% + CF\% + Ash\% + CF\ \%)$$

3.10. Statistical Analysis

All data gathered were subjected to statistical analysis using the Generalized Linear Mixed Model (GLIMMIX) procedure of the Statistical Analysis System Institute (SAS) version 9.4 (SAS Institute, 1994). The main and interactive effects of particle size and levels of wheat bran on the

parameters mentioned were assessed. Significant mean differences were separated using Tukey's test at a 5% probability level.

CHAPTER FOUR

RESULTS

4.1. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Mortality

At the end of the experimental period, 17 mortalities out of 300 birds were recorded. This amounted to an overall percentage mortality of 5.67%. No evidence of treatment-related effects were observed. Post mortem autopsies carried out indicated yolk sac infection and chronic respiratory tract disease as causes of observed mortalities.

4.2. Effect of dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Apparent Nutrient Digestibility

The digestibility coefficients for dry matter (DM), organic matter (OM) and crude protein (CP) are shown in Table 4.1. The digestibility coefficients of DM, OM and CP averaged 74.86, 43.15 and 70.15% across treatments. No main or interactive effect between WB level and maize particle size on any of the parameters were observed.

4.3. Effect of dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Overall Growth Performance (day one to day 42)

Table 4.2 shows the effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on growth performance from day 1 to day 42. A WB level effect ($p=0.02$) was observed on ADFI by day 42. The ADFI of birds on diets with 6/13%WB (104.55g) and 10/17%WB (104.64g) was significantly higher ($p\leq0.05$) than that of birds on 8/15%WB (94.90g). In addition, a significant effect ($p\leq0.05$) on ADFI caused by an interaction between the WB level effect and maize particle size was observed.

Table 4. 1:Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on dry matter, organic matter and crude protein digestibility

Treatment	%Wheat bran		Maize particle size	%		
	Starter	Finisher		Dry matter	Organic matter	Crude protein
T1	6	13	Fine	73.61	32.82	69.54
T2	8	15	Fine	74.83	45.48	70.23
T3	10	17	Fine	74.05	46.30	71.00
T4	6	13	Coarse	74.15	45.88	68.97
T5	8	15	Coarse	81.28	51.28	74.08
T6	10	17	Coarse	71.22	37.13	67.07
Pooled SEM[1]				4.52	6.08	4.01
				Main effect		
%Wheat bran						
	Starter	Finisher				
	6	13		73.88	39.35	69.26
	8	15		78.06	48.38	72.15
	10	17		72.64	36.71	69.04
SEM				2.26	4.30	2.84
Maize particle size						
			Fine	74.16	41.54	70.26
			Coarse	75.55	41.43	70.04
SEM				1.85	3.50	2.32
Source of variation				P-value		
Wheat bran * Maize particle size				0.37	0.06	0.63
Wheat bran				0.25	0.17	0.69
Maize particle size				0.60	0.98	0.95

SEM[1]: Standard error of means

Feed intake of birds on T4 [(coarse + 6/13%WB); 107.15g] and T6 [(coarse + 10/17%WB); 106.73g] was significantly higher ($p \leq 0.05$) that that of birds on T5 [(coarse + 8/15%WB); 89.29g] but similar ($p \geq 0.05$) to that of birds on T1 [(fine + 6/13%WB); 101.96g], T2 [(fine + 8/15%WB); 100.49g] and T3 [(fine + 10/17%WB); 102.55g].

Table 4. 2: Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on overall growth performance (day 1 to day 42)

				Grams			
Treatment	%Wheat bran		Maize particle size	Average daily feed intake	Average daily gain	Feed conversion ratio	Final body weight
	Starter	Finisher					
T1	6	13	Fine	101.96^{ab}	57.17	1.81^{ab}	2578.4
T2	8	15	Fine	100.49^{ab}	57.25	1.76^{ab}	2612.3
T3	10	17	Fine	102.55^{ab}	54.58	1.90^{b}	2519.5
T4	6	13	Coarse	107.15^{a}	60.01	1.78^{ab}	2695.9
T5	8	15	Coarse	89.29^{b}	54.64	1.66^{a}	2531.8
T6	10	17	Coarse	106.73^{a}	60.66	1.77^{ab}	2726.0
Pooled SEM[1]				3.67	2.20	0.05	100.34
				Main effect			
%Wheat bran							
	Starter	Finisher					
	6	13		104.55^{a}	58.59	1.79	2637.2
	8	15		94.90^{b}	55.95	1.70	2572.0
	10	17		104.64^{a}	57.62	1.83	2622.8
SEM				2.49	1.63	0.04	107.60
Maize particle size							
			Fine	101.67	56.33	1.82^{b}	2570.1
			Coarse	101.06	58.44	1.73^{a}	2651.3
SEM				2.12	1.39	0.03	63.46
Source of variation				P-value			
Wheat bran * Maize particle size				0.05	0.20	0.04	0.41
Wheat bran				0.02	0.55	0.07	0.82
Maize particle size				0.84	0.30	0.05	0.38

SEM[1]: Standard error of means; Means in a column with different superscripts are significantly different at $p \leq 0.05$

There were no main or interactive effect ($p \geq 0.05$) between WB level and maize particle size on ADG and BW throughout the experimental period. However, maize particle size effect on FCR was significant (P=0.05) on FCR with birds fed coarse maize particles (1.73) being the most efficient in converting feed to gain as compared to birds fed fine maize particle sizes (1.82). In addition, a significant response (P=0.04) to the interaction between WB level and maize particle size on FCR was observed. Birds on T5 (1.66) were the most efficient in converting feed to gain at the end of the trial. Their FCR performance was similar ($p \geq 0.05$) to that of birds T1 (1.81), T2

(1.76), T4 (1.78) and T6 (1.77) but higher (p≥0.05) than that of birds T3 (1.90). Figures 4.1, 4.2 and 4.3 illustrates the trend in ADFI, ADG and FCR from day 0 to day 42.

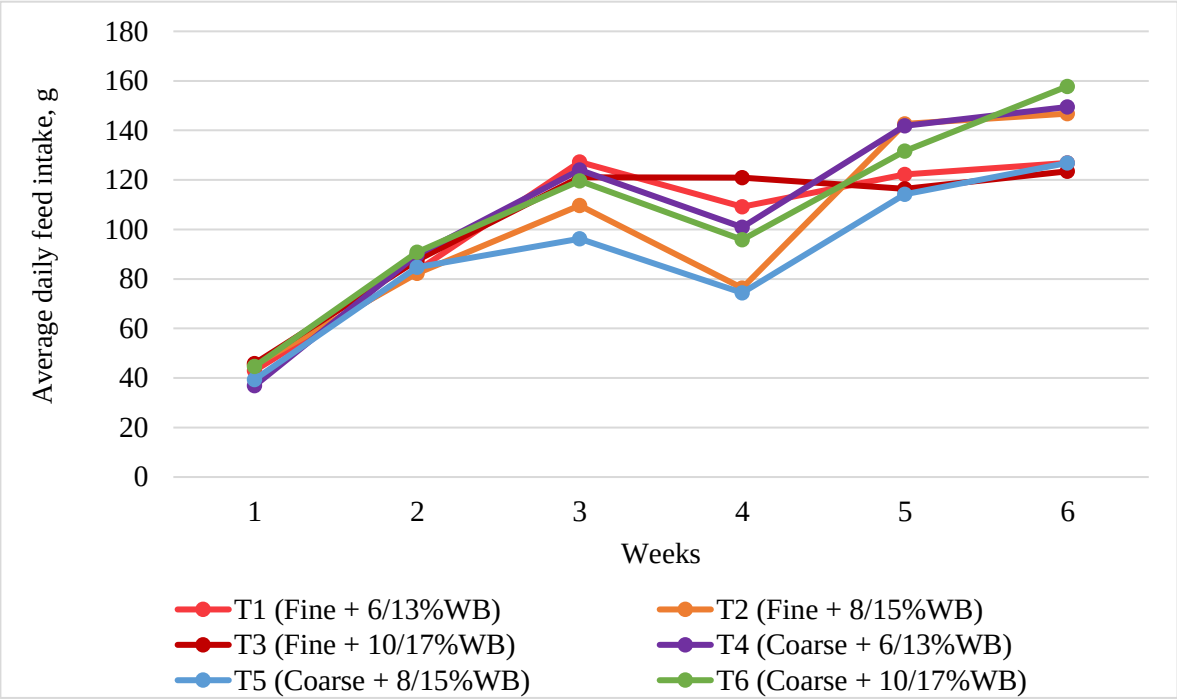

Figure 4. 1: Trends in average daily feed intake

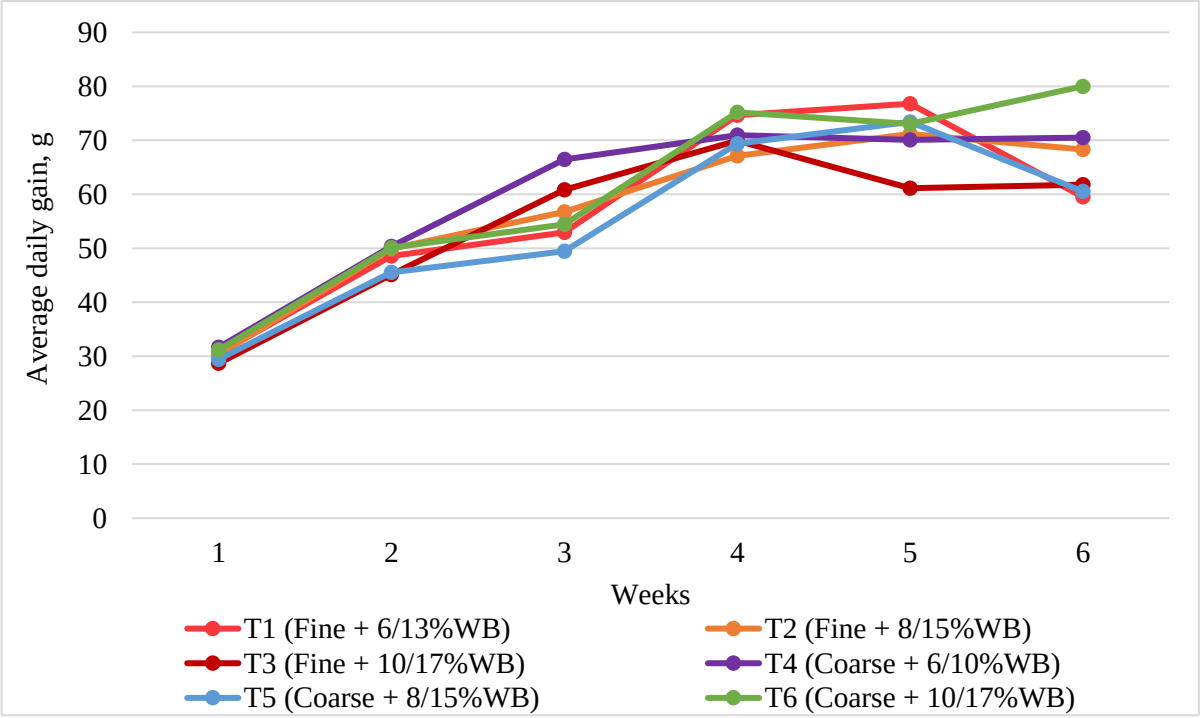

Figure 4. 2: Trends in average daily gain

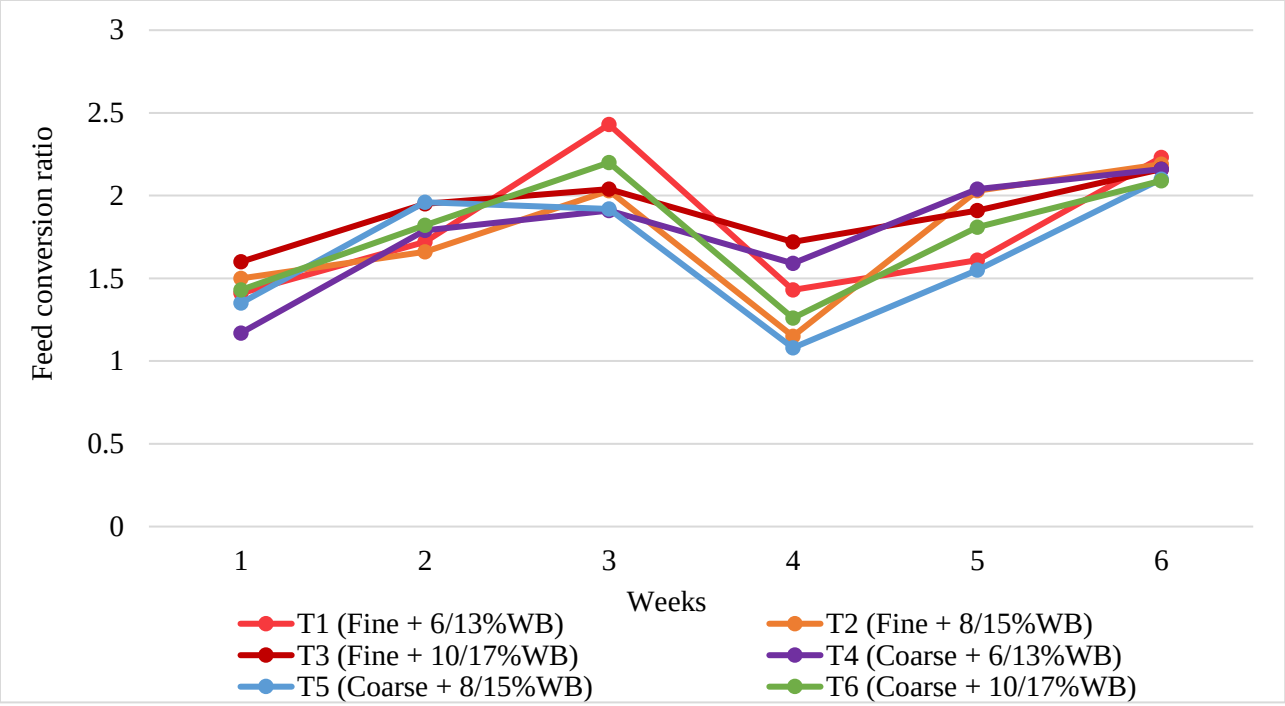

Figure 4. 3: Trends in feed conversion ratio

4.4.1. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Growth Performance during the Starter Phase (day one to day 21)

The effect of diet on growth performance of during the starter phase is presented in Table 4.3. No WB level or maize particle size effect was observed on ADFI, ADG, FCR and BW by day 21. On the other hand, a significant interaction ($p \leq 0.05$) between WB level and maize particle size was observed on ADG, FCR and BW at the end of the starter phase. Birds on T4 [(coarse + 6%WB); 48.81g] were the heaviest ($p \leq 0.05$) with birds on T5 [(coarse + 8%WB); 40.57g] having the least weight gain. The ADG values of T1 [(fine + 6%WB); 44.02g), T2 [(fine + 8%WB); 45.65g], T3 [(fine + 10%WB); 44.88g] and T6 [(coarse + 10%WB); 45.23g] were similar ($p \geq 0.05$) to that of T4.

Also, birds on T4 (1.56) converted feed to gain efficiently during the starter phase and their performance was similar ($p \geq 0.05$) to that of birds on T1 (1.85), T2 (1.73), T5 (1.83) and T6 (1.82) but higher ($p \leq 0.05$) than that of birds on T3 (1.86). The interactive effect on BW ($p=0.04$) followed a similar trend as that of ADG and FCR with birds on T4 (1215.94g) and T2 (1152.00g) having the highest BW with birds on T5 (1057.60g) having the lowest.

Table 4. 3: Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on growth performance during the starter phase (day 1 to day 21)

			Grams			
Treatment	%Wheat Bran	Maize particle size	Average daily feed intake	Average daily gain	Feed conversion Ratio	Body weight
T1	6	Fine	84.53	44.02^{ab}	1.85^{ab}	1104.6^{ab}
T2	8	Fine	79.06	45.65^{ab}	1.73^{ab}	1152.0^{a}
T3	10	Fine	84.83	44.88^{ab}	1.86^{b}	1128.4^{ab}
T4	6	Coarse	78.71	48.81^{a}	1.56^{a}	1215.9^{a}
T5	8	Coarse	76.26	40.57^{b}	1.83^{ab}	1057.6^{b}
T6	10	Coarse	85.00	45.23^{ab}	1.82^{ab}	1131.5^{ab}
Pooled SEM[1]			3.45	1.60	0.08	34.42
			Main effect			
%Wheat bran						
	6		81.62	46.41	1.71	1160.3
	8		77.66	43.11	1.78	1103.5
	10		84.92	45.05	1.84	1130.0
SEM			3.45	1.13	0.05	36.91
Maize particle size						
		Fine	82.81	44.85	1.82	1128.3
		Coarse	79.99	44.87	1.74	1134.1
SEM			1.99	0.92	0.04	21.77
Source of variation			P-value			
Wheat bran * Maize particle size			0.70	0.02	0.04	0.04
Wheat bran			0.13	0.14	0.22	0.35
Maize particle size			0.33	0.99	0.21	0.85

SEM[1]: Standard error of means; Means in a column with different superscripts are significantly different at $p \leq 0.05$

4.4.2. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Growth Performance during the Finisher Phase (day 22 to day 42)

The effect of WB level and maize particle size on growth performance during the finisher phase is shown in Table 4.4. Maize particle sizes were kept constant with WB level increasing from 6, 8 and 10% to 13, 15 and 17%. On an average, the ADFI, ADG, FCR, BW values across treatment were 120.99g, 69.64g, 1.78 and 2610.67g respectively. The values obtained on growth performance of birds during the finisher phase were comparable ($p \geq 0.05$) with no WB level, maize particle size or interactive effects being observed.

Table 4. 4: Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on growth performance during the finisher phase (day 22 to day 42)

			Grams		
Treatment	%Wheat Bran	Maize particle size	Average daily feed intake	Average daily gain	Feed conversion Ratio
T1	13	Fine	119.39	70.32	1.76
T2	15	Fine	121.93	68.86	1.79
T3	17	Fine	120.26	64.28	1.93
T4	13	Coarse	130.75	70.53	1.93
T5	15	Coarse	105.14	67.79	1.57
T6	17	Coarse	128.46	76.08	1.72
Pooled SEM[1]			5.82	3.89	0.11
				Main effect	
%Wheat bran					
	13		125.07	70.43	1.84
	15		113.54	68.33	1.68
	17		124.36	70.18	1.83
SEM			4.31	2.88	0.08
Maize particle size					
		Fine	120.53	67.82	1.83
		Coarse	121.45	71.47	1.74
SEM			3.67	2.46	0.06
Source of variation				P-value	
Wheat bran * Maize particle size			0.07	0.27	0.16
Wheat bran			0.14	0.86	0.26
Maize particle size			0.86	0.31	0.34

SEM[1]: Standard error of means

4.5. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Relative Weights of Carcass Parameters

The effects of dietary treatments on carcass parameters are presented in Tables 4.5. By day 42, the average live, de-feathered and dressed weights of experimental birds were 3070.00, 2791.67 and 2309.67g with no WB level or maize particle size been observed (P≥0.05).

Table 4. 5: Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on relative weights of carcass parameters

				grams			%[1]					
Trt[2]	%WB[3]		PS[6]	Live weight	Defeat.[7] weight	Dressed weight	Breast	Back	Wings	Thighs	Drum -sticks	Abd.[8] fat
	S[4]	Fi[5]										
T1	6	13	F[9]	3084.0	2800.0	2356.0	37.09	18.01	10.03	13.24	12.14	0.53
T2	8	15	F	3026.0	2748.0	2342.0	36.27	18.25	10.07	13.69	12.26	0.57
T3	10	17	F	3224.0	2626.0	2252.0	35.57	19.32	10.96	14.43	12.61	0.58
T4	6	13	C[10]	3030.0	2988.0	2206.0	34.10	18.52	10.81	14.36	13.13	0.69
T5	8	15	C	2892.0	2678.0	2248.0	33.14	18.98	11.05	14.72	13.03	0.78
T6	10	17	C	3164.0	2910.0	2454.0	35.14	17.51	10.79	14.51	12.75	0.70
Pooled SEM[11]				173.66	96.93	209.85	28.13	13.78	7.10	10.09	8.53	0.87
Main effect												
%Wheat bran												
	S	Fi										
	6	13		3057.00	2894.00	2231.00	34.68	18.50	10.42	14.10	12.64	0.65
	8	15		2959.00	2713.00	2295.00	34.70	18.61	10.56	14.21	12.65	0.67
	10	17		3194.00	2768.00	2353.00	35.35	18.42	10.87	14.47	12.68	0.64
SEM				122.80	68.54	148.39	19.89	9.74	5.02	7.14	6.03	0.61
Maize particle size												
			F	3111.33	2724.67	2269.33	36.31	18.53	10.36	13.79	12.34	0.56
			C	3028.67	2858.67	2316.67	54.34	29.86	16.34	22.42	19.63	1.45
SEM				100.26	55.97	121.16	16.24	7.96	4.10	5.83	4.93	0.50
Source of variation						P-value						
WB * PS				0.97	0.19	0.56	0.49	0.34	0.41	0.40	0.39	0.37
Wheat bran				0.41	0.18	0.85	0.46	0.38	0.45	0.45	0.42	0.40
Maize particle size				0.57	0.10	0.78	0.44	0.32	0.32	0.31	0.31	0.22

%[1]: Absolute weights were expressed as percentage of dressed weight; Trt[2]: Treatment; WB[3] Wheat bran; S[4]: Starter; Fi[5]: Finisher; PS[6]: Maize particle size; Defeat[7]: Defeathered; Abd[8]: Abdominal fat; F[9]: Fine; C[10]: Coarse; SEM[11]: Standard error of the means

Furthermore, no interactive or main effects ($p \geq 0.05$) were observed on the relative weights of the breast, back, wings, thighs and drumsticks of all birds. No WB level ($p=0.40$) or maize particle size ($p=0.22$) effect on relative abdominal fat weight was observed.

4.6. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Relative Organ Weights

The full and empty intestines, empty crop, full proventriculus, full gizzard, heart and liver of experimental birds were not significantly ($p \geq 0.05$) affected by the level of WB and maize particle size as shown in Table 4.6. On the other hand, there was a significant interaction ($p=0.02$) between the level of WB and maize particle size on relative empty gizzard weight of birds. Birds on T4 [(coarse + 6/13%WB); 2.00%] had heavier empty gizzards ($p \leq 0.05$) than birds on T1 [(fine + 6/13%WB); 1.39%], T2 [(fine + 8/15%WB); 1.49%], T3 [(fine + 10/17%WB); 1.41%] and T5 [(coarse + 8/15%WB); 1.57%] but similar ($p \geq 0.05$) to birds on T6 [(coarse + 10/17%WB); 1.80%]. Birds on T1 (fine + 6/13%WB; 1.39%) had the lowest empty gizzard relative weight. In addition, a maize particle size effect ($p \leq 0.0001$) on the relative empty gizzard weight was observed. Birds on coarse diets (T2, T5 and T6) had heavier empty gizzards than birds on fine diets (T1, T3 and T4).

4.7. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Caecal Short-Chain Fatty Acids Profile and Concentration (SCFAs)

Amongst the three caecal SCFAs analysed, acetic acid (approximately 8147.14 µg/g) was predominant in the caecal contents of all birds. This was followed by butyric (approximately 128.96 µg/g) and propionic acids (approximately 73.29 µg/g).

Table 4. 6: Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on relative organ weights

				%[1]							
Trt[2]	%Wheat bran		PS[5]	Empty Crop	Full provent[6]	Full gizzard	Empty gizzard	Full intest[7]	Empty intest[8]	Heart	Liver
	S[3]	F[4]									
T1	6	13	Fine	0.38	0.49	2.75	1.39^{c}	5.17	3.09	0.48	1.79
T2	8	15	Fine	0.45	0.40	2.58	1.49^{bc}	5.32	2.98	0.47	1.72
T3	10	17	Fine	0.42	0.51	2.87	1.41^{bc}	5.05	2.89	0.43	1.68
T4	6	13	Coarse	0.43	0.49	3.31	2.00^{a}	5.46	3.71	0.54	1.82
T5	8	15	Coarse	0.43	0.36	2.82	1.57^{bc}	4.81	2.98	0.44	1.65
T6	10	17	Coarse	0.45	0.47	2.98	1.80^{ab}	4.84	2.86	0.49	1.95
Pooled SEM[9]				0.06	0.11	0.20	0.09	0.46	0.29	0.04	0.19
						Main effect					
%Wheat bran											
	S	F									
	6	13		0.41	0.49	3.03	1.69	5.32	3.40	0.51	1.80
	8	15		0.44	0.38	2.70	1.53	5.06	2.98	0.46	1.69
	10	17		0.44	0.60	2.93	1.61	4.95	2.87	0.46	1.82
SEM				0.04	0.08	0.14	0.06	0.33	0.20	0.03	0.14
Maize particle size											
			Fine	0.42	0.54	2.73	1.43^{b}	5.18	2.98	0.46	1.73
			Coarse	0.43	0.44	3.03	1.79^{a}	5.04	3.18	0.49	1.81
SEM				0.04	0.07	0.12	0.52	0.27	0.17	0.03	0.11
Source of variation							P-value				
Wheat bran * Maize particle size				0.88	0.44	0.53	0.02	0.69	0.45	0.45	0.67
Wheat bran				0.84	0.17	0.26	0.21	0.72	0.18	0.46	0.76
Particle size				0.78	0.26	0.08	≤.0001	0.71	0.40	0.43	0.63

%[1]: Absolute weights expressed as percentage of live weight; Trt[2]: Treatment; S[3]: Starter phase; F[4]: Finisher phase; PS[5]: Maize particle size; Full provent[6]: Full proventriculus; Full intest[7]: Full intestines; Empty intest[8]: Empty intestines; SEM[9]: Standard error of the means

Also, for each SCFAs, there was no interaction between WB level and maize particle size. Figures 4.6, 4.7 and 4.8 show the observed trend in the concentration of acetic, propionic and butyric acids across treatments.

Again, amongst treatments, there was no linear increase ($p \geq 0.05$) in concentration of any of the analysed caecal SCFAs in response to the increasing level of WB as shown in Table 4.5. There were some numerical increases in the concentration of acetic, propionic and butyric acid of birds fed coarse diets (T4, T5 and T6) (Table 4.7).

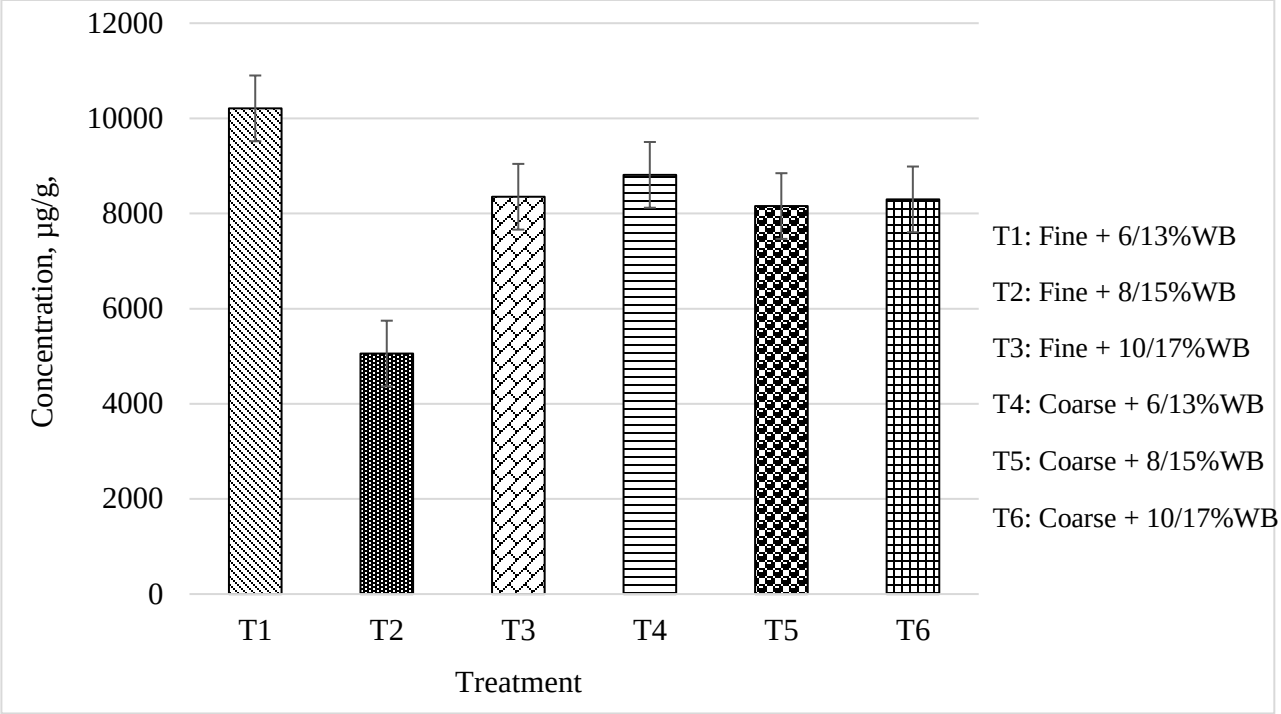

Figure 4. 5: Acetic acid concentration in caecal content

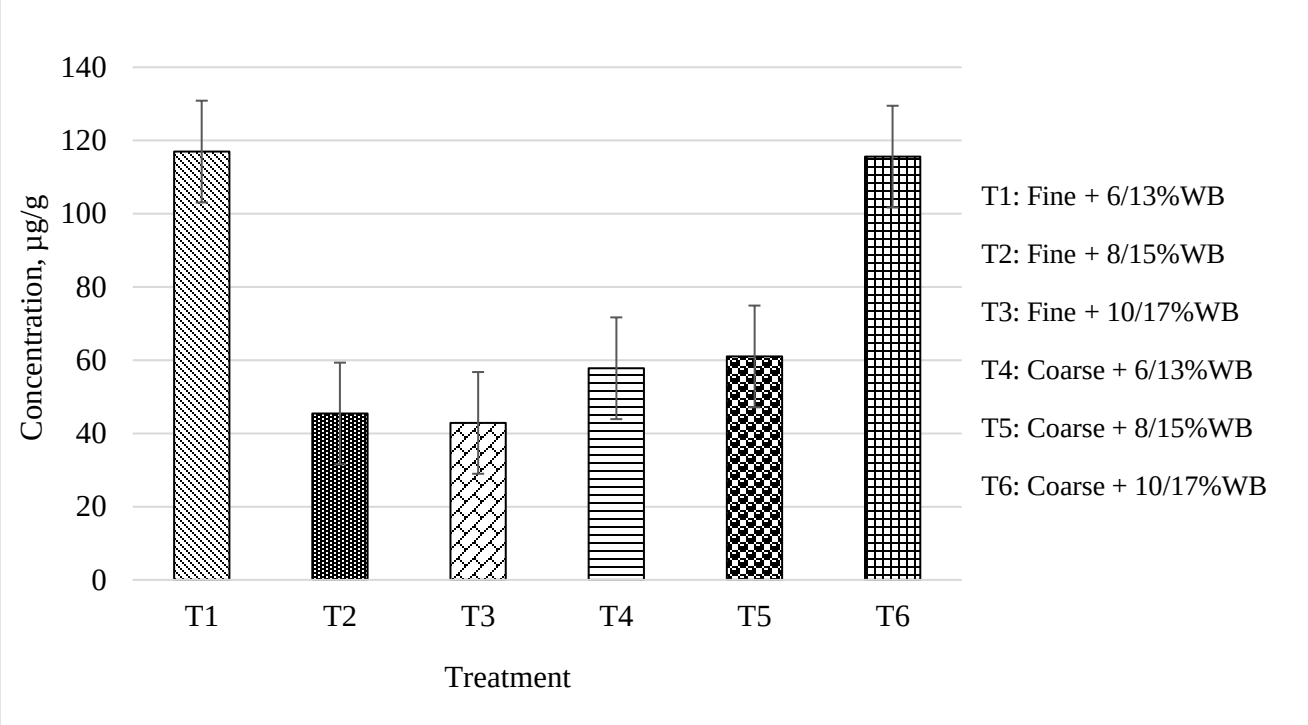

Figure 4. 4: Propionic acid concentration in caecal content

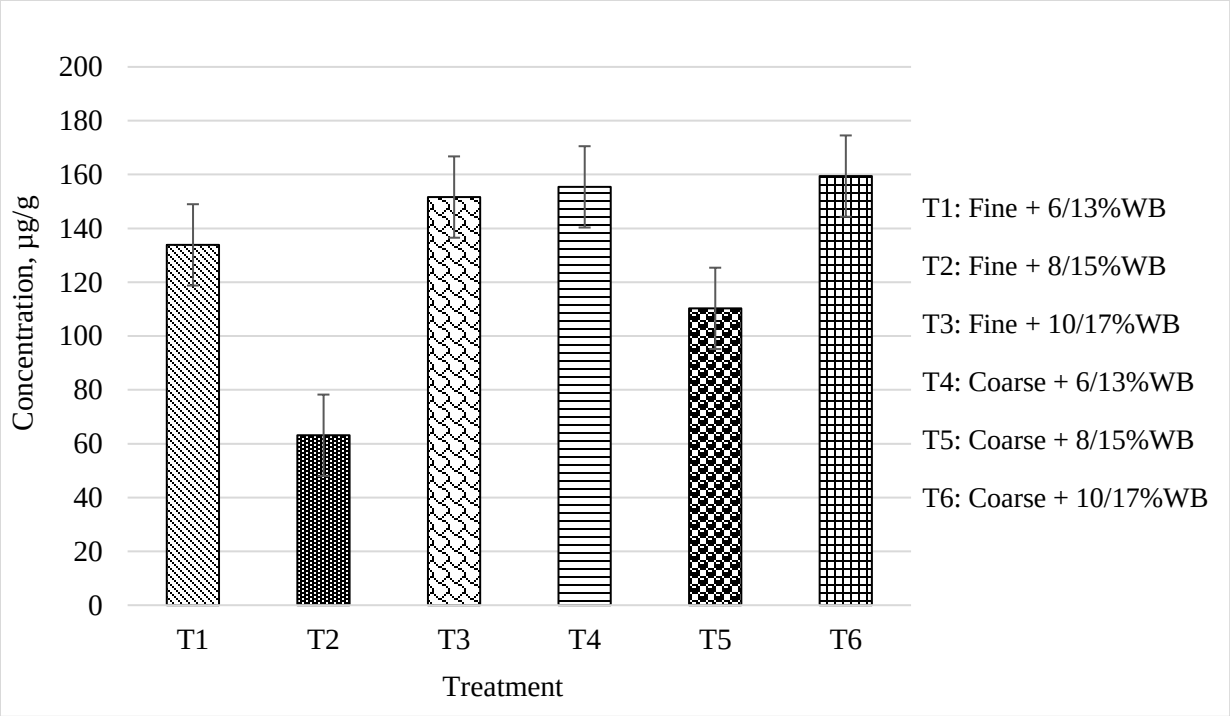

Figure 4. 6: Butyric acid concentration in caecal content

Table 4. 7: Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on caecal short-chain fatty acids concentration

		µg/g		
Main effect		Acetic acid	Propionic acid	Butyric acid
%Wheat bran				
Starter	Finisher			
6	13	9510.72	87.39	144.65
8	15	6606.17	53.23	86.71
10	17	8324.29	53.23	155.53
SEM[1]		1216.47	24.73	19.60
Maize particle size				
Fine		7872.48	68.43	116.22
Coarse		8421.63	78.14	141.71
SEM		933.24	20.56	16.00
Source of variation			P-value	
Wheat bran		0.26	0.26	0.63
Maize particle size		0.70	0.70	0.75

SEM[1]: Standard error of means

4.8. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Caecal pH and Microbial Count

Figure 4.9 shows caecal pH values obtained from the interaction between WB level and maize particle size across treatments. There was no significant WB-effect (p=0.63) or maize particle size effect (p=0.82) on caecal pH (Table 4.8).

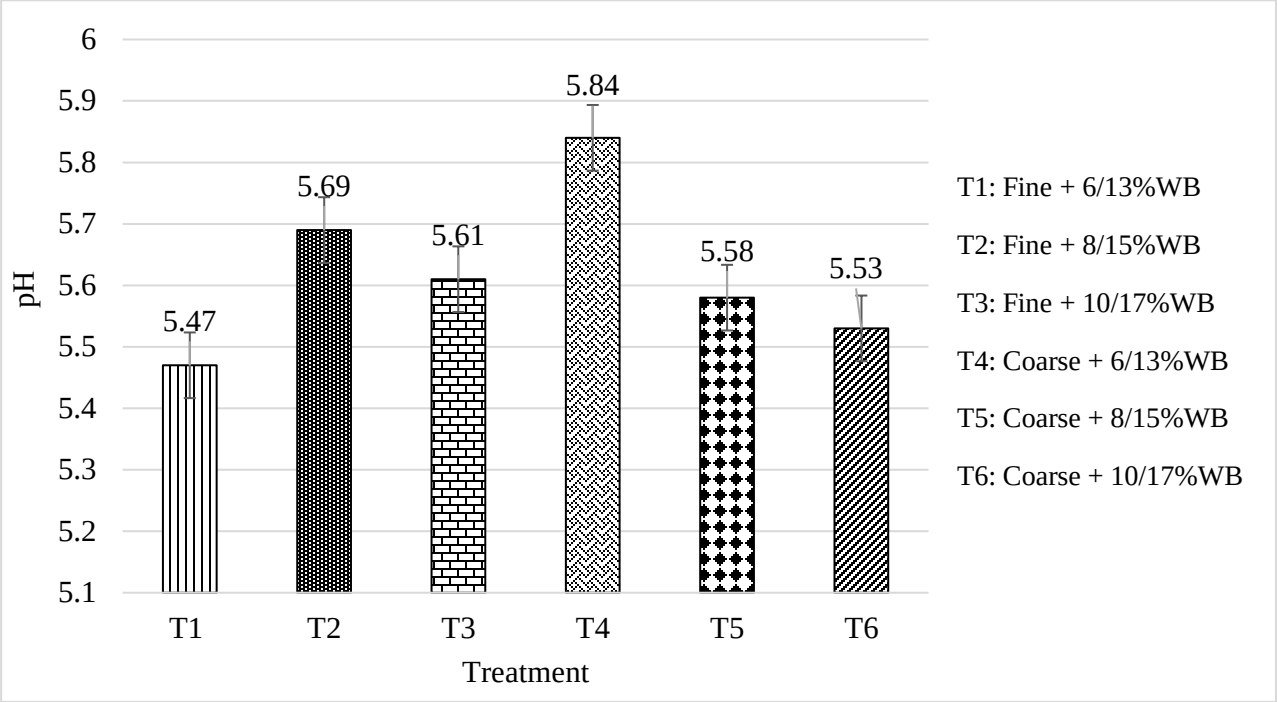

Figure 4. 7: Caecal pH values

Also, analysis of the caecal content showed a relative abundance of *Lactobacillus spp.* (average of 9.40 $\log_{10}$ cfu/ml) as compared to *E. coli* (average of 6.78 $\log_{10}$ cfu/ml) across treatment.

No significant interactive effect (p=0.15) was identified as shown in Figure 4.10. In addition, there was no linear response in the count of *E. coli* and *Lactobacillus spp*. as the level of WB increased neither was the effect of maize particle size significant (p≥0.05) (Table 4.9).

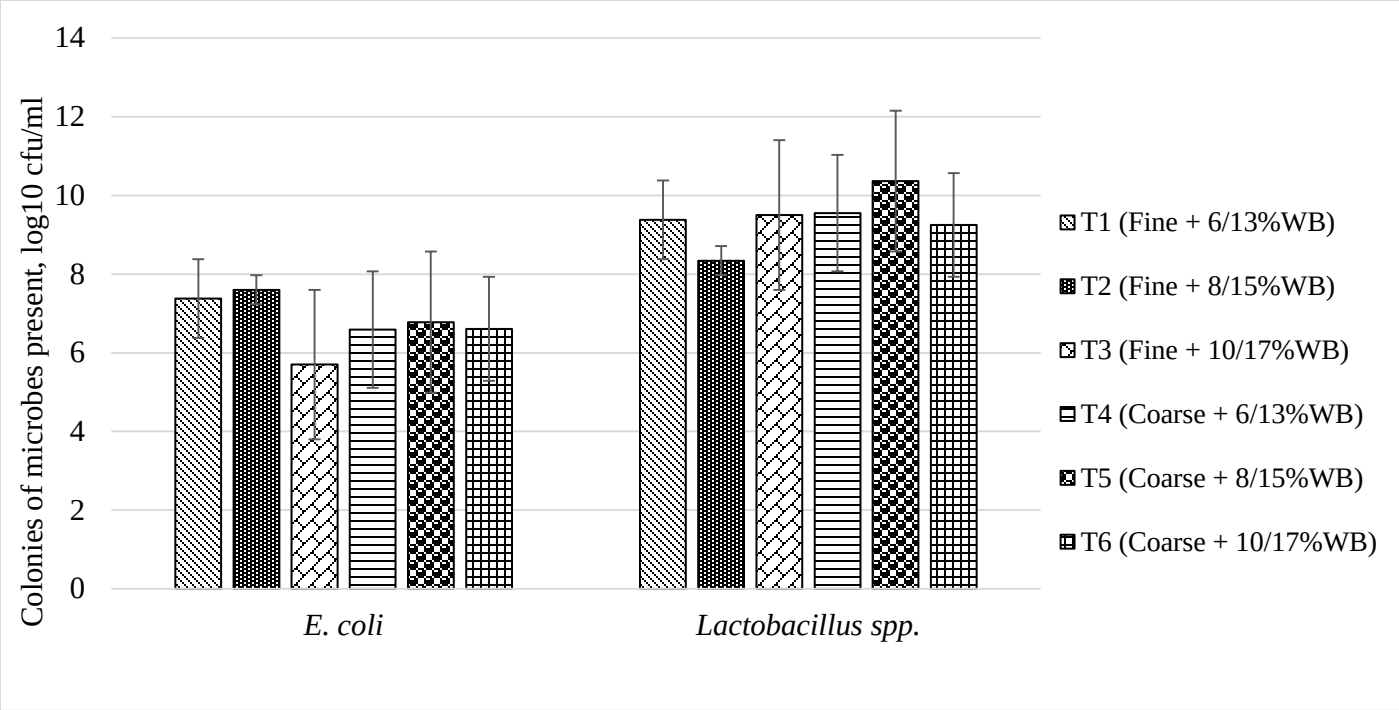

Figure 4. 8: Interactive effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on caecal microbial count

Table 4. 8: Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on caecal pH and microbial population

			log_{10} cfu/ml	
Main effect		pH	*Escherichia coli*	*Lactobacillus spp.*
%Wheat bran				
Starter	Finisher			
6	13	5.65	6.99	9.47
8	15	5.63	7.19	9.35
10	17	5.57	6.16	9.37
SEM[1]			0.45	0.42
Maize particle size				
Fine		5.59	6.89	9.07
Coarse		5.65	6.66	9.72
SEM		0.08	0.36	0.34
Source of variation			P-value	
%Wheat bran		0.63	0.25	0.98
Maize particle size		0.82	0.66	0.19

SEM[1]: Standard error of means

4.9. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Intestinal Morphometric Measurements

Table 4.9 shows the effect of dietary treatments on morphological structures in the jejunum and ileum. Results obtained showed no significant effect of WB level and maize particle size on the villi height (VH), crypt depth (CD) and villi height: crypt depth (VH: CD) ratio in the jejunum and ileum.

Table 4. 9: Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on morphology of the jejunum and ileum

Parameter				Jejunum			Ileum		
Treatment	% Wheat bran		Maize particle size	Villi height (µm)	Crypt depth (µm)	VH:CD[1]	Villi height (µm)	Crypt depth (µm)	VH:CD
	Starter	Finisher							
T1	6	13	Fine	801.25	156.39	5.58	726.42	297.56	4.68
T2	8	15	Fine	669.57	132.13	5.18	596.66	141.39	4.24
T3	10	17	Fine	625.73	157.25	4.51	587.37	146.56	4.46
T4	6	13	Coarse	656.55	118.97	5.54	592.28	112.26	5.31
T5	8	15	Coarse	740.56	144.59	5.29	671.75	121.38	5.62
T6	10	17	Coarse	630.73	127.60	4.94	638.02	118.58	5.44
Pooled SEM[2]				59.62	20.87	0.58	53.04	67.65	0.63
						Main effect			
%Wheat bran									
	Starter	Finisher							
	6	13		728.90	137.68	5.56	659.36	204.91	5.00
	8	15		705.06	138.36	5.23	634.21	132.57	4.93
	10	17		628.23	142.43	4.73	612.70	131.38	4.95
SEM				42.16	14.76	0.41	37.51	47.84	0.44
Maize particle size									
			Fine	698.85	148.59	5.09	636.82	195.17	4.46
			Coarse	675.95	130.39	5.26	634.02	117.40	5.46
SEM				34.42	12.05	0.34	30.62	39.06	0.36
Source of variation						P-Value			
Wheat bran * Maize particle size				0.20	0.45	0.92	0.12	0.40	0.83
Wheat bran				0.23	0.97	0.37	0.68	0.47	0.99
Maize particle size				0.64	0.30	0.72	0.95	0.17	0.06

VH:CD[1]: Villi height to crypt depth ratio; SEM[2]: Standard error of the mean

In the jejunum, the VH, CD and VH: CD averaged 687.40µm, 139.49µm and 5.17. On an average, the ileal measurements for VH, CD and VH: CD were 635.42µm, 156.29µm and 4.69. Histopathogical images of the jejunal and ileal segments of birds on dietary treatments are shown in Plates 1 and 2 respectively.

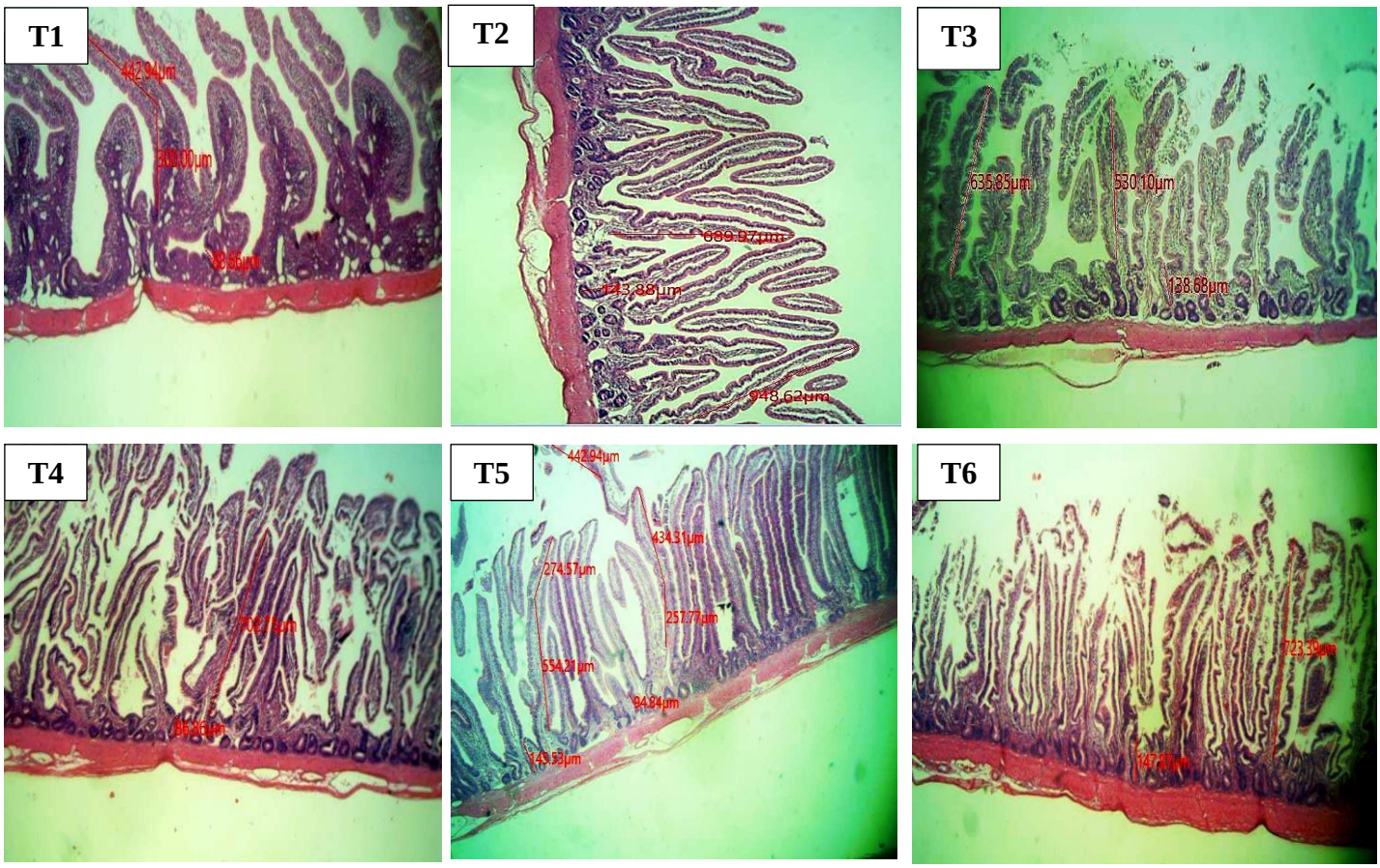

Plate 4. 1: Histopathogical images of the jejunal segments of birds on dietary treatments

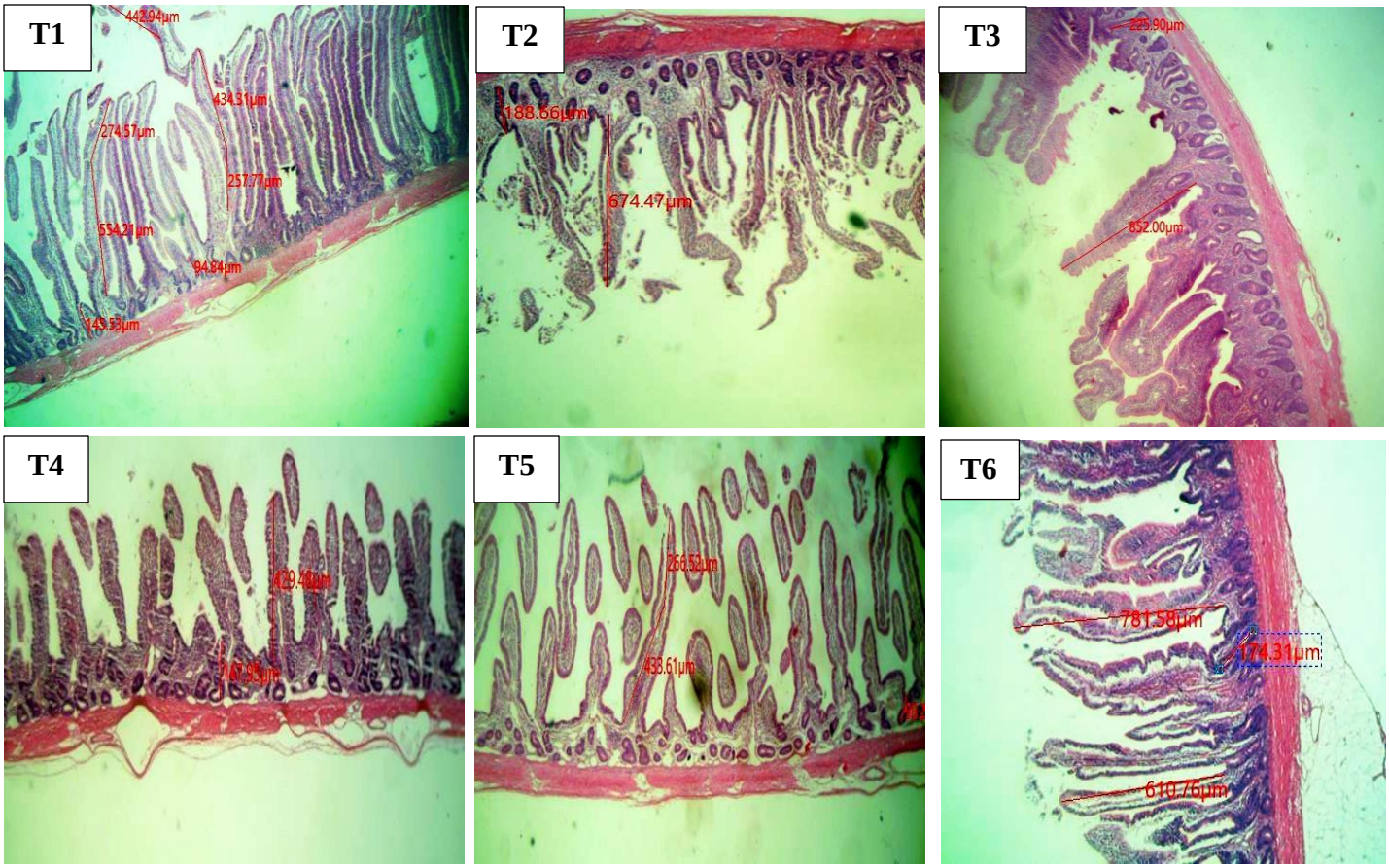

Plate 4. 3: Histopathogical images of the ileal segments of birds on dietary treatments

4.10. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Blood Haematological Indices and Serum Glucose

Table 4.10 shows the effect of the dietary treatments on red blood cells (RBC), haematocrit (Hct), haemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) across treatments. The effect of WB level and maize particle size on RBC, HGB, Hct, MCV, MCH and MCHC count across treatments was comparable ($p \geq 0.05$). Maize particle size significantly ($p \leq 0.05$) influenced glucose levels in the blood with birds fed coarse maize particles (9.72mmol) having a lower serum glucose content

as compared to birds fed fine maize particles (11.29mmol) (Table 4.11). However, no response (p=0.22) to increasing level of WB was observed.

Table 4. 10: : Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on blood haematology and glucose levels

Trt[1]	%WB[2]		PS[3]	RBC[4] (10^{12}/L)	HGB[5] (g/dL)	Hct[6] (%)	MCV[7] (fL)	MCH[8] (pg)	MCHC[9] (g/dL)	Glucose (mmol)
	Starter	Finisher								
T1	6	13	Fine	2.95	12.80	34.48	111.90	43.43	38.75	11.34
T2	8	15	Fine	3.03	13.48	27.82	114.42	44.68	39.00	12.04
T3	10	17	Fine	2.99	10.60	32.90	115.85	43.75	37.75	10.50
T4	6	13	Coarse	2.86	12.60	34.55	116.87	44.00	37.67	9.82
T5	8	15	Coarse	2.91	12.92	33.40	116.46	44.34	38.40	10.02
T6	10	17	Coarse	2.97	12.98	35.02	118.10	43.64	36.60	9.32
Pooled SEM[10]				0.34	3.05	4.22	0.34	0.98	1.96	0.63
Main effects										
%Wheat bran										
	Starter	Finisher								
	6	13		2.91	12.70	33.15	114.38	43.71	32.21	10.58
	8	15		2.97	13.20	31.15	115.44	44.51	38.70	11.03
	10	17		2.98	11.79	34.79	116.98	43.70	37.18	9.91
SEM				0.11	0.70	2.16	1.39	0.79	0.40	0.45
Maize particle size										
			Fine	2.99	12.29	33.98	114.06	43.95	38.50	11.29[a]
			Coarse	2.91	12.83	32.08	117.14	43.99	37.56	9.72[b]
SEM				0.09	0.61	1.90	1.22	0.70	0.36	0.36
Source of variation				P-value						
Wheat bran * Maize particle size				0.58	0.55	0.50	0.77	0.93	0.88	0.80
Wheat bran				0.90	0.39	0.52	0.51	0.73	0.06	0.22
Maize particle size				0.94	0.33	0.45	0.09	0.97	0.08	0.01

Trt[1]: Treatment; WB[2]: Wheat bran; PS[3]: Particle size; RBC[4]: Red blood cell; HGB[5]: Haemoglobin; Hc[6]: Haematocrit; MCV[7]: Mean corpuscular volume; MCH[8]: Mean Cell Haemoglobin; MCHC[9]: Mean Cell Haemoglobin Concentration; SEM[10]: Standard error of the means
Means in a column with different superscripts are significantly different at $P \leq 0.05$

4.11. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Immune Status

Results obtained for differential white blood cell count showed a relative abundance of heterophils (52.53%), lymphocytes (33.83%), eosinophils (9.58%), monocytes (3.93%) and basophils

Table 4. 11: Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on white blood cell differentials and immune relative organ weight (expressed as a percentage of live weight)

Trt[1]				%							
	%Wheat bran		PS[2]	Bas[3]	Eosi[4]	Mono[5]	Hetero[6]	Lymp[7]	H:L[8]	Thymus	Spleen
	Starter	Finisher									
T1	6	13	F[9]	0.00	12.00	2.75	45.00	40.25	1.33	0.21	0.10
T2	8	15	F	0.40	7.60	4.20	58.00	29.80	2.02	0.20	0.11
T3	10	17	F	0.00	8.20	3.40	55.60	32.60	1.84	0.17	0.18
T4	6	13	C[10]	0.00	10.33	4.00	47.67	38.00	1.51	0.50	0.35
T5	8	15	C	0.17	9.17	4.83	55.33	30.50	1.98	0.14	0.17
T6	10	17	C	0.20	10.20	4.40	53.60	31.80	1.81	0.23	0.14
Pooled SEM[11]				0.15	0.95	0.95	5.06	5.06	1.69	0.12	0.10
Main effect											
%Wheat bran											
	Starter	Finisher									
	6	13		0.00	11.17	3.38	46.33	39.13	1.42	0.36	0.23
	8	15		0.28	9.20	4.52	56.67	30.15	2.00	0.17	0.14
	10	17		0.10	8.38	3.90	54.60	32.20	1.83	0.20	0.16
SEM				0.11	1.14	0.64	3.42	2.86	0.23	0.09	0.07
Maize particle size											
			F	0.20	3.45	9.27	52.87	34.22	1.73	0.19	0.13
			C	0.06	4.41	9.90	52.20	33.43	1.77	0.29	0.22
SEM				0.10	0.57	1.02	3.04	2.53	0.21	0.07	0.06
Source of variation				P-value							
WB * PS				0.79	0.95	0.59	0.88	0.95	0.96	0.38	0.35
Wheat bran				0.26	0.54	0.33	0.18	0.16	0.32	0.28	0.70
Particle size				0.31	0.25	0.67	0.88	0.83	0.90	0.35	0.29

Trt[1]: Treatment; PS[2]: Maize particle size; Bas[3]: Basophils; Eosi[4]: Eosinophils; Mono[5]: Monocytes; Hetero[6]: Heterophils; Lymp[7]: Lymphocytes; H:L[8]: Heterophils to lymphocytes ratio: F[9]: Fine; C[10]: Coarse; SEM[11]: Standard error of means

(0.13%), in descending order (Table 4.11). The values obtained for eosinophils, monocytes, heterophils, lymphocytes and heterophils: lymphocytes ratio for all birds were not significantly affected ($p \geq 0.05$) by WB level or maize particle size or an interaction between them. Similarly, no interactive effect or WB-effect was observed on the immune organs.

4.12. Economics of Production

The cost of feed per kilogram for diets for both starter and finisher phase are shown in Table 4.13. There was a linear increase in cost of feed per kilogram as the wheat bran inclusion level increased.

The effect of dietary treatments on selected cost variables is shown in Table 4.14. It cost Gh¢ 0.40 and Gh¢ 0.24 lesser to produce a kilogram of the final body weight (FBW) and dressed weight (DW) respectively of birds on T3 as compared to birds on the control diet. Also, the cost/kg weight gain, total feed cost/kg of final body weight and total feed cost /kg of dressed weight reduced as WB level increased.

Table 4. 12: Cost of feed per kilogram of dietary treatments (GH¢)

Treatment	%Wheat bran		Maize particle size	Cost/kg of feed	
	Starter	Finisher		Starter	Finisher
T1	6	13	Fine	1.95	2.12
T2	8	15	Fine	1.98	2.15
T3	10	17	Fine	2.01	2.18
T4	6	13	Coarse	1.95	2.12
T5	8	15	Coarse	1.98	2.15
T6	10	17	Coarse	2.01	2.18

Table 4. 13: Effect of dietary fibre level (using wheat Bran as a DF source) and maize particle size on feed cost variables (GH¢)

Treatment	%Wheat bran		Maize particle Size	Cost/kg of feed consumed/bird		Overall cost of feed consumed /bird	Cost/kg weight gain	Total feed cost/ kg	
	Starter	Finisher		day 1 to day 21	day 22 to day 42			FBW[1]	DW[2]
T1	6	13	Fine	3.92	5.10	9.01	3.90	2.94	3.84
T2	8	15	Fine	3.60	5.12	8.72	3.63	2.88	3.73
T3	10	17	Fine	3.47	4.37	7.85	3.44	2.48	3.49
T4	6	13	Coarse	3.53	5.24	8.77	3.52	3.06	3.94
T5	8	15	Coarse	3.79	4.96	8.75	3.70	3.05	3.93
T6	10	17	Coarse	3.92	5.48	9.40	3.71	2.98	3.86

FBW[1]; DW[2]; Dressed weight

CHAPTER FIVE

DISCUSSION

5.1. Percentage mortality

Dietary or environmental factors can lead to the incidence of infections and diseases in broilers (Martínez *et al*., 2015). This weakens the immune system leading to a possible occurrence of morbidity or mortality. In this trial, the 17 mortalities recorded were well spread across treatments and amounted to an overall mortality rate of 5.67%. Post mortem autopsies carried out did not indicate feed components as the cause of death. Also, hygienic practices such as the provision of clean water and feed, maintenance of a good environment is critical in order to promote healthy development during broiler production. All these were duly observed throughout the trial. Stress that arises form growth and environmental adaptation could be a contributing factor to the observed results. In addition, breeders such as Hy-line and Dekalb have indicated an acceptable

weekly morality rate of 0.8-1.5% in broiler production **(**Hy-Line, 2009; Lohmman, 2009; Dekalb, 2009). Based on this, the estimated maximum mortality rate for six weeks is 6.9%. By day 42, 5.67% mortality rate was recorded. This shows that increasing WB levels with fine or coarse maize particles did not adversely affect the health status of growing broilers. The findings in this study is similar to that of Idan (2019) and Martínez *et al.* (2015) who fed up to 12% and 200g/kg WB respectively to poultry. In their work, mortality rate was no attributed to dietary effects.

5.2. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Apparent Nutrient Digestibility

Determining the bioavailability of nutrients in feed ingredients is necessary in order to optimize feed efficiency and reduce feed cost (Ravindran, 2013). A decrease in nutrient digestibility in monogastric (broiler) diets has been linked to dietary fibre levels (Wilfart *et al*, 2007; Urriola *et al*., 2009). As an anti-nutritional factor, dietary fibre is able to encapsulate dietary nutrients through the formation of complexes, which hinders nutrient digestion and absorption (Bindelle *et al.*, 2008; Mateos *et al.*, 2012). In this study, increasing the levels of dietary fibre during the finisher phase increased the crude fibre content in experimental diets (Table 3.3 and Table 3.4). However, the increased dietary fibre levels did not adversely affect digestibility coefficients of DM, OM and CP. This may be attributed to the effect of fibre type, level of inclusion and maize particle sizes used in this study. Contrary to the traditional view about dietary fibre, inclusion of dietary fibre especially insoluble fibre (such as in WB) in broiler diets increases gizzard activity as fibre is hard to grind (Mateos *et al.*, 2012). This may have resulted in an enhanced GIT motility, an increase in the secretion of digestive enzymes in the pyloric region, HCL production and microbial fermentation, which lower gut pH (Zaefarian *et al.*, 2016; Kheravii *et al.*, 2018). All these beneficial effects may have accounted for the observed similarities in the digestibility coefficients. In addition, when gut pH is low, pepsin stimulation and mineral digestibility are enhanced which may have accounted for the observed similarities in CP and OM digestibility coefficients. Adibmoradi *et al.* (2016) accounted improvements in nutrient digestibility when birds were fed rice hulls (insoluble fibre) at an inclusion rate of 1.5% as compared to 0.75% to an increase in secretion of HCL as DF levels increased. Similar to the results of this study, González-Alvarado *et al.* (2010) reported an improvement in the digestibility coefficients of dry matter, organic matter

and soluble ash when oat hulls (30g/kg) were included in broilers diets as compared to sugar beet pulp (30g/kg). In contrast, Khempaka *et al.* (2009) observed a reduction in dry matter and organic matter digestibility as the inclusion level of dried cassava pulp increased from 8 to 16% in broiler diets. In this trial, including WB up to 17% did not serve as an anti-nutritional factor, rather, the presence of WB in the GIT created a favourable environment for digestive enzymatic activities.

5.3. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Overall Growth Performance (day one to day 42)

The type of fibre and level of inclusion can reduce or promote feed intake in broilers (Mateos *et al.*, 2012). An increase in fibre content may result in the dilution of dietary energy (Classen, 2017). This leads to an increase in feed intake by broilers in order to meet their daily nutrient and energy needs (Nahashon *et al.*, 2006). In this study, all experimental diets were formulated to be isocaloric and isonitrogenous as WB was not a feed additive. As such, the observed significant WB level effect on ADFI by day 42 can be attributed to the influence of the insoluble nature of the fibre type in WB on digesta passage rate as well as an increase in the physical capacity of the birds' GIT. Wheat bran is characterized by a faster digesta passage rate, which normally results in early gastric emptying, which may lead to a higher feed intake (Montagne *et al.* 2003; Hetland *et al.* 2004) as observed in this study. Also, birds naturally eat to 'gut fill' with a positive correlation exiting between feed intake and the physical capacity of a bird's GIT (Nir *et al.*, 1994a; Ferket and Gernat, 2006). In this study, the WB effect on ADFI was highest in birds on diets with the highest WB inclusion level; 10 and 17%WB at starter and finisher phases respectively. This implies that the presence of structural components such as high levels of WB at both starter and finisher phases caused a progressive increase in the physical capacity of the GIT of birds; thus, enabling an increase in feed intake. The trend in the interactive effect on ADFI can be attributed to the trend

in WB effect observed in this study. In contrast to the findings of this trial, Donkoh *et al.*, (1999) observed a linear reduction in ADFI as WB level (150g, 250g, 350g and 450g) increased in a broiler trial (21-56 days). However, result obtained by Adibmoradi *et al.* (2016) when insoluble fibre was fed to broilers is in agreement with the findings of this study.

As birds grow, their gape develops and mature enabling them to consume the preferred larger feed particles (Amerah *et al.*, 2007a). As such, feed intake of birds on diets with moderate or large feed particles is expected to increase as they grow. The lack of maize particle size effect at the various WB levels suggests that although the GIT of birds fed coarse maize particles become progressively adapted to the nature of their diet, the rate of feed intake was not high enough to elicit a significant difference in ADFI. Results obtained corroborates that of Rubio *et al.* (2020) who reported no maize particle size effect on day 42 when maize particles of sizes: 615, 863, 1644 and 2613µm were fed to broilers. Furthermore, Oppong-Sekyere *et al.* (2012) reported similarities in feed intake when broilers were fed fine (713.82µm) and coarse (1462µm) maize particles.

Weight gain in broilers is a function of the dietary protein content (Beski *et al.*, 2015). Wheat bran has a good amount of nutrients such as protein, amino acids, and a good source of B vitamins, which maximizes growth (Prueckler *et al.*, 2014). On the other hand, WB is mainly made up of arabinoxylans and a lesser fraction of beta-glucans and cellulose (Chalamacharla *et al.*, 2018). These components can negatively influence the bioavailability of dietary nutrients to birds, hence impairing nutrient digestion, utilization and weight gain (Woyengo *et al.*, 2008). Results obtained on ADG by day 42 indicates that increasing the level of WB in this study did not affect the bioavailability of nutrients needed for growth. Also, this assertion is evidenced by the observed similarities in nutrient digestibility coefficients (Table 4.1). The fermentation of DF is known to produce SCFAs, which serves as energy for villi and crypt cells responsible for nutrient digestion

and absorption (Taheri *et al.*, 2016). This may also have accounted for the improved ADG at day 42. These results are contrary to the findings of Sanchez *et al.* (2019) who observed a decrease in weight gain when birds were fed insoluble fibre (11% rice bran). However, the findings of Hetland and Svihus (2001) agrees with that of this trial. In their trial, insoluble fibre (oat hulls) had no negative effect on weight gain of broilers and this was attributed to the increase in gut physical capacity of the birds.

Feed conversion ratio is a measure of how much feed consumed is efficiently converted into body tissues (Kantanka, 2013). The inclusion of dietary fibre and medium to large feed particles in broiler diets is said to enhance grinding action and musculature development of the gizzard (Svihus, 2011). A well-developed gizzard enhances feed utilisation due to the associated improvements in digestive processes such as increase in retention time, HCL secretion, digesta reflux and microbial fermentation (Hetland *et al.*, 2003; Sacranie *et al.*, 2012). The improved FCR of birds fed coarse maize particles by day 42 (Table 4.2) can be attributed to the functional and well-developed nature of their gizzards. In addition, less energy is needed for feeding and grinding when the gizzard is adequately developed (Amerah *et al.*, 2007a). This makes more energy available for growth and development as observed in birds fed coarse maize particles. Naderinejad *et al.* (2016) and (Kheravii *et al.*, 2017a; Kheravii *et al.*, 2017b) observed improvements in FCR when maize particle size was increased in broilers which agrees with the findings of this trial. In addition, supplementing broilers diets with 50% fine and coarse particles each improved FCR and ADG as compared to feeding fine particles alone (Xu *et al.*, 2015b). The maize particle size effect on FCR positively influenced the observed significant interaction by day 42 (Table 4.2).

5.4. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Growth Performance during the Starter Phase (day one to day 21)

Voluntary feed intake in broiler chicks can be influenced by age, dietary particle size and fibre level in diets (Amerah *et al.*, 2007b; Zaefarian *et al.*, 2016). Broiler chicks may have difficulties in swallowing and grinding diets with high fibre levels and larger feed particle sizes (Ravindran *et al.* 2006). This can be attributed to their smaller gape dimensions and the undeveloped nature of the gizzard's myelinated muscles and koilin layer (Kiarie and Mills, 2019). However, the similarities in ADFI from day 1 to day 21 suggests that the two maize particle sizes did not impair swallowing and subsequent feed intake. Furthermore, the results indicate an adaptation of the gizzard of chicks on coarse maize particles and highest WB level to the dietary components. This may have enhanced quick and effective grinding which in turn prevented excessive or reduced feed intake. This confirms the assertion made by Mateos *et al.* (2012) that supplementing broiler diets with moderate amounts of dietary fibre and coarse feed particles prevents feed wastage and increases productivity. The results obtained corroborates that of González-Alvarado *et al.* (2007) who observed no effect on ADFI when 3% oat hulls were included in broiler diets from day 1 to day 21.

The negative effect of dietary fibre inclusion on voluntary feed intake in broiler chicks is said to result in a proportional reduction in ADG and FCR especially from day 0 to day 21 (Pacheco *et al.*, 2018). However, the improved ADG from day 0 to day 21 can be attributed to the beneficial effect coarse maize particles and moderate amounts of WB had on the development of digestive organs and processes, which enhance early weight gain. This explains the higher weight gain observed in birds fed 6%WB with coarse maize particles.

Reports from studies indicate a better FCR for broilers fed coarse maize particles (Amerah *et al.*, 2007b; Nir *et al.*, 1995) or brans (Taheri *et al.*, 2016). Results from this study confirms the assertion that dietary manipulation of feed particle size can be a potential nutritive strategy used to mitigate the negative effects dietary fibre may have on broiler growth (Amerah *et al.*, 2007a; Amerah *et al.*, 2007b; Kheravii *et al.*, 2018). In contrast, Pacheco *et al.* (2018) indicated a reduction in ADG when birds were fed maize particles larger than 1000µm as energy for growth is diverted into the grinding of coarser maize particles. The improved FCR of birds on T4 shows that ADFI from day 1 to day 21 was efficiently translated in ADG. Also, the observed results could be due to a reduction in the viscosity of the digesta liquid phase which enabled efficient nutrient digestion and utilization (Yasar, 2003). The inclusion of brans and coarse particles in diets makes the digesta spongy, which increases the permeability of the digesta to digestive enzymes due to the available spaces between the digesta particles (Lentle, 2005; Sarikhan *et al.*, 2010). These results corroborate that of Taheri *et al.* (2016) who observed an improvement in FCR when broiler diets were supplemented with 4 and 8%WB. Wheat bran is known for its bulk effect on the GIT hence feeding high amounts may cause laxative effects in broilers (Hyatt *et al.*, 2012). The improved BW by day 21 demonstrates that increasing WB level with fine or coarse maize particle sizes did not promote residues of unhealthy compounds in the gut to impair growth and development in experimental birds by day 42. Khempaka *et al.* (2009), observed a linear decrease in the body weight of broilers as the inclusion rate of insoluble fibre (dried cassava pulp) increased from 8 to 16%.

5.5. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Growth Performance during the Finisher Phase (day 22 to day 42)

In order to maximize broiler growth performance and productivity, the composition of diets must be adjusted as broilers age. From day 22 to day 42, maize particle sizes were kept constant with

WB level increasing from 6, 8 and 10% to 13, 15 and 17%. The crude fibre content in broiler diets as they grow is critical to their optimal development (Mateos *et al.*, 2012). The lack of adverse effect of the increased crude fibre content (Table 4.4) with fine or coarse maize particles on ADFI, ADG and FCR from day 22 to 42 can be attributed to a balance in digesta passage rate. Wheat bran is known for a faster digesta passage and an increase in WB level is expected to cause an increase in digesta mass and passage in the gut (Taheri *et al.*, 2016). An increase in digesta mass may also lead to the occurrence of abrasive effects on intestinal walls leading to a reduction in nutrient digestion and absorption (Montagne *et al.*, 2003). Current results show that the increase in WB level (dietary fibre) did not impair digestive processes and nutrient utilization.

5.6. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Carcass Parameters

Optimal development of carcass traits especially prime cuts (such as thighs, breasts and back of broilers) is important, as they are indicators of the economic value of broilers (Nkukwana *et al.*, 2014). The level of dietary protein accretion in broilers directly influences the development of prime cuts (Beski *et al.*, 2015). However, high levels of fibre in broiler diets potentially reduces the development of carcass traits (Bindelle *et al.*, 2008). This is because of the linear decrease in the digestibility and absorption of nutrients essential for the development of muscles and tissues (Oloruntola *et al.*, 2016). Despite the significant differences observed in ADFI by the day 42 (Table 4.2), crude protein, organic matter and dry matter digestibility (Table 4.1) were not impaired. This may explain the similarities in final body weights by day 42 and subsequent similarities in carcass traits. Furthermore, since the experimental diets were formulated to be isocaloric and isonitrogenous, the similarities in protein levels and energy levels may have influenced the similarities in carcass traits. Contrary to the findings of this trial, Shirzadegan and Taheri (2017)

observed an improvement in the relative weights of the breast, drumsticks, thigh, abdominal fat, heart and liver when alfafa meal or rice bran were included in broiler diets (up to 6%).

The quality of broiler mash diets can be evaluated by the effect of dietary particle size on carcass traits (Aguzey *et al*., 2018). Birds fed medium to large maize particles tend to consume a more balanced nutrient profile due to the lack of particle size preference (Parsons *et al.*, 2006). The lack of any adverse effect of maize particle size on carcass parameters suggests that the maize particle sizes used in this trial encouraged the consumption of a more balanced nutrient profile. The similarities in the effect of maize particles on carcass parameters is consistent with results of Ribeiro *et al.* (2002) (337 to 936µm maize particles) and Benedetti *et al.* (2011) (460 to 870µm maize particles). On the other hand, Parsons *et al.* (2006) noted a linear reduction in breast weight as maize particle sizes (781, 950, 1,042, 1,109, and 2,242µm) increased. They explained this to be due to the diversion of digestible energy from breast growth to gizzard growth and development.

5.7. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Relative Organ Weights

The gizzard responds to changes in dietary composition by structural adaptation (Kheravii *et al.*, 2018). The presence of structural components such coarse particles and dietary fibre in the gizzard might have led to increase in the contraction rate of the myelinated muscles and koilin layer of the gizzard in order to accommodate the extra grinding resulting from the digesta texture (Parsons *et al.*, 2006; Svihus, 2011). The higher relative weight of birds fed coarse maize particles can be attributed to the progressive increase in gizzard musculature of these birds to be able to break down ingested feed. This might have resulted in the increased relative weight of the empty gizzards, which is essential for an efficient nutrient digestibility (Kheravii *et al.*, 2018). Similar to the results

of this study, Jacobs *et al.* (2010) and Xu *et al.* (2015b) observed an increase in gizzard weights when maize particle size increased in broiler diets. In addition, Nir and Pitchi (2001) reported a positive correlation between relative gizzard weights and feed particle size in mash diets as observed in the trial. The observed significant interactive effect on the relative empty gizzard weights can be explained by the influence of maize particle size. Findings of this trial are similar to those of Xu *et al.* (2015c) where gizzard weights increased linearly as maize particle sizes moved from 422 to 431, 471, 509, 542, and 640µm.

An increase in the musculature of digestive organs such as the crop, proventriculus and intestines is also a physiological response to the extended period of time a mass of ingested feed has to stay in these sections of the GIT (Rodríguez *et al.*, 2006; Savon *et al.*, 2006). Despite the significant differences ($p \leq 0.05$) in fibre intake by day 42, the absence of the aforementioned phenomenon demonstrates feed intake of the various treatments was not high enough to effect a marked increase in the relative empty weights of these organs. By contrast, Mazhari *et al*. (2015) recorded higher relative intestinal weights in broilers fed 600g/kg wheat screenings as compared to those fed 150, 300 and 450g/kg. Current results are however similar to those of this study, Khempaka *et al.* (2009) observed no differences in organ weights when up to 16% insoluble fibre (cassava pulp) was fed to broilers. Weights organs such as pancreas, liver and kidney are indices of toxicity in broilers (Obun *et al.*, 2011). Hence, an increase in their weights may indicate an increase in detoxification activities due to the presence of harmful compounds or anti-nutrients in the feed (Adesehinwa *et al.*, 2011; Nortey *et al.*, 2015). The observed similarities in relative liver weights is suggestive of absence or low levels of toxic particles in the experimental diets. Similar results were obtained by Aguilar *et al.* (2011). In contrast, López and Baião (2004) and Fronte *et al.*

(2013) linked feeding of coarse diets to an increase in liver weights of broilers which they attributed to the effect of feed particle spectra on the musculature of the liver.

5.8. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Caecal Short-Chain Fatty Acids (SCFAs) Profile, pH and Microbial Population

The production rate and profile of SCFAs in the broiler gut is influenced by, the substrate available, age, breed and intestinal length (Walugembe, 2013). Arabinoxylan oligosaccharides produced from bactericidal degradation of WB are a very good source of butyric acid (Aoe *et al.*, 2018). However, results obtained from this study showed a relative abundance of acetic (AA), butyric (BA) and propionic (PA) acid, in a decreasing order. This can be attributed to the effect of substrate (diet), microbial diversity and degree of disassociation on SCFAs. Wheat bran serves as a suitable substrate for the proliferation of a wide range of indigenous microbes due to its diverse NSPs composition (Deroover *et al.*, 2017). Microbial degraders such as *Bifidobacteria* and *Lactobacillus spp*. can produce acetic and lactic acids respectively using WB as a substrate (De Maesschalck, 2015). On the other hand, through cross feeding, butyrate-producing bacteria such as *Faecalibacterium* and *Roseburia*, can convert acetic and lactic acids to butyric acid (Moens *et al.*, 2016; Vermeulen *et al.*, 2018). This suggests that the levels of SCFAs produced in this study might have been more dependent on the relative populations of microbes present in the caeca as suggested by Moens *et al.* (2016). Also, acetic acid (4.75) has a lower degree of disassociation (pKa) as compared to butyric (4.82) and propionic (4.88) acids (Hajati and Rezaei, 2010). The caecal pH values obtained in this trial ranged from 5.47 to 5.84 and this may have enhanced a faster disassociation of butyric and propionic acids. The trend in the concentrations of caecal SCFAs in this study is similar to that observed by Walugembe (2013) and Shang *et al.* (2020).

The pH along the GIT varies with the caecal pH of a matured broiler ranging from 5.8 to 6.8 (Hajati, 2018). The caecal pH values obtained at the end of the trial ranged from 5.47 to 5.84 with no treatment effect. Moreover, the observed similarities in caecal pH can be attributed to the lack of differences in the effects of WB level and maize particle size on the concentrations of acetic, butyric and propionic acids in the caeca. Charbeneau and Roberson (2004), Singh *et al.* (2014) and Naderinejad *et al.* (2016) demonstrated a lack of particle size effect on gizzard pH when mash diets were fed to broilers compared to pelleted diets.

Supplementation of broiler diets with insoluble dietary fibre has been suggested to be a readily available substrate for microbial fermentation (Jha *et al.*, 2019). However, the extent in gut pH reduction resulting from fibre fermentation determines the microbial profile and count (Fuller, 2001; Kheravii *et al.*, 2018). According to Hajati (2018), the proliferation of *Lactobacillus spp.* increases at a pH range of 5.4 to 6.4 while *E. coli* survive at a pH range of 6 to 8. The lack of increase or decrease in the counts of *Lactobacillus spp.* and *E. coli* respectively as WB level increased suggests that, the microbial fermentation at the lowest level of WB inclusion was enough to cause a reduction in gut pH. This may have resulted in the creation of a favourable acidic environment for the early seeding of *Lactobacillus spp.* and reduction in the survival rate of *E. coli*. The observed trend in microbial population can also be attributed to effect of caecal SCFAs on gut microbial profile. Short chain fatty acids have the ability exert toxic effects on pathogenic bacteria through their antimicrobial properties but creates a suitable environment for the multiplication of beneficial bacteria (Kheravii *et al.*, 2018).

An increase in the level of fibre and feed particle size in broiler diets is proportional to an increase in microbial fermentation which results in a higher proliferation of beneficial bacteria, increased SCFAs concentration and reduction in gut pH (Kheravii *et al.*, 2018). WB level and maize particle

size had no effect on the digesta after grinding in the gizzard in the current study. Hence, differences in digesta particle size which enhanced relative gizzard weights might have evened out between treatments by the time the digesta reached the caeca and may explain the similarities in caecal SCFAs concentrations, pH and microbial count. This agrees with reports of Hetland *et al.* (2002) and Hetland *et al.* (2004) who indicated a lack of influence of dietary particle size on the digesta after it leaves the gizzard. However, work done by Lentle (2005) contradicted this assertion stating the presence of coarser particles in broiler digesta after ingested feed passed through the gizzard. These differences in observations can be attributed to differences in feed uniformity and particle size ranges.

5.9. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on the Jejunal and Ileal Morphology

The morphology of the villi and crypts in the small intestines are baseline indicators of feed efficiency and overall gut health (Wang and Peng, 2008; Xu *et al.*, 2015a). The villi facilitate nutrient digestion and absorption through the luminal absorptive area (Silva *et al.*, 2007; Biasato *et al*, 2018) while the crypt depth (CD) development is suggestive of intestinal maturation and crypt-cell turnover rate (Miles *et al.*, 2006). The supplementation of broiler diets with insoluble fibre and large dietary particles enhances histopathological changes in the intestines through the production of SCFAs, which simulate the intestinal cells (Sarikhan *et al.*, 2010). The similarities in the effect of WB level on villi height (VH) and villi to crypt depth ratio (VH: CD) implies that the digestive and absorptive function of the jejunum and ileum was maintained. Results obtained on CD of the jejunal and ileal segments is suggestive of a normal energy expenditure in villi synthesis across treatments. Physiologically, the changes in the morphology of the small intestines is a result of the progressive effort to adapt to dietary composition in order to improve feed intake

and nutrient absorption (Mourão *et al.*, 2008). The lack of response in VH, CD and VH: CD as WB level increased implies that, the differences in WB levels were not high enough to trigger structural adaptations in order for nutrient digestion and absorption to be maintained. Feeding moderate or coarse particles of an insoluble fibrous diet increases digesta retention time (Kheravii *et al.*, 2018). This leads to longer contact period between digesta and intestinal mucosa resulting in longer proliferative activity of the villi (Dahlke *et al.*, 2003). In this study, the similarities in the relative weights of the full intestines is suggestive of similarities in digesta retention across treatments. The similarities in digesta retention time could have accounted for the similarities observed in the effect of maize particle size on VH, CD and VH: CD. These results are similar to reports of Amerah *et al.* (2007b) and Zang *et al.* (2009) which indicated the absence of maize particle size effect on gut morphology.

5.10. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size Blood Haematological Indices and Serum Glucose

The physiological and health condition of a bird is largely influenced by nutrition, drugs, pathological effects, environmental effects, age and breed (Gnonlonfin *et al.*, 2012; Ekunseitan *et al.*, 2013). Wheat bran (WB) is rich in antioxidants (flavonoids, phenolic acids, tocopherols, lignans, phytosterols and carotenoids), vitamin B complex and other bioactive compounds (Belobrajdic and Bird, 2013; Chalamacharla *et al.*, 2018). Coupled with the positive effect it has on the development of a healthy gut, WB exerts a very good degree of antimicrobial and anti-inflammatory properties on the overall health of broilers. Nutrients such as protein, iron, copper, vitamin B2, B6, B12 and folic acid are essential in the normal production of RBC (Nyaulingo, 2013). The RBC values obtained at the end of the trial fell within the normal physiological range of 1.93 to 3.5 10^{12}/L (Jain, 1993). This implies that RBCs were effectively produced and utilized

for metabolic and chemical activities. A high haematocrit level indicates toxicity reduction and a better feed efficiency in broilers (Mitruka and Rawnsley, 1977; Onu and Aniebo, 2011). The haematocrit values of birds on the test diets were similar to that of birds on the control diet and fell within the normal haematocrit range for broilers [(22.0-35.0 %), (Jain, 1993)]. This implies that oxygen and carbon dioxide transport was carried out efficiently during processes such as nutrient digestion and utilization. The normal haematocrit levels are in sync with the improvement in FCR observed by day 42 (Table 4.2). This corroborates the findings of Donkoh *et al.* (2003) who reported no adverse effect on haematocrit when broiler diets were supplemented with insoluble fibre. The genetic makeup of broilers as well as environmental conditions may affect the MCV of birds (Onyishi *et al.*, 2017). Similar to RBC and haematocrit values, MCV values of experimental birds were similar across treatments and did not deviate from the normal range. This is an indication that good management practices were observed throughout the trial. A deviation of HGB values from the normal range (7.0 – 13.0 g/dL) is suggestive of an anaemic condition and poor heart, liver or marrow function in broilers (Olagunju *et al.*, 2013). The HGB value of birds on the control diet (13.48 g/dL) deviated slightly from the normal range for broilers however, it was similar to the HGB values of birds on the test diets. The observation in birds on the control diet is suggestive of a compensatory mechanism to increase the oxygen levels in the blood. However, none of the mortalities, which occurred in birds on the control diet was attributed to an anaemic or organ malfunction. The dietary energy available to a bird after digestion is measured by its serum glucose levels (Ofori, 2015). Serum glucose level in birds on fine diets was higher than that of birds on coarse diets. This may be attributed to the fact that birds on coarse diets may have used some of their energy in adjusting to the particulate nature of the feed during the early stages of their life.

5.11. Effect of Dietary fibre level (using wheat Bran as a DF source) and Maize Particle Size on Immune Status

Basophils are responsible for histamine secretion (Vulevic *et al.*, 2013) and are rarely found in the blood of chickens (Hidanah *et al.*, 2018). However, basophil levels increase when there is stress due to high environmental temperature (Onyishi *et al.*, 2017; Hidanah *et al.*, 2018). This could have accounted for the presence of basophils in the blood of birds on T2, T5 and T6. Eosinophils are released in response to parasitic infections (Irizaary-Rovira, 2004) while elevated levels of heterophils reflect the occurrence of either a parasitic or bacterial infection (Mitchell and Johns, 2008). The normal physiological range of eosinophils and heterophils percentages fort broilers is 1.5 to 6.0% and 15.0 to 40.0% respectively (Jain, 1993). The absence of WB level or maize particle size effect indicates that the level of inclusion was not high enough to lower the elevated levels of eosinophils and heterophils. Lymphocytes produce antibodies to fight chronic antigenic stimulations (Hidanah *et al.*, 2018) while monocytes serve as clearing agents in the blood which gets rid of dead cells or pathogenic microorganisms (Abbas *et al.*, 2014; Bayona *et al.*, 2017). The lymphocytes and monocytes values obtained in the study were lower than the normal minimal value of broilers [(45.0 to 70.00% and 5.0 to 10.0%); Jain, 1993]. However, no dietary effect was observed.

According to Elmore (2006a and 2006b) the spleen and thymus are essential in accessing the response of the immune system to dietary components. As such, compensatory hypertrophy or cellular infiltration of the spleen and thymus by immune cells may occur as a response to infections or recovery in birds (Eshak *et al.*, 2016). The similarities in the relative weights of the spleen and thymus of birds on the control diet and birds on the other test diets suggest that the test diets were nutritionally adequate to support and maintain the health of the birds. The antioxidant, anti-

inflammatory and microbial growth-promoting effect of WB helps maintain gut health and immune status (Stevenson *et al.*, 2012). Work done by Rodríguez *et al.* (2006) and González-Alvarado *et al.* (2007) demonstrated the similarities in the relative weight of the spleen when different fibre sources were fed to layers and broilers respectively.

5.12. Economic Evaluation

Economic returns in broiler production can be improved through feed cost reduction, an optimal growth performance and flexibility in diet formulation (Costa *et al*. 2008). Feed cost is mostly reduced when AFIs partially or fully replace traditional feed ingredients in broiler diets (Onunkwo and George, 2015). The significant differences ($P \leq 0.05$) observed in the cost/kg/FC/bird (GH¢) during the starter phase can be attributed to the observed trend in ADFI during the starter phase. This in turn may have influenced the trend of significance in the overall cost of FC consumed/bird (GH¢). Even though there were significant differences in the cost per weight gain of birds, it did not translate onto the total FC/kg/FBW (GH¢) and total FC/kg/DW (GH¢). There was a linearly reduction as WB levels increased in total FC/kg/FBW (GH¢) and total FC/kg/DW (GH¢). It cost Gh¢ 0.40 and Gh¢ 0.24 lesser to produce a kilogram of the FBW and DW respectively of birds on T3 than those on the reference diet. The reduction in cost could be attributed to the relatively cheaper cost of wheat bran. Although the reduction seems small, it is important when large volumes of feed are being manufactured.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

1. Broilers were able to efficiently digest and utilize diets containing up to 17%WB with fine (725µm) or coarse (1178µm) maize particles which is a good indicator broilers' ability to physiologically adapt to the nature of their diets.
2. Inclusion of up to 17%WB with fine (725µm) or coarse (1178µm) maize particles in broiler diets increased overall feed intake and improved feed conversion ratio with birds fed coarse particles showing a better growth performance.
3. Feeding broilers diets containing up to 10%WB with coarse maize particles (1178µm) during the starter phase did not adversely affect average daily feed intake but improved average daily gain and feed conversion ratio. This shows diets influence good broiler weight gain as early as during the starter phase.
4. Feed cost reduced as the levels of wheat bran increased with fine (725µm) or coarse (1178µm) maize particles with no adverse effect on overall growth performance and health.
5. The carcass yield of experimental birds was not negatively affected by the inclusion of up to 17%WB with fine (725µm) or coarse (1178µm) maize particles.
6. There was no proportional increase in caecal butyric acid concentration as WB level increased with fine (725µm) or coarse (1178µm) maize particles. Rather, there were higher concentrations of acetic acid in caecal content.
7. There was no linear increase or decrease in the counts of *Lactobacillus spp*. or *E. coli* respectively as %WB increased with fine (725µm) or coarse (1178µm) maize particles.

Rather, there was a relative abundance of *Lactobacillus spp* which influenced the overall gut health of the broilers.

8. Increasing the levels of wheat bran with fine (725µm) or coarse (1178µm) maize particle did not linearly reduce caecal pH even though an acidic environment was created.
9. Feeding broilers up to 17%WB with fine (725µm) or coarse (1178µm) maize particles did not adversely affect villi height, crypt depth and villi to crypt depth ratio of the jejunum and ileum. Hence, effect nutrient absorption and utilization enhanced overall growth performance.
10. The experimental treatments used in this study had no adverse effects on the haematological indices, serum glucose content and immune status of broilers, which is a good indication of good health.

6.2. Recommendations

1. Feed manufacturers can include up to 17% wheat bran with fine (725µm) or coarse (1178µm) maize particles in broiler diets in order to cut down feed cost, promote growth and gut health.

2. Further studies should be carried out to establish a relationship, if any, between the roles dietary particle size uniformity and differences in geometric mean diameter play in broiler growth performance and gut health.

3. Further investigations should be conducted to determine if:

    a. The influence of dietary particle size on broiler growth and gut health is affected by feed form.

b. Varying the dietary particle size and levels of inclusion of other commonly used agro-by products such cassava peels, copra cake and palm kernel cake in Ghana will enhance broiler growth, gut health and cost-effectiveness.

c. Dietary fibre level (using wheat Bran as a DF source) s and dietary particle size has any influence on microbial diversity. This will enable effective formulation of diets, which will influence the ratio of gut microbes in order for specific short-chain acids to be produced.

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