Effect of pre-pelleting whole corn inclusion on growth performance, processing yield, meat quality, gut microbiome, and digestive organ development at different grow-out phases of broilers.

by

Fozol Korim Ovi

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 12, 2020

Keywords: Whole Corn, Pre-pelleting inclusion, Feed conversion ratio, Carcass yield, Breast meat yield, Intestinal microbiome

Approved by

Dr. Wilmer J. Pacheco, Chair, Extension Specialist and Assistant Professor of Poultry Science
Dr. Ken Macklin, Extension Specialist and Professor of Poultry Science
Dr. Charles Starkey, Assistant Professor of Poultry Science

ABSTRACT

Corn is typically ground before its incorporation into broiler diets. However, grinding is the second most expensive cost center of feed manufacturing after pelleting. Therefore, feeding whole corn can be an alternative to reduce the grinding cost without a negative effect on broiler performance. Since, corn kernel size is often too big to be included in poultry diets without grinding, inclusion of whole corn prior to pelleting can utilize the compressive force of pellet-mill to break down the corn kernel. Furthermore, whole corn inclusion might have some benefits on broiler performance and processing yield similar to whole wheat inclusion. Two experiments were conducted to evaluate the effects of pre-pelleting whole corn inclusion on growth performance, processing yield, meat quality, organ development, and intestinal microbiome of broilers. Both experiments consisted of 4 dietary treatments with 10 replicate pens per treatment and 25 broilers per pen. In the first experiment, treatment diets consisted 0, 2.5, 5, and 7.5% whole corn that replaced ground corn and was provide from 1 to 42 d of age. In the second experiment, whole corn was included as 0, 3, 6, and 9% of the total diet from 14 to 42 d, following a common starter diet fed in crumbled form from 1 to 14 d. In both studies, feed intake and body weight (BW) were determined at 14, 28, 42 d and feed conversion ratio (FCR) were adjusted by adding the weight of the mortality to the BW of live birds. At 43 d, 10 birds/pen were processed for yield determination. Cooking loss and coloration of breast meat fillets were evaluated in each treatment group of broilers during the first experiment after 48 h of water chilling. In addition, two-birds per pen were euthanized by CO2 asphyxiation at 42 d during the second experiment to determine the weight of crop, proventriculus, gizzard, liver and ceca and was expressed as a ratio of total live BW. Intestinal microbiome was analyzed at 42 d during the first experiment, however in the second experiment microbiome was analyzed at 28 d. Data were statistically evaluated using ANOVA test through GLM procedure of SAS and means were separated by Tukey HSD test. In first experiment, 7.5% whole corn inclusion significantly improved FCR from 28 to 42 d of age ($P<0.05$; 1.94 vs. 2.00). Broilers fed diets with 5% whole corn had higher breast meat yield than broilers fed 7.5% ($P<0.05$; 29.11 vs. 28.40 %) but was similar to broilers fed diets without whole corn. However, inclusion of whole corn did not influence cooking loss and color of breast meat ($P>0.05$). In addition, diets with 7.5% whole corn showed a trend towards increased *Faecalibacterium* ($P=0.07$) and decreased *Lactobacillus* ($P=0.08$) in cecal microbiota compared to 5% whole corn. In second experiment, the inclusion of whole corn did not influence BW, feed consumption, and FCR ($P>0.05$) from 14 to 42 d of age. However, broilers fed diets with 9% whole corn had higher carcass yield ($P<0.05$; 77.32 vs 77.86) than birds fed diets without whole corn. In the ileum, relative abundance of obligatory anaerobes decreased when 3% pre-pelleting whole corn was included, followed by a significant increase in diets containing 6 and 9% pre-pelleting whole corn inclusion ($P<0.05$). The inclusion of whole corn did not influence the relative weight of liver, gizzard, crop, and ceca ($P>0.05$). However, relative proventriculus weight decreased in birds fed diets with 9% whole corn inclusion compared to birds fed diets with 0% whole corn ($P< 0.05$; 0.28 vs 0.35%). Results of these studies indicated that up to 5% whole corn can be included in starter feed and 9% in grower and finisher feeds without any negative effect on broiler performance.

ACKNOWLEDGEMENTS

I am grateful to graduate school of Auburn university for sponsoring this research project. I highly appreciate the contribution of my advisor Dr. Wilmer Javier Pacheco. Without his guidance and encouragement this project could not be successfully completed. I also express my deep gratitude to my committee members, Dr. Charles Starkey and Dr. Ken Macklin for their thoughtful advice and support to this project. I acknowledge the helping hand of the members of Pacheco lab, Starkey lab, Dozier lab, Morey lab in different stages of data collection and experimentation. A special thanks to Mitchel Pate, director of Charles C. Miller Jr. poultry Research and Education Center for facilitating this research. I am also thankful to all technician and workers of Charles C. Miller Jr. poultry Research and Education Center for their tireless efforts and dedication to ensure successful completion of this project.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Corn is the most cultivated cereal grain of the world (Awika 2011; USDA WASDE, 2020). It contains the highest metabolizable energy among all cereal grains (Barzegar et al., 2019) nearly 3,360 kcal/kg (NRC., 1944; NRC 1988). Due to its high and consistent energy content corn has been used as an economical feed ingredient in livestock diets (Larbier and Leclercq 1994) and has been established as energy standard for other cereals and cereal by products (Leeson and Summers., 2009). Corn is typically ground prior to its incorporation in broiler diets. Grinding is important to improve mixability (Koch 2002), pellet quality (Angulo et al., 1996), feed utilization (Douglas et al., 1990) and to increase surface area to facilitate digestive enzyme attachment (Goodband et al., 1995; Mavromichalis 2000). However, grinding is the second most expensive cost center after pelleting (Deaton et al., 1989) and excessive grinding reduces grinding capacity (Wondra et al.,1995) and increases energy consumption (Reece et al., 1986b; Amerah et al., 2007a).

Chickens can detect particle size differences using mechanoreceptors in their beak (Gentle 1979) and prefer coarse particles over finely ground feed particles at all ages (Schiffman 1968; Portella et al., 1988). Coarse particles (>1mm) facilitate gizzard development (Svihus, 2011) and their absence or limited inclusion can impede proper development and natural functionality of gizzard (Svihus et al., 2002; Zaefarian et al., 2016). However, the benefits of coarse particle are limited in pelleted and crumbled feed due to additional grinding that occurs during pelleting as feed is pressed through the pellet die to form the pellet (Zaefarian et al., 2016). Pre-pelleting whole grain inclusion may withstand this grinding effect and overcome this limitation of coarsely ground feed. Whole wheat has been used in poultry diets as early as 1951 (Ewing, 1951) and diet containing whole wheat has been reported to have higher apparent metabolizable energy

(McIntosh et al., 1962; Svihus and Hetland, 2001) and apparent ileal digestibility of starch and dry matter (Svihus and Hetland, 2001; Svihus, 2011) compared to ground wheat diet, resulting lower FCR (Plavnik et al., 2002; Truong et al., 2017). Research with whole corn inclusion is sparse, despite having the capacity to reduce grinding cost and some potential benefit to broiler performance similar to whole wheat. Kernel size of corn is typically considered too big to be included in broiler diet without grinding. Inclusion of whole corn prior to pelleting could be a viable alternative. Pre-pelleting whole corn inclusion can utilize the additional grinding effect of pellet-mill described in Zaefarian et al. (2016) to break the kernel down and insert small pieces of broken kernel inside pellet. Singh et al. (2014b) used 0, 15, 30, 45 and 60% whole corn in broiler diet from 1 to 21 d and reported increased apparent metabolizable energy up to 30% whole corn inclusion. The authors also reported a linear increase of relative gizzard weight and decrease of proventriculus weight as the concentration of whole corn was increased. In the follow up study Singh and Ravindran (2019) used 0 or 11.5% whole corn in association with 3 kernel types of corn (hard, semi hard and soft) and reported increased relative gizzard weight without effecting BW, feed intake or FCR. Lu et al. (2011) fed Yang-zhou geese with a diet containing 64% whole corn from 8 to 28 d and 61.5% whole corn from 29 to 70 d and reported greater gizzard weight and marginally significant improvement in FCR (P=0.07) between 50 to 70 d in geese fed diet containing whole corn compared to geese feed diets with ground corn.

Research presented herein consisted of 2 experiments with the objective to determine the effect of pre-pelleting whole corn inclusion in broiler diet at different growout phases (starter, grower and finisher period). The first experiment was conducted using 0, 2.5, 5 and 7.5% whole corn replacing ground corn in broiler diets to determine its effect on broiler performance, intestinal microbiota and carcass characteristics from 1 to 42 d. In the second experiment, whole corn was used as 0, 3, 6 and 9% of diet between14 to 42 d following a common starter feed from 1 to 14 d. In addition to the parameters of first experiment, the effects of pre-pelleting whole corn inclusion on feed particle size, pellet quality and digestive organ development was evaluated in the second experiment.

KNOWLEDGE GAP IN LITERATURE

The optimum concentration of whole corn inclusion prior to pelleting has not yet been established for commercial broilers. Singh et al. (2014b) reported inclusion of 15% whole corn can limit feed intake of broilers resulting poor growth performance from 1 to 21 d despite increasing relative gizzard weight and apparent metabolizable energy. Singh and Ravindran (2019) reported higher relative gizzard weight for 11.5% whole corn inclusion from 1 to 21 d, however this increased gizzard weight neither improved nor worsen broiler growth performance. This finding contradicts with Svihus (2011) where the author speculated better performance resulted from increased gizzard weight and functionality stimulated by coarse particles in diet. Both of the previous experiments with whole corn inclusion in broiler diet (Singh et al., 2014b; Singh and Ravindran 2019) were conducted at early rearing phases from 1 to 21d. However, Lott et al. (1992) reported limiting effects of coarse particle on feed intake of broilers at early age. Portella et al. (1988) reported that despite affinity towards coarse particle exhibited at all ages, the actual capacity of coarse particle consumption increases with the increase of beak size at advance age. Effect of age was not considered during previous experiments with whole corn inclusion. Broiler performance might be benefited from gradual increase of whole corn concentration from starter phase to grower and finisher phase.

Therefore, during the first experiment only 0, 2.5, 5 and 7.5% whole corn was used to replace ground corn which was kept persistent throughout the rearing phase (1 to 42 d). However, in a second experiment a common starter feed without whole corn was provided in crumble form at starter period (1 to 14 d) before the birds were provided with diets containing 0, 3, 6 and 9% whole corn. Benefits of gradual increment of whole corn concentration can be better understood by comparing results of these two experiments, which can assist in establishing optimum level of whole corn inclusion in starter, grower and finisher periods.

LITERATURE REVIEW

1.1 Corn usage in poultry diets:

Corn is the most cultivated cereal grain in the world (Awika, 2011). It is considered a caryopsis that originated from wild-pod popcorn which was first domesticated in North America (Wet and Harlan, 1972). Currently, United States is the highest corn producing and corn consuming country in the world (USDA WASDE, 2020). Corn is the most cultivated cereal grain in USA (USDA, National agricultural statistical service 2019) and contains the highest apparent metabolizable energy (AME) (Barzegar et al., 2019; NRC 2012) among all cereal grains. Nearly 65% of total corn produced in the world is utilized in livestock feeding (FAO., 2005) and a major portion is used by the poultry feed industry. In poultry diets, 1 kilogram of corn supplies nearly 3,360 kcal/kg of metabolizable energy (NRC., 1944; NRC 1988), which makes it an economical ingredient in livestock diets (Larbier and Leclercq 1994). Furthermore, due to its high and consistent energy content, corn has been established as a standard of energy content for other cereals and cereal by products (Leeson and Summers., 2009).

1.2 Chemical composition of Corn

Corn represents the major energy source in poultry feeds (Dei, 2017) accounting for approximately 60% of required dietary energy (Islam et al., 2018). Indeed, feed costs represent between 60 and 70% of total broiler production costs and the majority of those cost are associated with purchasing and grinding corn (Thirumalaisamy 2016). Chemical composition of corn and its nutritive value is influenced by kernel type, kernel color, moisture content, drying temperature, grinding method and particle size (Amerah et al., 2007a). Chemical composition of corn kernel primarily determines its nutritive value (Gehring et al., 2013) and greatly influences growth performance and processing yield of chickens. A mature corn kernel has three major components: endosperm, germ, pericarp (Figure 1.). Each component of corn kernel has different nutritional composition (Table 1.1). Endosperm is the principal component of corn kernel and it greatly influences overall nutrient composition of corn (Watson 1994; Hopkins et al., 1974).

Table 1.1: Nutrient content of different components of corn kernel on dry matter basis.

Corn component	[a]Kernel	[b]Starch	[b]Sugar	[b]Oil	[b]Protein	[b]Ash
	--(% DM basis)------------------------------------					
Endosperm	82.9	87.6	0.6	0.8	8.0	0.3
Germ	11.1	8.3	10.8	33.2	18.4	10.5
Pericarp	5.2	7.3	0.3	1.0	3.7	0.8
Tip cap	0.8	5.3	1.6	3.8	9.1	1.6
Overall kernel	100	73.4	1.9	4.4	9.1	1.4

[a] Kling. (1991), [b]Earle et al. (1944)

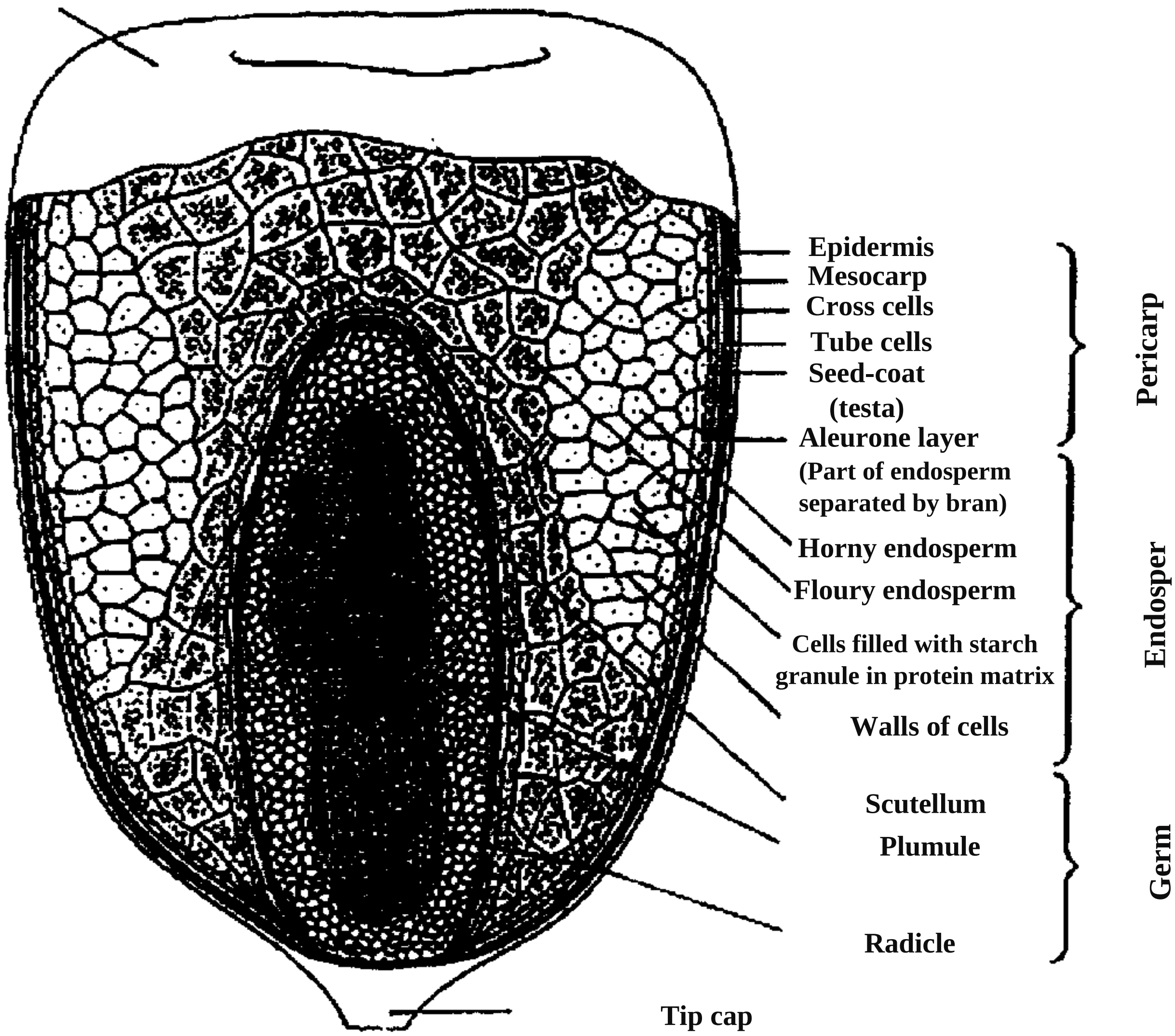


Figure 1.1: Longitudinal cross section of corn kernel in 30X magnification and simplified. (source: www.fao.org)

1.2.1 Carbohydrates

Corn has a high concentration of digestible starch and low concentration of fiber and pentosans (table 1.2). Majority of starch is embedded in the protein matrix of the endosperm (Earle et al., 1944; Watson 2003; Dei, 2017), which has two markedly distinct parts, horny endosperm and floury endosperm. Starch in horny endosperm is almost entirely composed of highly branched amylopectin (Kling 1991). Whereas, floury endosperm contains roughly 73% amylopectin and 27% non-branching amylose (MacDonald et al., 1988). However, differences in starch composition have minimum effect on the nutritive value of corn used in poultry diets (Singh and Ravindran 2019; Larbier and Leclercq, 1994).

Table 1.2: Concentration of different carbohydrates in corn kernel on dry matter basis.

Carbohydrate	Concentration (% DM)
Starch	77
Pentosan	5
Sugar	2
Crude fiber	1.2

MacDonald et al. (1988)

1.2.2 Protein

In corn, the majority of proteins are stored in the germ (Landry and Moureaux 1980; Ryšavá 1994) which can be as high as 18.4% (Earle et al., 1944). However, since germ represents only 11.1% of the corn kernel (Kling 1991), the average protein content of corn is around 9.1% (Earle et al., 1946). Corn with 85% dry matter contains around 8.5% crude protein (Leeson and Summers, 2009). Cowieson et al. (2019) quantified the amino acid content in corn and determined their apparent ileal digestibility by feeding a corn-based diet to Ross 308 male broilers (table 1.3). According to the authors, threonine had the lowest digestibility (48.9%), followed by lysine, aspartic acid, glycine, and serine. Whereas leucine, glutamic acid, alanine, methionine, proline, phenylalanine, and arginine of corn had considerably greater digestibility with the highest digestibility recorded is 84.3% in leucine. Remaining amino acids in corn had an intermediate digestibility.

Table 1.3: Amino acid content of corn-based diet and their subsequent apparent ileal digestibility.

Amino acid	Content (g/kg)	Digestibility%	Digestible AA[1]%
Arginine	4.02	78.5	0.32
Histidine	2.27	78	0.18
Isoleucine	2.65	70.5	0.19
Leucine	9.23	84.3	0.77
Lysine	2.38	56.4	0.13
Methionine	1.69	79.8	0.13
Phenylalanine	3.87	79.2	0.31
Threonine	2.84	48.9	0.14
Valine	3.85	70.2	0.27
Alanine	5.52	80.1	0.44
Aspartic acid	5.34	60.9	0.33
Cysteine	2.09	74.4	0.16
Glycine	3.23	63.5	0.21
Glutamic acid	14.57	83.3	1.21
Proline	6.84	79.3	0.54
Serine	3.76	66.6	0.25
Tyrosine	3.14	74.3	0.23

Cowieson et al. (2019)

1.2.3 Lipids

Lipid content of corn has been estimated between 3.8% (Leeson and Summer, 2009) to 4.4% (Earle et al., 1944) with the majority being stored in germ (table 1.4). Corn contains low concentration of long chain saturated fatty acids (Palmitic acid, stearic acid) and high concentration of unsaturated fatty acids such as oleic acid, linoleic acid (Martinez et al., 1996) (table 1.4) which makes the corn oil a desirable fatty acid source.

Table 1.4: Different fatty acids concentration in corn kernel determined on dry matter basis.

Fatty acid	Structure (Total No Carbon: No. of double bond)	Concentration (%DM)
Palmitic acid	16:0	11.3
Stearic acid	18:0	3.0
Oleic acid	18:1	43.4
Linoleic acid	18:2	41.8
Linolenic acid	18:3	0.6

Martinez et al. (1996)

1.2.4 Vitamins and Minerals

Corn is a good source of vitamins (table 1.5) and contains 0.6 to 2.1 mg/gm of alpha tocopherol (vitamin E) (Morrison 1977). However, concentration of vitamin E increases as the kernel matures. Vitamin A and carotenoids are also present in sizeable amount particularly in yellow corn varieties (Egesel et al., 2003; FAO 1992). Lutein was the most predominant xanthophyll followed by zeaxanthin and cryptoxanthin (Muzhingi et al., 2008). Other carotenoids like trans-ß-carotenes, 9-cis-ß-caroteins, 13-cis-ß-caroteins were also present in a reasonable amount.

Table 1.5: Vitamin content of yellow dented corn in dry matter basis.

Vitamin	Units	Avg	Range
A	mg/kg	2.5	
E	IU[1]	30	
Thiamine (mg/kg)	mg/kg	3.8	3.0-8.6
Riboflavin	mg/kg	1.4	0.25-5.6
Pantothenic acid	mg/kg	6.6	3.5-14
Biotin	mg/kg	0.08	
Folic acid	mg/kg	0.3	
Choline	mg/kg	567	
Niacin	mg/kg	28	9.3-70
Pyridoxine	mg/kg	5.3	

Watson (1994)

[1]IU= 1.49 × (mg of α tocopherol per kg + mg of γ tocopherol per kg × 0.1)

Corn contains a high concentration of water-soluble vitamins like thiamine, niacin, pyridoxin, and choline. However, more than 80% of niacin present in corn is bound as undigestible niacytin (Watson 1994), which can only be available to chicken after alkaline treatment (Harper et al., 1958).

Corn contains 1.4% ash on DM basis with the highest concentration present in germ. Potassium, phosphorous and magnesium are the most predominant minerals, whereas calcium and zinc are present in relatively low concentration (table 1.6; IITA-1982; Asiedu 1991; Prasanthi et al., 2017). However, up to 72.4% of phosphorous is present in phytate form, which is biologically unavailable to chicken (Watson 1994) without phytase supplementation.

Table1.6: Mineral content in corn kernel determined on dry matter basis.

Minerals	Concentration (mg/ 100gmDM)
Calcium	6.0
Phosphorus	300.0
Magnesium	160.0
Sodium	50.0
Potassium	400.0
Chlorine	70.0
Sulphur	140.0
Iron	2.5
Manganese	6.8
Copper	4.5

IITA-1982

1.3 Physical Characteristics of corn:

Interactions between physical characteristics and chemical composition are often overlooked (Gehring et al., 2013) while considering nutritive value of feedstuffs. Based on kernel characteristics, corn can be classified as flinty, floury, opaque, dent, and sweet corn (Watson 1994). Flinty corn has a strong round kernel due to the rigidity of its protein matrix in the endosperm (Dei 2017). Whereas, floury corn has soft endosperm and is native to North America. Opaque corn is a variety of floury corn where the protein matrix surrounding the starch in endosperm is substantially thinner and gets ruptured while drying, resulting the formation of a translucent air pocket that acts as a point of weakness (Duvic 1961). Dent corn was originated from crossing of floury and flinty corn and has characteristic depression on the crown, formed during drying of matured kernel (Watson 1994). According to Li et al. (2014), corn varieties have significantly different energy content and metabolizable energy. Feeding broilers with diets containing flinty corn between 0 to 7 d resulted in a slower starch digestion rate and lower BW gain than broilers fed diet with dented corn (Grbesa and Kiš, 2005).

Kernel hardness is influenced mainly by corn genetics, which is considered an innate characteristic of corn derived from the proportion of floury and flinty endosperm (Watson 1994). Kernel hardness influences milling characteristics and particle size distribution. With hard corn varieties yielding coarser grind with irregular particle shape compared to soft corn varieties (Cordova et al., 2020). Kernel hardness also influence broiler performance, gastro-intestinal tract development, and feed utilization. Benedetti et al. (2011) reported higher gizzard weight, DM digestibility, and lower nitrogen excretion in broilers fed diets with hard corn instead of dent corn from 1 to 21 d. Collins et al. (2001) used two varieties of yellow dented corn with varying size and kernel hardness (small and hard, large and soft) in broiler diets from 1 to 49 d and reported a higher feed intake, BW gain and chilled carcass weight in broilers fed the hard corn variety. Sing and Ravindran (2019) fed broilers diets containing hard, semi-hard and soft corn in combination with 0 or 11.5% whole corn inclusion from 1 to 21 d and reported a higher relative gizzard weight and better FCR in broilers fed diets with hard and semi hard corn compared to broilers fed diets with soft corn.

Moisture content of corn directly influences its nutrient composition and milling characteristics. Islam et al. (2015) deemed corn with 16% moisture unsuitable for both mash and pellet feed due to its lower nutrient density. Probst et al. (2013) milled corn with around 10, 16 and 20% moisture and reported poor particle size uniformity and higher energy consumption when corn with 20% moisture was milled compared to corn with 10% moisture. Furthermore, hardness typically decreases as moisture content increases (Armstrong et al., 2007), particularly when the moisture contents varies from 10 to 13%. Acceptable moisture concentration in corn is considered at 15% (Leeson and Summer 2009).

Temperature and relative humidity of air used during corn drying influences kernel quality (Li et al., 2014). According Fairchild et al. (2005), corn should be dried with a minimum of 82°C air temperature with 30 cubic feet per minute (CFM) airflow whereas, the optimum level should be 93°C with maximum of 180 CFM airflow. According to the authors, grain temperature is the limiting factor in air temperature during drying as kernel quality declines if the grain temperature exceeds 40°C. However, kernel quality may decrease at lower temperatures (20 to 60C) resulting increased kernel breakage (Gunasekaran and Paulsen 1985) and poor grinding uniformity (Floyd 1994).

1.4 Grinding method:

The grinding method influences energy consumption, average particle size, particle size uniformity, and broiler performance. The most common equipment used for grinding are hammermills and roller mills. Although hammermills typically consume more energy than roller mills (Vukmirovic et al., 2016; Thomas et al., 2018), they are the preferred grinding equipment in commercial multi-species feed manufacturing facilities due to their capacity to produce a wide range of particle sizes and their ease of maintenance (Koch 2002). Roller mills are more energy efficient and produce a more uniform particle size with a narrower standard deviation of particle size distribution than hammermills, which make them the preferred equipment for mash diets (Naylor and Smith 1931; Vukmirovic et al., 2016; Thomas et al., 2018). However, roller mills are not efficient in grinding fibrous materials. Hammermill consists a row of rotating hammer blades suspended from a central rotating shaft enclosed within a metal casing containing a screen with perforated holes (Lyu et al., 2020). Grinding occurs due to impact of rapidly moving hammer tip with slowly moving grains (Koch., 2002). In roller mills particle size reduction occurs by shear force, as the grain passes through a narrow space between pairs of rollers with grooves and corrugations (Koch 2002). Modern roller mills often consist of multiple pairs of rolls where top pair crack corn, intermediate pair crimps and the bottom pair controls the desired particle size (Rydel et al., 2005).

1.5 Feed particle size:

Particle size of ingredients and finished feed has gained interest as an alternative to improve performance and nutrient availability (Amerah et al., 2007b). Chickens has a mechanical grinder, “gizzard” in their digestive system, which reduces feed particle size, regulates feed-flow throughout the gastrointestinal tract, and contributes to physical and chemical degradation of nutrients (Svihus, 2011). Chickens detect particle size differences using mechanoreceptors in their beak (Gentle 1979) and prefer coarse over finely ground feed particles (Schiffman 1968; Portella et al., 1988). Coarse particles (>1mm) facilitate gizzard development (Svihus, 2011) and their absence or limited inclusion can impede development and natural functionality of gizzard (Svihus et al., 2002; Zaefarian et al., 2016). However, bird age must be considered to define the optimum inclusion of coarse particles (Portella et al., 1988). During pre-starter and starter periods, a high inclusion of coarse particles can reduce BW and lead to poor FCR by limiting feed intake and diverting a greater portion of dietary energy to gizzard contraction (Lott et al., 1992; Singh et al., 2014a).

Geometric mean diameter of particles (GMD) is typically used to measure average particle size of feed and feed ingredients which is expressed as microns (μm) whereas the range of variation in particle size distribution is described as geometric standard deviation

(GSD). Particle size is determined by sieving 100 g feed sample (Baker and Herman 2002) through a stack of sieves (descending order) with designated screen size (ASTM-E11) placed on a mechanical shaker for 10 min. Weight of particles retained on each sieve is used to calculate GMD and GSD using the formula:

$$\mathbf{d}_{gw} = \mathbf{log^{-1}} \left[\frac{\sum(\mathbf{W}i \log \mathbf{d}i)}{\sum \mathbf{W}i}\right] \quad \text{and} \quad \mathbf{S_{gw}} = \mathbf{log^{-1}} \left[\sqrt{\frac{\sum \mathbf{W}i\,(\log \mathbf{d}i - \log \mathbf{d}gw)}{\sum \mathbf{W}i}}\right]$$

(ASABE standard, S319.4 2009)
where,
d_{gw}= geometric mean diameter of particles
S_{gw}= Geometric standard deviation of particles
d_i= diameter of particle retained in $_i$th sieve
W_i= Weight of particle retained in $_i$th sieve

1.5.1 *Particle size on growth performance:*

Particle size of feed and feed ingredients is shown to influence BW, feed intake, FCR of broilers (Table 1.7). Svihus (2011) recommended to include 20 to 30% coarse particle (>1mm) in broiler diets to stimulate gizzard development. However, effects of corn particle size on broiler performance are more evident in mash diets (Reece et al., 1985; Svihus et al., 2004b; Chewing et al., 2012).

Silva et al. (2018) used a meal particle size of 650 and 850 μm in pelleted or expanded broiler diets from 1 to 42 d of age and reported no significant influence of meal particle size on feed intake, BW, and FCR of broilers. However, Rubio et al. (2020) used corn particle size of 1644μm instead of 615μm in pre-pelleted meal diets and reported increased feed intake and FCR in broilers between 28 to 42 d of age. Similarly, Benedetti et al. (2011) evaluated the effect of corn particle size of (460, 730, and 870 μm) and corn type (hard and dented) on broiler performance from 1 to 42 d and reported higher feed intake and FCR, and lower BW in broilers as corn particle size increased from 460 to 870 μm. Parsons et al. (2006) evaluated the effect of corn particle size (781, 950, 1042, 1109 and 2242 μm) and feed form (mash, soft pellets and hard pellets) from 21 to 42 d of age and reported higher feed intake and increased FCR when corn particle size exceeded 1042 μm in mash diets. Xu et al. (2015a) used 0, 25 and 50% coarse corn (1362μm) to replace fine corn (294μm) content in broiler diets, which resulted a particle size of 432, 541 and 640 μm in pre-pelleting mash diet, which was then pelleted and offered to caged broilers from 1 to 50 d. These authors reported higher BW and lower FCR in broilers fed diets with 25% and 50% coarse corn replacing finely ground corn. Xu et al. (2015b) used a coarse (1,642μm) and fine corn (229μm) in 0, 10, 20, 30 and 50% ratio to obtain a particle size of 422, 431, 471, 509, 542, and 640 μm in meal diet which was then provided to broilers either as mash or crumbled form from 1 to 14 d of age. The authors reported lower feed intake and BW as well as higher FCR when particle size of mixed diet was 542 and 640μm. Reece et al. (1985) milled corn using hammermill or roller-mill to obtain 814 and 1343 μm corn particle size, which was then incorporated into mash, pelleted or crumbled diets from 1 to 47 d. Authors reported higher feed intake, BW and lower FCR at 21 d in broilers fed mash diets with 1343μm corn particle size compared to broilers fed mash diet with 814μm corn particle size. Similar result was reported by Svihus et al. (2004b) with ground wheat, milled at different hammermill and roller mill setup which was provided to broilers as mash (280, 300, 310, 420 and 590 μm) or pelleted diet (220, 240, 250, 280 and 300 μm) from 11 to 30 d of age, resulting higher BW in broilers fed mash diet with 590 μm particle size compared to mash diet with 420μm particle size.

Chewing et al. (2012) analyzed the effect of meal particle size (300 and 600 μm) and feed form (pellet or mash) on growth performance of broilers from 0 to 44 d and reported higher feed intake and FCR in broilers fed diets with 600 μm corn particle size. Mingbin et al. (2015) conducted a similar experiment using corn particle size of 573, 865, and 1027μm in mash or crumble feed from 1 to 40 d age. The authors reported, an initial increase in BW and FCR at 21 d with corn particle size of 865 and 1027μm, however by 40 d of age growth performance of broilers evened out among all treatment groups. Lott et al. (1992) used two hammermill screen sizes (3.1 8 and 9.59 mm) for corn milling, resulting an average particle size of 710 and 1,173μm in ground corn, which was then used in crumbled diets fed to broilers from 1 to 21 d. Broilers fed diets containing corn grind with an average particle size of 1,173 μm had lower BW and higher FCR than broiler fed diets containing corn with an average particle size of 710 μm. Singh et al. (2014a) used 0, 15, 30, 45, 60% coarsely ground corn to obtain 578, 726, 877, 987, and 1,172 μm particle size in mashed broiler diets which was fed from 11 to 35 d. Authors reported a positive linear correlation between feed intake and mash particle size, while FCR showed a quadratic effect, increasing up to 877 μm before declining. However, Jacob et al. (2010) used 557, 858, 1210, and 1387 μm corn particle size in mash broiler diets from 1 to 21 d without any significant effect on BW and FCR.

Table 1.7: Effect of particle size on feed intake, body weight and FCR of broilers.

Particle size (µm)	Feed intake (g/bird)	Body weight (g/bird)	FCR (g:g)	Source
Corn grind of	28 to 42 d	At 42 d	At 42 d	Rubio et al. (2020)
615	2,742[b]	3,231	1,575[bc]	
863	2,828[ab]	3,294	1,574[c]	
1644	2,920[a]	3,333	1,599[a]	
2613	2,802[ab]	3,250	1,597[ab]	
Corn grind of	At 21 d	At 21 d	At 21 d	Benedetti et al. (2011)
460	1230[b]	929[a]	1.39[b]	
730	1279[a]	914[ab]	1.48[a]	
870	1275[ab]	913[b]	1.47[a]	
Corn type				
Dent	1276	924	1.46	
Hard	1247	914	1.44	
Corn	At 21 d	At 21 d	At 21 d	Reece et al. (1985)
814	832[1]	582[b]	1.43[a]	
1343	889[1]	635[a]	1.40[b]	
Mash particle size	11 to 30 d	11 to 30 d	11 to 30 d	Svihus et al. (2004b)
280	1840	1076[ab]	1.72	
300	1849	1089[ab]	1.70	
310	1720	1029[ab]	1.68	
420	1695	993[b]	1.71	
590	1845	1135[a]	1.64	
Pellet particle size	11 to 30 d	11 to 30 d	11 to 30 d	
220	2133	1361	1.57	
240	2149	1348	1.60	
250	2095	1333	1.58	

280	2118	1350	1.57	
300	2076	1315	1.58	
Feed form	Broiler at 44 d	Male at 44 d	Broiler of 44 d	Chewing et al. (2012)
Mash	4,964^{B}	2,733^{B}	2.02^{A}	
Pellets	5,372^{A}	3,227^{A}	1.94^{B}	
Particle size				
300	5,098^{b}	2,981	1.90^{B}	
600	5,372^{a}	2,979	1.97^{A}	
Feed form×meal particle size				
Mash-300	4,776^{B}	2,739	1.94^{B}	
Mash-600	5149^{AC}	2,726	2.11^{A}	
Pellet-300	5,423^{A}	3,224	1.87^{C}	
Pellet-600	5,320^{AD}	3,231	1.84^{C}	
Corn particle size in mash	1 to 21 d	1 to 21 d	1 to 21 d	Mingbin et al. (2015)
537	859^{2,a}	1168^{3,a}	1.361^{a,b}	
865	859^{2,a}	1155^{3,a}	1.346^{b}	
1027	859^{2,a}	1170^{3,a}	1.360^{a,b}	
Corn particle size in crumble	1 to 21 d	1 to 21 d	1 to 21 d	
537	764.4^{2,c}	1056^{3,c}	1.384^{a}	
865	816.9^{2,b}	1121^{3,b}	1.372^{a,b}	
1027	831.6^{2,b}	1121^{3,b}	1.386^{a}	
Corn particle size in crumble	1 to 21 d	1 to 21 d	1 to 21 d	Lott et al., 1992
710	1048	748.6^{a}	1.40^{b}	
1,173	1032	729.2^{b}	1.42^{a}	
Meal particle size	11 to 35 d	11 to 35 d	11 to 35 d	Singh et al., 2014a
578	2,673^{L}	1,595^{Q}	1.693^{Q}	
726	2,889^{L}	1,648^{Q}	1.754^{Q}	

877	2,896[L]	1,707[Q]	1.768[Q]	
987	2,882[L]	1,787[Q]	1.679[Q]	
1,172	2,901[L]	1,733[Q]	1.689[Q]	
Meal particle size	At 42 d	At 42 d	At 42 d	Xu et al. (2015a)
432	5,257	2,929[b]	1.82[A]	
541	5,350	3,118[a]	1.86[B]	
640	5,154	3,059[a]	1.82[B]	
Corn particle size	0 to 21 d	0 to 21 d	0 to 21 d	Jacob et al. (2010)
557	1172[1]	849	1.38[5]	
858	1216[1]	863	1.41[5]	
1,210	1238[1]	820	1.51[5]	
1,387	1198[1]	856	1.40[5]	
Diet type	At 14 d	At 14 d	At 14 d	Xu et al., 2015b
Mash	160[B]	168[B]	1.37[A]	
Crumble	179[A]	200[A]	1.29[B]	
Meal particle size	At 14 d	At 14 d	At 14 d	
422	190[A]	190[A]	1.30[c]	
431	187[AB]	187[AB]	1.35[a]	
471	189[A]	189[A]	1.31[bc]	
509	184[ABC]	184[ABC]	1.33[abc]	
542	178[BC]	178[BC]	1.35[a]	
640	175[C]	175[C]	1.35[a]	
Corn particle size of	21 to 42 d	21 to 42 d	21 to 42 d	Parson et al., 2006
781	2739[4,b]	1578[4]	1.92[5,b]	
950	2871[4,b]	1590[4]	1.94[5,b]	
1,042	2852[4,b]	1629[4]	1.93[5,b]	
1,109	2854[4,b]	1576[4]	1.97[5,a]	
2,242	3123[4,a]	1610[4]	2.08[5,a]	

Diet particle size	1 to 42 d	1 to 42 d	1 to 42 d	Silva et al., 2018
650	4,457	2817	1.63	
850	4,455	2796	1.64	

[1]Obtained by multiplying FCR with body weight.

[2]Obtained by multiplying average daily feed intake by the number of days reared.

[3] Obtained by multiplying average daily gain by the number of days reared

[4] Obtained by dividing the total feed intake of a pen by the total number of birds in a pen (23).

[5] FCR = $\frac{1}{\text{Feed efficiency}}$

[L] Linear effect

[Q] Quadratic effect

[a,b,c] Column with different superscript differ significantly (P<0.05)

[A,B,C] Column with different superscript differ significantly (P<0.01)

1.5.2 Particle size and processing yield:

Effects of feed particle size on processing yield are inconclusive (Table 1.8). Mingbin et al. (2015) formulated diets with corn particle size of 537, 865 and 1027 µm and fed them to broilers as mash (1 to 40 d) or as a combination of crumbles (1 to 21 d) and pellets (21 to 40 d). Particle size of corn did not influence carcass yield, breast meat yield and thigh yield at 41 d of age. Singh et al. (2014a) used 0, 15, 30, 45, 60% coarse corn in broiler diet resulting a particle size of 578, 726, 877, 987, and 1,172 µm in meal diets and between 11 to 35 d and reported a linear decrease in breast meat yield with the increase of meal particle size. Silva et al. (2018) used 650 and 850µm meal particle size in pelleted or expanded broiler feeds from 1 to 42 d age and reported no significant difference in carcass yield, breast and tender yield, drum yield, thigh yield and abdominal fat at 42 d. Similar results were reported by Benedetti et al. (2011) where diet particle size (460, 730 and 870µm) did not influence carcass yield, breast meat yield and drum and thigh yield at 42 d

Table 1.8: Effect of particle size on carcass yield, breast meat yield, tender yield, drum and thigh yield of broilers.

Particle size (μm)	Carcass Y.[1] (%)	Breast meat Y.[2] (%)	Tender Y.[3] (%)	Drum Y[4]. (%)	Thigh Y[5]. (%)	Source
Crumble-Pellet	At 41 d	At 41 d	N/A	Drum and Thigh at 41 d		Mingbin et al. (2015)
Fine (corn 537μm)	75.89	19.44		24.91		
Medium (corn 865μm)	75.33	19.04		24.86		
Coarse (corn 1027μm)	75.02	18.98		24.69		
Mash	At 41 d	At 41 d		Drum and thigh at 41 d		
Fine (corn 537μm)	75.25	18.63		25.11		
Medium (corn 865μm)	74.19	19.02		23.79		
Coarse (corn 1027μm)	75.04	19.24		24.40		
Corn grind of	At 42 d	At 42 d	N/A	Drum and thigh at 42 d		Benedetti et al. (2011)
460	71.82	37.91		31.96		
730	71.85	37.47		32.28		
870	72.36	37.37		32.37		
Corn type	At 42 d	At 42 d		Drum and thigh 42 d		
Dent	72.08	37.61		32.24		
Hard	71.94	37.56		32.16		
Meal particle size	At 35 d	At 35 d	N/A	N/A	N/A	Singh et al. (2014a)
578	72.7	16.6^{L}				
726	72.5	16.5^{L}				

877	72.2	15.5^{L}			
987	71.2	15.3^{L}			
1,172	72.6	15.3^{L}			
Meal particle size	At 42 d	Breast and tender at 42 d	At 42 d	At 42 d	Silva et al. (2018)
650	78.32	28.65	9.90	10.44	
850	77.86	28.43	9.83	10.41	

[4]Drum and thigh yield

1Carcass yield

2 Breast meat yield

3Tender yield

1.5.3 Particle size and digestive organ development:

Broilers fed coarse particles have shown a positive effect on gizzard and proventriculus development, particularly in mash feed (Table 1.9). Benedetti et al. (2011) used 460, 730 and 870 μm corn particle size in broiler diets from 1 to 42 d and reported higher combined weight of proventriculus and gizzard at 11 and 42 d in diets with 730 and 870 μm corn particle size. Svihus et al. (2004b) used a meal particle size of 280, 300, 310, 420 and 590 μm in mash diets from 11 to 30 d and reported higher gizzard weight in broilers fed diets with 420 and 590 μm meal particle size instead of 280, 300 and 310 μm. Chewing et al. (2012) used corn particle size of 300 and 600 μm in pelleted and mashed broiler diets from 1 to 44 d and reported higher relative gizzard weight in broiler fed diets with 600 μm corn particle size in both pelleted and mash form. Singh et al. (2014a) used 0, 15, 30, 45 and 60% coarse corn (1,695μm) to increase the particle size meal diet to 578, 726, 877, 987 and 1172 μm and fed to broilers from 11 to 35 d. They reported a linear increase of relative gizzard weight at 35 d as diet particle size increased, whereas proventriculus weight showed a quadratic effect by increasing up to 726 μm particle size before declining with 1172 μm particle size. Xu et al. (2015a) used 0, 25 and 50% coarse corn to produce mash diets with 432, 541 and 640 μm meal particle size which was fed to caged broilers from 1 to 50 d. They reported significantly greater gizzard weight and gizzard-proventriculus ratio at 42 d when 541 and 640 μm particle size was used. Jacob et al. (2010) used 557, 858, 1210 and 1387 μm corn particle size in mashed broiler diet from 1 to 21 d and reported significantly higher relative gizzard weight for feeding diet containing corn particle size of 858, 1210 and 1387 μm. Xu et al. (2015b) used a meal particle size of 422, 431, 471, 509, 542, and 640 μm in mash and crumbled diets from 1 to 14 d of age, and reported an increased relative gizzard weight as meal particle size of mash feed was increased to 471, 509, 542 and 640 μm. Parson et al. (2006) used corn particle size of 781, 950, 1042, 1109 and 2242 μm in broiler diet that was provided as mash, soft pellet or hard pellet form between the age of 21 to 42 d and reported greater gizzard weight once corn particle size exceeded 1109 μm in mash feed.

Table 1.9: Effect of particle size on proventriculus and gizzard development of broilers.

Particle size(μm)	Gizzard R^1	Proventriculus R^2	Source
Corn particle size	Gizzard and proventriculus 42 d of age		Benedetti et al. (2011)
460	3.02[b]		
730	3.45[a]		
870	3.53[a]		
Meal particle size of mash	At 30 d	N/A	Svihus et al. (2004b)
280	1.49[b]		
300	1.44[b]		
310	A1.62[ab]		
420	1.76[a]		
590	1.75[a]		
Meal particle size of pellet			
220	1.11		
240	1.18		
250	1.21		
280	1.24		
300	1.25		
Corn particle size	At 42d	N/A	Chewing et al. (2012)
300-Mash	1.45[b]		
600-Mash	1.56[a]		
300-Pellet	1.05[d]		
600-Pellet	1.30[c]		

Corn particle size	At 41 d	At 41 d	Mingbin et al. (2015)
537	1.05	0.307	
865	1.09	0.315	
1027	1.16	0.314	
Meal particle size	At 35 d	At 35 d	Singh et al. (2014a)
578	1.42^{L}	0.318^{Q}	
726	1.57^{L}	0.326^{Q}	
877	1.65^{L}	0.359^{Q}	
987	1.61^{L}	0.359^{Q}	
1,172	1.64^{L}	0.332^{Q}	
Meal particle size	At 42 d	At 42 d	Xu et al. (2015a)
432	0.66^{C}	0.250	
541	0.76^{B}	0.318	
640	0.88^{A}	0.288	
Corn particle size	At 21 d	N/A	Jacob et al. (2010)
557	1.50^{c}		
858	1.94^{b}		
1,210	2.14^{a}		
1,387	2.20^{a}		
Meal particle size of mash	At 14 d	N/A	Xu et al. (2015b)
422	0.200^{C}		
431	0.210^{C}		
471	0.214^{BC}		
509	0.229^{B}		
542	0.252^{A}		
640	0.254^{A}		

Meal particle size in crumble	At 14 d		
422	0.175^{D}		
431	0.182^{D}		
471	0.182^{D}		
509	0.187^{D}		
542	0.189^{D}		
640	0.188^{D}		
Corn particle size of	At 42 d	N/A	Parson et al. (2006)
781	1.51^{b}		
950	1.54^{b}		
1,042	1.60^{b}		
1,109	1.61^{b}		
2,242	1.81^{a}		

[1]Gizzard R= Relative gizzard weight is the ratio of average empty gizzard weight and average live weight expressed as percent.

[2]Proventriculus R = Relative proventriculus weight is the ratio of average empty proventriculus weight and average live weight expressed as percent.

^{L} Linear effect

^{Q} Quadratic effect

^{a,b,c,d} Column with different superscript differ significantly (P<0.05)

^{A,B,C,D} Column with different superscript differ significantly (P<0.01)

1.5.4 Particle size on nutrient digestibility and metabolizable energy:

Effects of particle size on nutrient digestibility have yielded confounding results and likely have been influenced by birds age and methodology used for evaluation. Amerah et al. (2007a) could not detect any alteration on apparent ileal metabolizable energy (AME_n) between 17 to 21 d when wheat content of broiler feed was ground in a hammermill equipped with 3 and 7mm screens. However, Peron et al. (2005) used ground wheat to an average particle size of 380 and 955 μm between 7 to 21 d and reported higher apparent metabolizable energy, dry matter and ileal starch digestibility at 20 d in broilers fed diet with 380μm wheat particle size. Xu et al. (2015a) reported significantly higher apparent ileal digestibility of energy and nitrogen when pre-pelleting mash diet containing 25 and 50% coarse corn (meal particle size of 541 and 640μm) compared to diet without coarse corn (meal particle size 432μm). Singh et al. (2014a) used 0, 15, 30, 45 and 60% coarse corn (corn particle size 1,695μm) to obtain 578, 726, 877, 987, and 1172μm meal particle size in mash diets from 11 to 35 d. The authors reported a quadratic effect of meal particle size on AME and DM retention with the highest AME and DM was recorded in diet containing 30% coarse corn (meal particle size 877μm). Jacob et al. (2010) fed mash diets with an average corn particle size of 557, 858, 1210 and 1387 μm to broilers from 0 to 21 d and reported a linear decrease in metabolizable energy as corn particle size increased.

1.6 Whole grain inclusion:

Historically early poultry rearing involved backyard birds reared almost exclusively on whole grain diets. Grinding feed ingredients was later adopted to increase mixability (Koch 2002), pellet quality (Angulo et al., 1996), feed utilization (Douglas et al., 1990) and to increase surface area to facilitate digestive enzyme attachment (Goodband et al., 1995; Mavromichalis 2000). However, grinding is the one of the most expensive cost centers in feed manufacturing only second to pelleting (Deaton et al., 1989) and excessive grinding reduces grinding capacity (Wondra et al.,1995) and increases energy consumption in feed mill (Reece et al., 1986b). Moreover, coarse particles are necessary for gizzard development (Svihus et al., 2002; Zaefarian et al., 2016) and as much as 20 to 30% coarse particles (>1mm) are required in broiler diets to maintain the natural functionality of gizzard (Svihus, 2011). Coarsely ground corn and wheat can be used to increase particle in mash size mash diets, however the additional grinding that occurs during pelleting and crumbling process limits their effectiveness in pelleted and crumbled feeds (Zaefarian et al., 2016).

Pre-pelleting inclusion of whole grains can be an effective alternative to increase particle size and reduce grinding cost. Whole wheat has been used in poultry diets as early as 1951 (Ewing, 1951) and reported to have higher apparent metabolizable energy (McIntosh et al., 1962; Svihus and Hetland, 2001) and apparent ileal digestibility of dry matter (Svihus and Hetland, 2001; Svihus, 2011). However, until recently the inclusion of whole corn was not considered despite being the principal ingredient in poultry diets in the United States. The kernel size of corn is typically considered too big to be included in poultry diets without grinding. Among other grains, whole sorghum and whole barley (Biggs and Parsons 2009; McIntosh et al., 1962; Taylor and Jones 2004; Jacob and Parsons 2013) have also been used in broiler feeds.

1.6.1 Methods of whole grain feeding:

Rose (1996) first described three key methods of whole wheat inclusion in broilers diets; free choice feeding, mixed feeding, and sequential feeding. In free choice feeding, birds are provided with ad libitum whole grain in a container while other ingredients (a protein source, and a vitamin mineral mixture) simultaneously present in separate containers. This feeding system based on the notion of "nutritional wisdom" where chicken is considered to have the capability to make the wise decision regarding their nutrient requirement while feeding (Forbes and Shariatmadari 1994; Forbes and Covasa 1995). However, Rose et al. (1995) reported that, in free choice feeding birds typically chose a diet that gives rapid growth, thus they end up eating too much protein supplement but not enough whole grain (wheat), resulting in higher feed costs.

In in mix feeding whole grain are usually mixed with other ingredients and provided as mixed mash or in pellet form. In pelleted mixed feeds, inclusion of whole grain can be subdivided in pre-pelleting and post-pelleting inclusion. In sequential feeding method, whole grain and other ingredients are fed from same feeder but at two separate times. In each of this whole grain feeding methodology, remaining ingredients can be provided as protein concentrate, supplementary balancing diet or as a complete diet containing both whole grain and remaining ingredients. Among all those methodologies, pre-pelleting whole grain inclusion provides better control on feeding and has the least nutrient segregation (Singh et al., 2019).

1.6.2 Whole wheat in broiler diet:

Whole wheat is the most commonly used whole grain in broiler diets and has been used in broiler feeds under different feeding methods like free choice and mixed feeding (Table-1.10). Amerah et al. (2008) used ground wheat or whole wheat at 10% (7 to 21 d) and 20% (22 to 35 d) under two feeding systems (free choice and mixed feeding) and reported decreased feed intake and improved BW and FCR in mixed feeding broiler at 21 and 35 d. However, carcass yield and breast meat yield were improved in broilers feed ground wheat whereas inclusion of whole wheat in both methods improved relative gizzard weight. Truong et al. (2017) used a control diet with ground wheat (0% whole wheat) and diets containing pre-pelleting and post pelleting inclusion (4.5, 9 and 18%) of whole wheat from 7 to 28 d and reported increased relative gizzard weight and decreased FCR for all diets containing whole wheat compared to control diet. Authors also reported reduced feed intake when 18% whole wheat added before and after pelleting and decreased BW when 18% whole wheat added as post-pelleting inclusion. Inclusion of 18% pre-pelleting whole wheat and 4.5, 9 and 18% inclusion of post pelleting whole wheat increased apparent ileal metabolizable energy. WU et al. (2004) conducted a 3×2 experiment with ground wheat, 20% pre-pelleting and 20% post-pelleting whole wheat inclusion in association with 0 or 1000 XU/Kg xylanase enzyme. The authors reported higher apparent metabolizable energy in ileum and decreased FCR when 20% whole wheat was included in both methods. Furthermore, feed intake decreased, and relative gizzard weight increased when 20% whole wheat was added after pelleting. Singh et al. (2019) used 0 and 20% whole wheat in broiler feeds included pre-pelleting or post-pelleting from 11 to 35 d and reported reduced feed intake and BW when 20% whole wheat was included in both methods, whereas, FCR was lower when 20% whole wheat was included post-pelleting. Furthermore, inclusion of whole wheat increased relative gizzard weight and decreased gizzard pH regardless of inclusion method. The authors also analyzed cecal microbiome at 35 d and reported decreased relative abundance of Clostridia and Campylobacteria spp. and marginal increase of Lactobacillus spp. when 20% whole wheat was added pre-pelleting and post-pelleting. Plavnik et al. (2002) included 0, 10 and 20% whole wheat to replace ground wheat in broiler diets form 6 to 46 d and reported higher BW and relative gizzard weight as well as lower FCR when 10 and 20% whole wheat was used to replace ground wheat. Taylor and Jones (2004) conducted a 3×2 experiment using ground wheat and 20% pre-pelleting whole wheat or whole barley plotted against 0 or 650 BGU/g β-glucanase in broiler diet from 5 to 42 d of age. Authors reported greater relative gizzard weight and lower relative proventriculus weight when 20% whole wheat was included whereas BW and FCR remained unaffected by whole wheat inclusion. Similarly, Svihus et al. (2004a) used 0 and 37.5% whole wheat in broiler diets from 11 to 20 d and reported no significant effect on BW, feed intake and FCR of broilers despite an increased apparent metabolizable energy evaluated from 13 to 15 d for whole wheat inclusion. Inclusion of whole wheat at higher concentration at early age might have some detrimental effect as described in Biggs and Parson (2009). The authors included 0, 5, 10, 15 and 20% whole wheat in pelleted broiler diet from 1 to 21 d in the first experiment and 0, 20, 35 and 50% whole wheat in their follow up experiment. In the first experiment, despite an increased apparent metabolizable energy and relative gizzard weight of broilers, there was no significant effect on BW, feed intake and FCR for whole wheat inclusion (0, 5, 10, 15, 20%). However, in the follow up experiment BW decreased and FCR increased when 50% whole wheat was added to diets from 1 to 21 d.

Table 1.10: Effects of whole wheat inclusion on growth performance, processing yield, apparent metabolizable energy, and relative weight of digestive organs

Whole wheat (WW)	Feed intake (g)	Body weight (g)	FCR[1] (g:g)	Carc. Y[3]. (%)	Breast Y[4]. (%)	Ten. Y[5]. (%)	AME[6] (Kcal/kg)	Prov. R[7]. (%)	Gizzard R[8]. (%)	Source
7 to 21 d	7 to 21d	7 to 21d	7 to 21d	At 35 d	At 35 d	N/A	N/A	At 35 d	At 35 d	Amerah et al. (2008)
Ground wheat (GW)	978[a]	681[a]	1.44[a]	71.7[a]	26.1[a]			0.04	0.30[b]	
Mixed feed. (10%)	1010[a]	717[a]	1.41[a]	69.9[b]	26.0[a]			0.01	1.00[a]	
Free choice (10)	870[b]	540[b]	1.61[b]	67.4[c]	24.6[b]			0.02	0.90[a]	
22 to 35 d	22 to 35d	22 to 35d	22 to 35d							
Ground wheat	2168[a]	1262[a]	1.79[a]							
Mixed feed. (20%)	2041[b]	1184[b]	1.77[a]							
Free choice (20)	1840[c]	1109[c]	1.71[b]							
11 to 35 d	11 to 35 d	11 to 35 d	11 to 35 d	N/A	N/A	N/A	N/A	N/A	At 35 d	Singh et al. (2019)
Ground wheat	3377[a]	1999[a]	1.689[ab]						0.690[b]	
20% pre-pellet WW	3065[b]	1776[b]	1.725[a]						0.897[a]	
20% post-pellet WW	2972[b]	1794[b]	1.656[b]						1.185[a]	
7 to 28 d	7 to 28 d	7 to 28 d	7 to 28 d	N/A	N/A	N/A	26 to 28d	N/A	At 28 d	Truong et al.,(2017)
Ground wheat	2466[a]	1638[a]	1.506[a]				2885[2,c]		1.45[e]	
Pre-P. inc.	Pre-P. Inc.	Pre-P. Inc.	Pre-P. Inc.				Pre-P. Inc		Pre-P. inc.	
4.5%WW replaced GW	2410[ab]	1667[a]	1.446[b]				2890[2,c]		1.52[dc]	
9.0%WW replaced GW	2421[ab]	1672[a]	1.449[b]				2884[2,c]		1.54[dc]	
18.0%WW replace GW	2358[bc]	1657[a]	1.423[b]				3026[2,b]		1.64[cb]	
Post-P. inc.	Post-P. inc.	Post-P. Inc.	Post-P. Inc.				Post-P. Inc		Post-P. inc.	
4.5%WW replaced GW	2391[abc]	1669[a]	1.433[b]				3028[2,b]		1.69[c]	
9.0%WW replaced GW	2366[bc]	1645[a]	1.439[b]				3074[2,ab]		1.81[b]	

18.0%WW replace GW	2305^{c}	1587^{b}	1.453^{b}				3138^{2,a}		2.00^{a}	
Trail-1:	Trail-1:	Trail-1:	Trail-1:	Trail-1:	Trail-1:	N/A	N/A	N/A	Trail-1:	Plavnik et al (2002)
6 to 46d	6 to 46d	6 to 46d	6 to 46d	N/A	N/A				6 to 46d	
Ground wheat	4633	2431^{b}	1.93^{1,a}						1,50^{b}	
10% WW replaced GW	4566	2525^{a}	1.81^{1,b}						1.51^{b}	
20% WW replace GW	4563	2494^{a}	1.82^{1,b}						1.65^{a}	
Trial-2:	Trial-2:	Trial-2:	Trial-2:	Trial-2:	Trial-2:				Trial-2:	
Ground wheat	5409^{a}	2676	2.08^{1,a}	67.9	15.1^{a}				1.05^{b}	
15% WW (21 to 45d)	5162^{b}	2666	1.94^{1b}	68.4	14.2^{b}				1.36^{a}	
5%+15% WW (1 to 21 d + 22 to 45 d)	5121^{b}	2623	1.96^{1,ab}	68.8	14.7^{ab}				1.27^{a}	
1 to 21 d	1 to 21 d	1 to 21 d	1 to 21 d	N/A	N/A	N/A	17 to 21 d	N/A	At 21 d	Wu et al. (2004)
Ground wheat	1267^{a}	800	1.589^{a}				2959^{2,c}		1.01^{b}	
20% WW Pre-P. Inc.	1265^{a}	831	1.524^{b}				3066^{2,b}		1.01^{b}	
20% WW post-P Inc.	1217^{b}	817	1.496^{c}				3136^{2,a}		1.75^{a}	
5 to 42 d	N/A	5 to 42 d	5 to 42 d	N/A	N/A	N/A	N/A	At 42 d	At 42 d	Tylor and Jones (2004)
Ground wheat		2,606	1.742					0.57^{a}	1.13^{a}	
20% WW replacing GW		2,603	1.739					0.41^{b}	1.18^{b}	

11 to 20 d	11 to 20 d	11 to 20 d	11 to 20 d	N/A	N/A	N/A	13 to 15 d	N/A	N/A	Svihus et al. (2004a)
Ground wheat	745	514	1.45[1]				3274[b]			
37.5% WW in diet	735	503	1.47[1]				3394[a]			
1 to 21 d	1 to 21 d	1 to 21 d	1 to 21 d	N/A	N/A	N/A	At 21 d	N/A	At 21 d	Biggs and Parson (2009)
0% Whole wheat	670	454	1.477[1]				3514[c]		2.07[c]	
5% Whole wheat	666	439	1.515[1]				3594[b]		2.14c	
10% Whole wheat	682	447	1.524[1]				3605[b]		2.27[b]	
15% Whole wheat	675	444	1.522[1]				3673[a]		2.36[ab]	
20% Whole wheat	670	438	1.529[1]				3668[a]		2.42[a]	
1 to 21 d	1 to 21 d	1 to 21 d	1 to 21 d							
0% whole wheat	680	442[a]	1.538[1,a]							
20% whole wheat	637	400[b]	1.592[1,a]							
35% whole wheat	691	436[a]	1.582[1,a]							
50% whole wheat	664	369[c]	1.789[1,b]							

[1]Feed conversion ratio= $\frac{1}{\text{Gain per feed}}$

[2] 1 Megajoule/kg = 239 Kcal/Kg

[3]Carcass yield

[4]Breast meat yield

[5]Tender yield

[6]Apparent metabolizable energy

[7]Relative proventriculus weight

[8]Relative gizzard weight

1.6.3 Whole corn inclusion:

Whole corn inclusion in broiler diet has the potential to reduce grinding cost by utilizing the additional grinding effect of pellet-mill described in Zaefarian et al. (2016). Whole corn inclusion may also have beneficial effects in growth performance, gizzard development and gut health of broiler similar to whole wheat inclusion. Unfortunately, there is not enough research conducted to evaluate this potential or to establish an optimum level of inclusion for whole corn due to its large kernel size, which is often considered too big to be included in broiler diet without previous grinding.

Singh et al. (2014b) used 0, 15, 30, 45 and 50% pre-pelleting whole corn in broiler diets from 1 to 21 d and reported a linear decrease in feed intake, BW, and relative proventriculus weight whereas, pellet hardness and relative gizzard weight linearly increase with increasing concentration of whole corn inclusion. The authors also reported a quadratic effect on pellet durability index and FCR which increased up to 45% whole corn inclusion then levelled out. Apparent metabolizable energy in ileum also showed quadratic effect by increasing up to 30% whole corn inclusion and then declining in diets containing 45 and 60% whole corn. Additionally, the authors also evaluated cecal microbiome of broilers fed diets with 0, 30 and 60% whole corn inclusion and reported a linear increase of *Lactobacillus spp.* and linear decrease of *Clostridium* and *Campylobacterium spp.*at 21 d as the whole corn inclusion increased from 0 to 30 and 60%. Singh and Ravindran (2019) conducted a 3×2 experiment with 3 corn kernel hardness plotted against 2 pre-pelleting whole corn inclusion (0 and 11.5%) in broiler diets from 1 to 21 d. Apart from increased relative gizzard weight, there was no effect on feed intake, BW, FCR, apparent metabolizable energy, starch and nitrogen digestibility in the diet with 11.5% whole corn inclusion compared to diet without whole corn.

Lu et al. (2011) fed Yang-zhou geese with a diet containing 64% whole corn from 8 to 28 d and 61.5% whole corn from 29 to 70 d and reported greater gizzard weight and marginally significant improvement in FCR (P=0.07) between 50 to 70 d in geese fed diet containing whole corn compared to geese feed diets with ground corn. The authors also reported a temporary reduction of feed intake and BW in geese fed diet containing whole corn during 8 to 49 d period however, this limiting effect of whole corn diminished by the end of 50 to 70 d period.

Table 1.11: Effects of whole corn inclusion on growth performance, processing yield, apparent metabolizable energy, and relative weight of digestive organs

Whole Corn (WC)	Feed intake (g)	Body weight (g)	FCR[1] (g:g)	Carc. Y[4]. (%)	Breast Y[5]. (%)	Tend. Y[6]. (%)	AME[7] (Kcal/kg)	Prov. R. (%)	Gizzard R[8]. (%)	Source
1 to 21 d	1 to 21 d	1 to 21 d	1 to 21 d	N/A	N/A	N/A	17 to 21 d	At 21 d	At 21 d	Singh et al. (2014b)
0% WC	1,303^{L}	1,005^{L}	1.304^{Q}				3,401^{q}	0.394^{L}	1.19	
15% WC	1,312^{L}	990^{L}	1.324^{Q}				3,446^{q}	0.433^{L}	1.44	
30% WC	1,214^{L}	919^{L}	1.334^{Q}				3,466^{q}	0.427^{L}	1.50	
45% WC	1,226^{L}	933^{L}	1.341^{Q}				3,424^{q}	0.457^{L}	1.57	
60% WC	1,136^{L}	857^{L}	1.341^{Q}				3,435^{q}	0.470^{L}	1.55	
1 to 21 d	1 to 21 d	1 to 21 d	1 to 21 d	N/A	N/A	N/A	At 21 d	At 21 d	At 21 d	Singh and Ravindran (2019)
0% WC	1286	980	1.319				14.38	0.401	1.44^{b}	
11.5% WC	1277	970	1.320				14.35	0.406	1.54^{a}	
8 to 28 d	8 to 28 d	8 to 28 d	8 to 28 d					At 28 d	At 28 d	Lu et al. (2011)
0% WC	1822^{2,a}	895^{3,a}	2.035^{1}					0.612	5.213^{b}	
64% WC	1751^{2,b}	861^{3,b}	2.034^{1}					0.625	5.728^{a}	
29 to 49 d	29 to 49 d	29 to 49 d	29 to 49 d					At 49 d	At 49 d	
0% WC	5752^{2,a}	2134^{3,a}	3.174^{1}					0.537^{b}	3.428^{b}	
61.5% WC	5480^{2,b}	1974^{3,b}	3.349^{1}					0.744^{a}	4.013^{a}	
50 to 70 d	50 to 70 d	50 to 70 d	50 to 70 d					At 70 d	At 49 d	
0% WC	10202^{2}	2761^{3}	7.027^{1}					0.368	3.626^{b}	
61.5% WC	9765^{2}	2778^{3}	5.344^{1}					0.349	4.335^{a}	

^{L}Linear effect

^{Q}Quadratic effect

[1]Feed conversion ratio = $\frac{1}{\text{Gain per feed}}$

[2]Feed intake = Average daily feed intake × number of days

[3]Body weight = Average daily gain × number of days

[4]Carcass yield

[5]Breast meat yield

[6]Tender yield

[7]Apparent metabolizable energy

[8]Relative proventriculus weight

[9]Relative gizzard weight

1.6.4 Other whole grain inclusion:

Among other grains, sorghum, and barley have been investigated for their potential to be added as whole grain in broiler diets. Taylor and Jones (2004) used 0 and 20% whole barley in combination with 0 or 650 BGU/g β-glucanase in broiler diet from 5 to 42 d of age. The authors reported increased relative gizzard weight and decreased proventriculus weight without any alteration of BW and FCR when broilers were fed diets containing whole barley instead of ground barley. Biggs and Parson (2009) initially used whole sorghum (10 and 20%) and whole barley (10 and 20%) in broiler diets from 1 to 21 d and compared the results with a corn soybean-meal based basal diet without any whole grain inclusion. Feed intake of birds was similar in all diets however, broilers fed diets containing 10 and 20% whole sorghum had significantly lower weight gain and higher FCR than broilers fed basal diets or the diet containing 10 and 20% whole barley. Broiler fed diets containing whole grain resulted higher relative gizzard weight and diet containing 20% whole barley had higher apparent metabolizable energy compared to basal diet. In the follow up study, the authors used whole wheat (20 and 35%), whole barley (10 and 20%) and whole sorghum (10 and 20%) in broiler diets from 1 to 21 d and again compared the results with a basal diet devoid of any whole grain. This time all whole grain containing diets resulted a significantly higher weight gain in broilers from 8 to 21 d period compared to basal diet. Diet containing 10% whole barley had the lowest FCR, whereas the diet with 35% whole wheat had the highest FCR and remaining diets resulted an intermediate FCR. Jacob and Parson (2013) conducted a 3×2 experiment with diets containing either finely ground corn (557μm), coarse corn (1,387μm) and 20% whole sorghum plotted against 0 or 15% DDGS inclusion. The authors reported higher feed intake and poor FCR in broilers fed 20% whole sorghum diet at 21 d, despite it containing the highest apparent metabolizable energy compared to diet formulated from fine or coarse corn. Furthermore, the diet containing coarse corn and whole sorghum found to increase relative gizzard weight of broilers compared to diet with finely ground corn.

1.7 References

Amerah A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007. Influence of feed particle size and feed form on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters. Poult. Sci. 86(12):2615-2623.

Amerah A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007a. Influence of feed particle size and feed form on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters. Poult. Sci. 86(12):2615-2623.

Amerah, A. M., and V. Ravindran. 2008. Influence of method of whole-wheat feeding on the performance, digestive tract development and carcass traits of broiler chickens. Anim. Feed Sci. Technol. 147:326-339.

Amerah, A. M., V. Ravindran, R. Lentle, and D. Thomas. 2007b. Feed particle size: Implications on the digestion and performance of poultry. World's Poult Sci. J. 63:439-455.

American Society for Testing and Materials. 2020. E11-20.

American Society of Agricultural and Biological Engineers standards. 2009. Method of determining and expressing fineness of feed materials by sieving. Am. Soc. Agric. Eng., St Joseph, MI S319.4.

American Society of Agricultural Engineers. 1983. Method of determining and expressing fineness of feed materials by sieving. Standard S319.2. Yearbook of Standards. St. Joseph, MO.

Angulo, E., J. Brufau, and E. Esteve-Garcia. 1996. Effect of a sepiolite product on pellet durability in pig diets differing in particle size and in broiler starter and finisher diets. Anim. Feed Sci. and Technol. 63: 25-34.

Armstrong, P. R., Lingenfelser, J. E., and Mckinney, L. 2007. The Effect of Moisture Content on Determining Corn Hardness from Grinding Time, Grinding Energy, and Near-Infrared Spectroscopy. Appl. Eng. Agric. 23(6):793-799.

Asiedu, M., R. Nilsen, O. Lie, and L. Einar. 1991. Effects of processing (sprouting and or fermentation) on sorghum and maize. I: proximate composition, minerals and fatty acids. Food Chem. 46(4):351-353.

Association of Official Agricultural Chemists. 2012. Official methods of analysis of AOAC International. 19th Edition. AOAC international, Gaithersburg, MD.

Awika, J. M. 2011. Major cereal grains production and use around the world. ACS Symposium series advances in cereal science: implications to food processing and health promotion. 1-13.

Baker, S. and T. Herrman. 2002. Evaluating particle size. MF-2051 Feed Manufacturing, Department of Grain Science and Industry, Kansas State University.

Barzegar, S., S. B. Wu, J. Noblet, and R. A. Swick. 2019. Metabolizable energy of corn, soybean meal and wheat for laying hens. Poult. Sci. 98(11):5876-5882.

Benedetti M. P. J. R. Sartori, F. B. Carvalho, L. A. Pereira, V. B. Fascina, A. C. Stradiotti, A. C. Pezzato, C. Costa, and J. G. Ferreira. 2011.Corn Texture and Particle Size in Broiler Diets. Brazilian J. Poult. Sci. 13(4): 227-234.

Biggs, P., and C. M. Parsons. 2009. The effects of whole grains on nutrient digestibilities, growth performance, and cecal short-chain fatty acid concentrations in young chicks fed ground corn-soybean meal diets. Poult. Sci. 88(9):1893-1905.

Campbell,G. M., P. J. Bunn, C. Webb, and S. C. W. Hook. 2001. On predicting roller milling performance: Part II. The breakage function. Pow. Technol.115(3):243-255.

Chewing, C. G., C. R. Stark, J. T. Brake. 2012. Effects of particle size and feed form on broiler performance. J. Appl. Poult. Res. 21:830-837.

Collins, N. E., E. T. Moran Jr., and H. L. Stilborn. 2001 Influence of yellow dent corn hybrids having different kernel characteristics yet similar nutrient composition on broiler production. Journal of Appl. Poult. Res. 10:228-235.

Cordova, H., E. Oviedo, I. Ospina, A. Fahrenhol, V. San-Martin, M. Chico, Y. Matta-Lozano, O. A. Diaz, and P. L. Sierra. 2020. Effects of corn kernle hardness and grain drying condition on particle size when grinding using a roller or hammer mill. Engormix-Animal feed. https://en.engormix.com/feed-machinery/articles/effects-corn-kernel-hardness-t44624.htm

Cowieson, A., J. O. Sorbara, G. Pappenberger, M. R. Abdollahi, F. F. Roos, and V. Ravindran. 2019. Additivity of apparent and standardized ileal amino acid digestibility of corn and soybean meal in broiler diets. Poult. Sci. 98(9):3722-3728.

Deaton, J. W., B. D. Lott, and J. D. Simmons. 1989. Hammer mill versus roller mill grinding of corn for commercial egg layer. Poult. Sci. 68:1342-1344.

Dei, H. K. 2017. Assessment of maize (Zea mays) as feed resource for Poultry. In poultry science. Manafi, M (Ed). https://www.intechopen.com/books/poultry-science/assessment-of-maize-zea-mays-as-feed-resource-for-poultry

Douglas, J. H., T. W. Sullivan, P. L. Bondf, F. J. Struwe, J. G. Baierl, and L. G. Robeson. 1990. Influence of grinding, rolling, and pelleting on the nutritional value of grain sorghums and yellow corn for broilers. Poult. Sci. 69:2150-2156.

Duvic, D. N. 1961. Protein granules of maize endosperm cells. Cereal Chem. 38:374-385.

Earle, F. R., J. J. Curtis, J. E. Hubbard. 1944. Composition of the components of corn kernel. Cereal Chem. 23(2):504-511.

Egesel, C. O., J. C. Wong, R. J. Lamber, and T. R. Rochford. 2003. Combining ability of maize inbreds for carotenoids and tocopherols. Crop Sci. 43(3):818-823.

Fairchild, F., J. Kice, G. T. Moorhead, L. P. Huff, L. G. McDaniel, M. B. Mac-Clancy, B. P. Ebner, L. Stevens, and B. Moeching. 2005. Bulk material handling and storage. In McEllhiney, R. R. (2005). Feed manufacturing technology 5th Ed. Schofield, K. E., Arlington, VA. American Feed Industry Association. 109-126.

Floyd, H. L. 1994. Harvesting and post harvesting management. In Watson, S. A. and Ramstad. E. P. 1994. Corn chemistry and technology. St. Paul, MN: AACC, American association of cereal chemists, Headquarters. Eagan, Minnesota. 53-79.

Food and agricultural organization of united nations. 1992. Maize in human nutrition. Chief editor, FAO. Rome.

Food and Agriculture Organization of united nations. 1981. Improvement of nutritional quality of food.

Food and agriculture organization of united nations. 2005. Statistical yearbook of FAO. FAO publications.

Forbes, J.M., E. Shariatmadari. 1994. Diet selection for protein by poultry. World's Poult. Sci. J. 50, 7-24.

Forbes, J.M., M. Covasa. 1995. Application of diet selection by poultry with particular reference to whole cereals. World's Poult. Sci. J. 51, 149-165.

Gabriel, I., S. Mallet., M. Leconte, A. Travel, and J. P. Lalles. 2008. Effects of whole wheat feeding on the development of coccidial infection in slow-growing broiler chickens. Archive Fur Geflugelkunde. 71:219-227.

Gehring, C. K., A. j. Cowieson, M. R. Bedford and W. A. Dozier. 2013. Identifying variation in the nutritional value of corn based on chemical kernel characteristics. World's Poult. Sci. J. 69 (2):299-311.

Gentle, M. J. 1979. Sensory control of feed intake. Page 259-273 in Food Intake Regulation in Poultry. K. N. Boorman ed. B. M. Freeman, Edinburg, Britain.

Goodband, R. D., M. D. Tokah, and J. L. Nelssen.1995. The effects of diet particle size on animal performance. MF-2050, Feed manufacturing. Department of grain science, Kansas state university. 1-6.

Grbesa, D and G. Kiš, 2005. Relation between endosperm and in vitro kinetic of starch digestibility in maize hybrids for broiler chickens. Ital. J. Anim. Sci. 4(3):139-141.

Gunasekaranm S., and M. R. Paulsen. 1985. Airflow resistance of mixtures of shelled corn and fines. ASAE 27:1512-1515.

Harper, A. E., B. D. Punekar, and C. A. Elvehjem.1958. Effect of alkali treatment on the availability of niacin and amino acids in maize. J. Nutr. 66(2):163-172.

Hopkins, C. G., L. H. Smith, and E. M. East. 1974. structure of the corn kernel and the composition of its different parts. 3rd ED. Dudley J. W. Madison. crop science society of America. Retrieved from: https://onlinelibrary.wiley.com/doi/abs/10.2135/1974.seventygenerations.c2

Ikram U., A. Mohammed, and F. Arifa. 2010. Chemical and nutritional properties of some maize (Zea mays L.) varieties grown in NWFP, Pakistan. Pak. J. Nutr. 9(11):1113-1117.

International institute of tropical agriculture. 1982. Maize crop: Maize production manual 1(8):1-2.

Islam, Md. S., Md. M. Haque, and Md. S. Hossain. 2015. Effect of Corn Moisture on the Quality of Poultry Feed. J. Poult. Sci. and Technol. 3(2):24-31.

Islam, S., M. Haque, and S. Hossain. 2018. Inspection method of corn for poultry feed. J. Poult. Sci. and Technol. 6(03):47-52.

Jacobs, C. M., P. L. Utterback, and C. M. Parsons. 2010. Effects of corn particle size on growth performance and nutrient utilization in young chicks. Poult. Sci. 89:539-544.

Jacobs, C., C. M. Parsons. 2013. The effects of coarse ground corn, whole sorghum, and a prebiotic on growth performance, nutrient digestibility, and cecal microbial populations in broilers fed diets with and without corn distillers dried grains with solubles. Poult Sci. 92(9): 2347-2357.

Kling I. G. 1991. Quality and Nutrition of Maize; IITA, international institute of tropical agriculture. research Guide 33. Ibadan. 9-21.

Koch, K. 2002. Hammermills and roller mills.MF-2048, Feed manufacturing. Cooperative extension service, Kansas state university. 1-5.

Landry, J. and T. Moureaux, 1980. Distribution and amino acid composition of protein groups located in different histological parts of maize grain. J. Agric. Food Chem. 28(6):1186-1191.

Larbier, M., B. Leclercq. 1994. Nutrition and feeding of poultry. Wiseman, J, and Trans. Ed. Loughborough: Nottingham university press.

Leeson, S., and J. D. Summers. 2009. Commercial Poultry Nutrition. Nottingham: Nottingham University Press.

Li, Q., M. Shi, C. Shi, D. Liu, X. Piao, D. Li, and C. Lai. 2014. Effect of variety and drying method on the nutritive value of corn for growing pigs. J. Anim. Sci. and Biotechnol. 5(1):18-25.

Lott, B. D., E. J. Day, J. W. Deaton, and J. D. May. 1992. The effect of temperature, dietary energy level, and corn particle size on broiler performance. Poult. Sci. 71:618-624.

Lu, J., X. L. Kong, Z. Y. Wang, H. M. Yang, K. N. Zhang, and J. M. Zou. 2011. Influence of whole corn feeding on the performance of digestive tract development and nutrient retention of geese. Poult. Sci. 90:587-594.

Lyu, F., M. Thomas, W. Hendriks, and A. V. D. Poel. 2020. Size reduction in feed technology and methods for determining, expressing and predicting particle size: A review. Animal Feed Sci. and Technol. 261:114347-114366.

MacDonald, P., R. A. Edwards, and J. F. Greenhalgh.1988. Animal Nutrition, 4th Ed. Essex: Longman Group Ltd: 479-481.

Martinez, B. F., P. E. Sevilla, and M. Bjarnason. 1996 Wet milling comparison of quality protein maize and normal maize. J. Sci. Food Agric. 71(2):156-162.

Mavromichalis, I., J. D. Hancock, B. W. Senne, T. L. Gugle, G. A. Kennedy, R. H. Hines, and C. L. Wyatt. 2000. Enzyme supplementation and particle size of wheat in diets for nursery and finishing pigs. J. Anim. Sci. 78(12):3086-3095.

McIntosh, J. L., S. J. Singer, L. R. Sibbald, and G. C. Ashton. 1962. Factors affecting the metabolizable energy content of poultry feeds 7. the effects of grinding, pelleting and grit feeding on the availability of the energy of wheat, corn, oats and barley 8. A study on the effects of dietary balance. Poult. Sci. 41:445-456.

Mingbin, L., L.Yan, Z. Wang, S. An, M. Wu and Z. Lv. 2015. Effects of feed form and feed particle size on growth performance, carcass characteristics and digestive tract development of broilers. Anim. Nutri. 1(3):252-256.

Morrison, W. R. 1977. Cereal lipids. Proceedings of Nutrition Society. 36 (1):143-148.

Muzhingi, T., K. Yeum, M. R. Russell, J. E. Johnson, J. Qin, and G. Tang. 2008. Determination of carotenoids in yellow maize, the effects of saponification and food preparations. Int. J. Vitam. Nutr. Res. 78(3):112-120.

National research council. 1944. Nutrients requirements of poultry, Washington. DC: National academic press.

National research council. 1988. Quality protein maize. Washington: National academy press.

National research council. 2012. Nutrient requirements of swine, 11th rev Ed. Washington, DC: National academic press,

Naylor, J. L. and R. Smith. 1931. Roller Mills vs. Hammermills on Corn. ASAE Paper No. 31-3023.

Neuffer, M. G., L. Jones, and M. S. Zuber. 1968. The mutants of maize. Crop science society of America.Madison, New York. 73-74.

Peron, A., D. Bastianelli, F. X. Oury, J. Gomez, and B. Carre. 2005. Effects of food deprivation and particle size of ground wheat on digestibility of food components in broilers fed on a pelleted diet. Br. Poult. Sci. 46:223-230.

Plavnik, I., B. Macovsky, and D. Sklan. 2002. Effect of feeding whole wheat on performance of broiler chickens. Anim. Feed Sci. and Technol. 96:229-236.

Portella, F. J., L. J. Caston, and S. Leeson. 1988. Apparent feed particle size preference by laying hens. Can. J Anim. Sci. 68:915-922.

Prasanthi, P. S., N. Naveena, M. R. Vishnuvardhana, and K. Bhaskarachar. 2017. Compositional variability of nutrients and phytochemicals in corn after processing. J Food Sci Technol. 54(5):1080-1090.

Probst, K. V., K. R. P. Ambrose, R. L. Pinto, P. Krishnakumar, and K. E. Ileleji. 2013. The effect of moisture content on the grinding performance of corn and corncobs by hammer milling. Transactions of the ASAE. American Society of Agricultural Engineers. 56(3):1025-1033.

Reece, F. N., B. D. Lott, and J. W. Deaton.1986b. The effects of hammer mill screen size on ground corn particle size, pellet durability, and broiler performance. Poult. Sci. 65:1257-1261.

Reece, F.N., B. D. Lott, and J. W. Deaton. 1985. The effects of feed form, grinding method, energy level, and gender on broiler performance in a moderate (21oC) environment. Poult. Sci. 64:1834-1839.

Rose, S.P. 1996. The use of whole wheat in poultry diets. World's Poult. Sci. J. 52, 59-60.

Rubio, A. A., J. B. Hess, W. D. Berry, W. A. Dozier and W. J. Pacheco. 2020. Effects of corn particle size on broiler performance during the starter, grower, and finisher periods. J. Appl. Poult. Res. 29:352-361.

Rydell, J., J. Lissolo, J. Guelden, S. L. Bates, R. Sample, R. Sellers, B. Burisek, Y. A. Tsien, and D. S. Brooks. 2005. Particle size reduction. In McEllhiney, R. R. 2005. Feed manufacturing technology 5th Ed. Schofield, K. E., Arlington, VA. American Feed Industry Association. 109-126.

Rysava, B. 1994. Proteins and Amino Acids in Maize Kernels. In: Bajaj Y.P.S. Ed. Maize. biotechnology in agriculture and forestry, vol 25. Springer, Berlin.

Schiffman, H. R. 1968 Texture preference in the domestic chick. J Comp. Phys. Psych. 66: 540-541.

Singh, Y., A. L. Molan, and V. Ravindran. 2019. Influence of the method of whole wheat inclusion on performance and caecal microbiota profile of broiler chickens. J of Appl. Anim. Nutr. 7:1-7.

Singh, Y., and V. Ravindran. 2019. Influence of feeding whole maize, differing in endosperm hardness, on the performance, nutrient utilisation and digestive tract development of broiler starters. J. Appl. Anim. Nutr. 7(1):1-10.

Singh, Y., V. Ravindran, T. Wester, A. Molan, and G. Ravindran. 2014a. Influence of feeding coarse corn on performance, nutrient utilization, digestive tract measurements, carcass characteristics, and cecal microflora counts of broilers. Poult. Sci. 93:607-616.

Singh, Y., V. Ravindran, T. Wester, A. Molan, and G. Ravindran. 2014b. Influence of prepelleting inclusion of whole corn on performance, nutrient utilization, digestive tract measurements, and cecal microbiota of young broilers. Poult. Sci. 93:3073-3082.

Svihus, B. 2011. The gizzard: Function, influence of diet structure and effects on nutrient availability. World Poult. Sci. J. 67(2):207-224.

Svihus, B., and H. Hetland. 2001. Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. Br. Poult. Sci. 42:633-637.

Svihus, B., E. Juvik, H. Hetland, and A. Krogdahl. 2004a. Causes for improvement in nutritive value of broiler chicken diets with whole wheat instead of ground wheat. Br. Poult. Sci. 45(1):55-60.

Svihus, B., H. Hetland, M. Choct, and F. Sundby. 2002. Passage rate through the anterior digestive tract of broiler chickens fed on diets with ground and whole wheat. Br. Poult. Sci. 43:662-668.

Svihus, B., K. H. Kløvstad, V. Perez, O. Zimonja, S. Sahlström, R. B. Schüller, W. K. Jeksrud and E. Prestløkken. 2004b.Physical and nutritional effects of pelleting of broiler chicken diets made from wheat ground to different coarseness by the use of roller mill and hammer mill. Anim. Feed Sci. and Technol. 117(3):281-293.

Taylor R. D. and G. P. D. Jones. 2004. The incorporation of whole grain into pelleted broiler chicken diets. II. Gastrointestinal and digesta characteristics. Brit Poult. Sci. 45(2):237-246.

Thirumalaisamy, G., J. Muralidharan, S. Senthilkumar, R. H. Sayee, and M. Priyadharsini. 2016. Cost-effective feeding of poultry. Int. J. Environ. Sci. and Technol. 5(6):3997-4005.

Thomas, M., M. Vri, T. Zandstra, A. F. B. Van-der-Poel. 2012. Grinding performance of wheat, maize and soybeans in a multicracker system. Animal Feed Sci. and Technol. 175 (3-4):182-192.

Thomas, M., W. H. Hendriks, and A. F. B. Van-der-Poel. 2018. Size distribution analysis of wheat, maize and soybeans and energy efficiency using different methods for coarse grinding. Animal Feed Sci. and Technol. 240:11-21.

Truong, H. H., A. F. Moss, S. Y. Liu, and P. H. Selle. 2017. Pre- and post-pellet whole grain inclusions enhance feed conversion efficiency, energy utilisation and gut integrity in broiler chickens offered wheat-based diets. Anim. Feed Sci. and Technol. 224:115-123.

United state department of agriculture, National agricultural statistical service. 2019. Crop production summery 2018.

United states department of agriculture. 2020. World agriculture supply and demand estimates. Washington: Economic research services.

Vukmirovic, D., J. Levic, A. Fistes, R. Colovic, T. Brlek, D. Colovic, and O.Djuragic. 2016. Influence of grinding method and grinding intensity of corn on mill energy consumption and pellet quality. Hem. Ind. 70(1):67-72.

Watson, S. A. 1994. Structure and composition. In Watson, S. A. and Ramstad. E. P. 1994. Corn chemistry and technology. St. Paul, American association of cereal chemists, Headquarters. Eagan, Minnesota. 53-79.

Watson. S. A. 2003. Description, development, structure, and composition of corn kernel. Corn chemistry and technology. American association of cereal chemist. 2(1):69-106.

Wet, J. M. J. D., and J. R. Harlan, 1972. Origin of maize: The tripartite hypothesis. Euphytica. 21(2):271-279.

Wondra, K. J., J. D. Hancock, K. C. Behnke, R. H. Hines, and C. R. Stark. 1995. Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. Journal of Animal Science 73:757-763.

Wu, Y. B., V. Ravindran, D. G. Thomas, M.J. Birtles, and W.H. Hendriks.2004. Influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digestive tract measurements and gut morphology of broilers. Brit. Poult. Sci. 45(3): 385-394.

Xie, L., Y. Yu, J. Mao, H. Liu, J. Hu, T. Li, R. Liu. 2017. Evaluation of biosynthesis, accumulation, and antioxidant activity of vitamin E in sweet Corn (Zea mays L.) during kernel development. International journal of molecular sciences, 18(12):2780-2790.

Xu, Y., C. R. Stark, P. R. Ferket, C. M. Williams, and J. T. Brake. 2015b. Effects of feed form and dietary coarse ground corn on broiler live performance, body weight uniformity, relative gizzard weight, excreta nitrogen, and particle size preference behaviors. Poult. Sci. 94:1549-1556.

Xu, Y., C. R. Stark, P. R. Ferket, C. M. Williams, W. J. Pacheco, and J. T. Brake. 2015a. Effect of dietary coarsely ground corn on broiler live performance, gastrointestinal tract development, apparent ileal digestibility of energy and nitrogen, and digesta particle size distribution and retention time. Poult. Sci. 94:53-60.

Zaefarian, F., M. Abdollahi, and V. Ravindran. 2016. Particle size and feed form in broiler diets: Impact on gastrointestinal tract development and gut health. World Poult. Sci. 72: 277-290.

EFFECTS OF PRE-PELLETING WHOLE CORN INCLUSION ON BROILER PERFORMANCE, INTESTINAL MICROBIOTA AND CARCASS CHARACTERISTICS

ABSTRACT

This study was conducted to evaluate the effect of whole corn inclusion before pelleting on performance, processing yield, and microbiome modulation of broilers from 1 to 42 d of age. One thousand male YPM × Ross 708 broilers were randomly distributed among four treatments with 10 replicate pens per treatment and 25 broilers per pen. Treatments consisted of four levels of whole corn (0, 2.5, 5.0, and 7.5%) replacing ground corn. Feed intake and BW were determined at 14, 28, and 42 d and FCR calculated by considering the weight of the mortality. At 42 d, ileal and cecal contents were analyzed to determine intestinal microbiota. At 43 d, 10 birds/pen were processed for yield determination. Data were evaluated using ANOVA test (PROC GLM) and means separated by Tukey HSD test. Inclusion of whole corn did not influence BW and feed intake (P>0.05) to 42 d of age. However, broilers consuming diets with 2.5, 5.0 and 7.5% whole corn had lower FCR from 28 to 42 d of age (1.93, 1.95 and 1.94 vs. 2.00, P<0.05) when compared to broilers fed diets with 0% whole corn. Broilers fed diets with 5% whole corn had higher breast meat yield (29.11 vs. 28.40 %, P<0.05) than broilers fed 7.5% whole corn, but yields were similar to broilers fed diets without whole corn. Diets with 7.5% whole corn showed a trend towards increased *Faecalibacterium* (P=0.07) and decreased *Lactobacillus* (P=0.08) in cecal microbiota compared to 5% whole corn. The results of this experiment indicated that up to 5% whole corn can replace ground corn in broiler diets from 1 to 42 d.

INTRODUCTION

Grinding is one of the most expensive procedure in feed manufacturing (Deaton et al., 1989) after pelleting. However, the majority of ingredients used in poultry diets requires some degree of grinding to improve mixing uniformity, reduce segregation after mixing, improve pellet quality (Koch, 1996) and to increase surface area between digesta and digestive enzymes for better digestion (Goodband et al., 2002). However, excessive grinding increases grinding costs, reduces grinding capacity (Wondra et al., 1995), and hampers gizzard development (Svihus et al., 2002). In fact, chickens have a grinding organ "gizzard" to grind coarse feed particles and increase their surface area to facilitate enzyme binding, nutrient digestion, and absorption (Svihus, 2011). Chickens can also detect particle size differences using mechanoreceptors in their beak (Gentle 1979) and have shown preferences toward coarse particles over finely ground feed particles (Schiffman 1968; Portella et al., 1988). However, coarse particles offered during pre-starter and starter phases can reduce feed intake and BW, which can lead to an increase in FCR by diverting a large portion of dietary energy to gizzard contraction in young broilers (Lott et al., 1992; Singh et al., 2014). Therefore, an optimum balance between grinding equipment and gizzard grinding is necessary for maximum growth performance. In previous trials, coarsely ground corn (Nir et al., 1994; Amerah et al., 2007; Xu et al., 2015;), cracked corn (Dozier et al., 2006; Clark et al., 2009) and whole grain inclusion (Svihus and Hetland, 2001; Amerah and Ravindran 2008; Singh et al., 2014; Singh et al., 2019; Singh and Ravindran 2019) have been used to increase particle size of feed. However, particle size reduction during pelleting and crumbling can limit effectiveness of coarse particles in pelleted feed (Zaefarian et al., 2006).

Among whole grains, whole wheat has been the main whole grain included in poultry diets, as early as 1951 (Ewing, 1951). However, until recently inclusion of whole corn was not considered despite being the principal ingredient in poultry diets in the United States. Kernel size of corn is typically considered too big to be included in poultry diets without grinding. However, including whole corn prior to pelleting can be an alternative to overcome this challenge. Additional grinding during pelleting and crumbling can be utilized to break the corn kernel and force it into pellets. Thus, whole corn inclusion can be an alternative to increase particle size inside pellets and reduce grinding costs by bypassing grinding equipment with the help of the pelleting process. Moreover, structural material inside the pellets can be used to stimulate gizzard development and reverse peristalsis, which can lead to better gut health and improved nutrient utilization (Gabriel et al., 2008). Therefore, this study was conducted to determine effects of pre-pelleting whole corn inclusion in broiler diets from 1 to 42 d of age on a corn and soybean-meal based diet.

MATERIAL AND METHODS

All procedures involving live birds were approved by Auburn University Institutional Animal Care and Use Committee (PRN 2017–3018).

Husbandry Practices

A total of 1000 YPM × Ross 708 male broiler chicks (Aviagen North America, Huntsville, AL) were obtained from a commercial hatchery at 1 d of age. Upon arrival, broilers were weighed and randomly distributed among 40 floor pens (25 birds per pen, 0.12 M^2/chick) in a solid-sided house with negative pressure ventilation system. Housing was equipped with exhaust fans, forced-air heaters, cooling pads, and electronic controllers to manage temperature and ventilation. Each pen was 150 cm in width, 200 cm in length, and 60 cm in height and was equipped with 5 nipple drinkers and 1 pan feeder that granted birds *ad-libitum* access to feed and water throughout the experimental period. Photoperiod was set at 23L:1D from 1 to 7 d, 21L:3D from 8 to 20 d and 16L:8D from 21 to 42 d. Environmental temperature was 34°C at placement, 31.3°C from 2 to 5 d, 29.4°C from 6 to 14 d, and 28.3°C from 15 to 23 d, 26.7ºC from 24 to 28 d and 23.8ºC from 29 to 42 d. Birds were inspected twice daily and room temperature, bird condition, mortality, and availability of feed and water were checked during each inspection. Feed intake, BW and FCR were determined at 14, 28, and 42 d during the experiment.

Feed Formulation, Manufacture, and Experimental Design

Starter, grower, and finisher diets were formulated to meet or exceed NRC suggested minimum nutrient requirement of broilers (NRC, 1994) (Table 1). Each pen was randomly assigned to 1 of 4 treatments (0.0, 2.5, 5.0, or 7.5%) with 10 replicate pens per treatment. Treatments had identical ingredient and nutrient composition apart from level of whole corn inclusion. Starter feed was provided in crumbled form, while grower and finisher feeds were provided as whole pellets of 4.0 mm diameter.

Corn was ground using a hammer mill (Model 11.5 × 38, Roskamp Champion, Waterloo, IA) equipped with 3.175 mm screens. Ground corn, whole corn, soybean meal, and additional dietary ingredients were mixed for 150 seconds (30 second dry and 120 second wet) in a twin shaft ribbon mixer (Model 726, Scott Equipment Co., New Prague, MN) to produce mash feeds, which were conditioned at 82°C for 45 seconds before forcing them through a ring die of 4.0 mm diameter using a pellet mill (Model 1112–4, California Pellet Mill Co., Crawfordsville, IN). Pellets were dried and cooled in a counterflow pellet cooler (Model CC0909, California Pellet Mill Co., Crawfordsville, IN) using ambient air. Starter feed was crumbled in a crumbler (Model 624SS, California Pellet Mill Co., Crawfordsville, IN) with a manual roll adjustment.

Initially, two feed samples of 500 g collected from each treatment were homogenized before nutrient analysis. Duplicate samples of 250 mg dried feed from each treatment were placed into aluminum foil and analyzed for crude protein (N x 6.25) by combustion method (AOAC 990.03) using Leco equipment (Leco Corporation, St. Joseph, MI).

***Measurement**s*

Broilers and feed were weighed at the beginning of experiments and at 14, 28, and 42 d to determine BW, feed intake, and FCR. Birds were observed twice a day, mortalities were removed daily, and their BW was considered to adjust FCR. On d 43, 10 birds from each pen were randomly selected and processed to determine carcass and parts yield. Feed was removed 10 h prior to processing but birds had access to drinking water during the feed removal period. On processing day selected birds were loaded into coops and transferred to Auburn University Pilot Processing Plant. Broilers were electrically stunned, exsanguinated, scalded, picked and eviscerated mechanically and placed on ice. Carcasses were static chilled for 4 h in ice water (4°C) and then excess water was drained for around 5 minutes before chilled carcass weights were determined. Chilled carcasses were deboned the following day by experience personnel utilizing stationary cones. During deboning, breast fillets (*Pectoralis major*) and tenders (*Pectoralis minor*) were weighted to determine parts yield. Carcass yield was calculated in relation to live BW of birds, whereas parts yield was calculated as a percentage of chilled carcass weight.

Cooking loss of breast meat fillets was measured according to methodology described by Lee et al. (2009). From each treatment 72 breast meat fillets were randomly collected and stored at 4°C for 4 d, followed by roasting at 200°C for 30 min in an air convection oven. Initial and final weight of breast fillets were measured and the difference was used to determine percentage of cooking loss.

$$\text{Cooking Loss} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100\%$$

Color of meat analyzed according to the methodology of Küçüközet and Uslu (2018) using CR-400 Minolta Chromameter, after a 48 h of water chilling following collection. Prior to any measurement, the chromameter was calibrated using a standard white plate. Data were collected using CIE Lab parameters, lightness (L*), redness (A*>0), greenness (A*<0), yellowness (B*>0), and blueness (B*<0) of cooked chicken meats were measured at 3 different spots of each fillet.

Intestinal Microbiota

Sample collection and analysis performed:

On d 42, two birds per pen were randomly selected, euthanized by cervical dislocation, and the entire gastrointestinal tract collected into a ziploc bag, immediately placed in ice, and taken to the laboratory. Ileal and cecal contents were gently squeezed and frozen at -20^{o}C for further analysis of ileal and cecal microbiota.

Analysis of ileal and cecal microbiota:

Sample preparation was done in accordance to Bortoluzzi et al. (2017) with a slight modification when using ileal digesta. Cecal content and ileal digesta were diluted in a 1:10 sterile phosphate buffer solution (PBS); 5 mL of mixed solution (ileal) and 1mL of mixed solution (cecal) were transferred to an adequate size tube, centrifuged for 3 minutes at 3,270 g, the supernatant discarded, and 200 µg of content used for DNA isolation.

DNA isolation of ileal and cecal digesta, and polymerase chain reaction (PCR) amplification and sequencing were done in accordance to Bortoluzzi et al. (2017). Sequencing of 16S rRNA was performed using the Illumina Miseq platform. Bioinformatic analysis was done according to Bortoluzzi et al. (2018). Briefly, sequences were paired-end and quality trimmed using Geneious (Newark, NJ). Operational taxonomic units (OTUs) were assigned at a 97% identity using SILVA database. Alpha (Chao 1, Observed species, Phylogenetic diversity (PD) of the Whole Tree, and Shannon indeces) and beta diversity indices were calculated using QIIME v1.9.1. Principal Coordinates Analysis (PCoA) was used to visualize the data.

Statistical Analysis

The experiment was conducted in a randomized block design where pen location was considered as blocking factor. Each treatment had 10 replicate pens and each individual pen was considered the experimental unit. Mortality data were subjected to arcsine transformation before analysis. Data were analyzed using one-way ANOVA in GLM procedure of JMP software under following model.

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

Where, Y_{ij} = observed response in birds of each pen; μ = is the overall mean; T_i = fixed effect of feed form treatment; and ε_{ij} = residual error.

Mean values among the 4 dietary treatments were compared using Tukey's honestly significant difference procedure and level of significance was considered at $P \leq 0.05$ unless otherwise indicated. For microbiome analysis, frequency of main bacterial groups observed in ileum and ceca was submitted to a non-parametric one-way ANOVA (Kruskal-Wallis test) and in case of significant difference, means were separated by Dunn's test.

RESULTS AND DISCUSSION

Growth performance data are presented in Table 2. Inclusion of whole corn did not influence BW gain, feed intake, and FCR among the treatments ($P > 0.05$) at 14, 28 or 42 d of age. However, there was a trend towards lower FCR ($P = 0.07$) in broilers fed diets with 2.5, 5.0, and 7.5% whole corn compared to broilers fed diets without whole corn at 42 d of age. In addition, broilers fed diets with 2.5, 5.0, and 7.5% whole corn had lower FCR ($P < 0.05$) during the finisher period from 28 to 42 d of age, compared to broilers fed diets without whole corn. Similar to this experiment, Wu et al. (2004) reported lower FCR and higher apparent metabolizable energy in broilers fed 20% pre-pelleting whole wheat inclusion in a wheat-based diet from 1 to 21 d of age. Singh and Ravindran (2019) reported the inclusion of 11.5% whole corn in broiler diets from 1 to 21 d had no influence on BW, feed intake, and overall FCR. However, in an earlier study, Singh et al. (2014) reported a linear decrease of feed intake and BW as the concentration of whole corn was increased (0, 15, 30, 45 and 60%) in broiler diets provided from 1 to 21 d as 3-mm pellets. The discrepancies observed when whole corn or wheat has been added in broiler diets is likely influenced by inclusion rate of the whole cereal grains, age of birds during the evaluation period and even the diameter of pellets fed, which can influence internal particle size of pellets.

Whole corn inclusion did not influence chilled carcass weight, carcass yield, and tender yield (Table 3). However, broilers fed diets with 5% of whole corn had higher breast meat yield (29.2 vs. 28.4%, $P < 0.05$) than broilers fed 7.5%, but similar to broilers fed diets without whole corn. Similar to this experiment, Wu and Ravindran (2004) used 10 and 20% pre-pelleting whole wheat in broilers from 1 to 35 d without any effect on carcass weight and carcass yield compared to diet without whole wheat. Plavnik et al. (2002) reported 6% reduction of breast meat yield with inclusion of 25% whole wheat in corn-wheat-SBM based diet. Amerah and Ravindran (2008) reported 4% lower carcass yield along with significant reduction in breast meat yield when broilers were fed 20% whole wheat from 22 to 35 d of age. According to Parsons et al. (2006), the reduction in breast meat can be a resulted from a higher amount of energy diverted to gizzard development and maintenance instead of breast development. However, inclusion of whole grains might also increase variation in particle size possibly causing nutrient segregation (Silva 1997) in pelleted diets leading to reduction in breast meat weight and yield.

Cooking loss and CIE lab color score of cooked breast meat is presented in Table 4. There were no significant differences in cooking loss and coloration of breast meat among treatments which implies that whole corn inclusion does not affect water holding capacity and visual appearance of meat. Previous research with whole grain inclusion only evaluated quantitative influence on breast meat yield and possibly it is the first time that effects of whole corn inclusion on breast meat quality was evaluated. Water holding capacity of meat is influenced by the presence of polar amino acids in actin-myosin protein of meat fiber (Zayas 1997), but diets used in this experiment were identical for all treatments as shown in Table 1. A wide range of post-processing factors like carcass aging (Karaoğlu et al., 2006), chilling method (Wang et al., 2000) and scalding temperature (Silva-Buzanello et al., 2019) can influence water holding capacity and meat coloration. These factors were cautiously kept constant while processing broilers of all treatments.

Inclusion of whole corn did not influence diversity of intestinal microbiota as there were no differences among experimental groups for alpha and beta diversity indices (Figure 1). Ileal microbiota was dominated by phylum *Firmicutes* (over 99%). Predominate genera found in ileal microbiota was *Lactobacillus* (84%), followed by *Streptococcus* (5.4%), and *Enterococcus* (4.7%) with no differences among treatments (P > 0.05; Figure 2A). Cecal microbiota was dominated by phylum *Firmicutes* (89%) consisting mainly of *Clostridiales* (19.3%), followed by *Ruminococcus* (12.3%), *Ruminococcaceae* (11.8%), and *Lachnospiraceae* (7%), with no differences among treatments (P > 0.05; Figure 2B). Cecal microbiota had a higher microbial diversity and showed a greater response with whole corn inclusion. Feeding 7.5% of whole corn, tended to increase *Faecalibacterium* (P = 0.07), and decrease *Lactobacillus* (P = 0.08) in cecal microbiota, when compared to 5% of whole corn inclusion. In a previous trial, Singh et al. (2014) reported a linear increase in *Lactobacillus* population with higher levels of pre-pelleting whole corn inclusion (0, 15, 30, 45 and 60%) in broiler diets at 21 d of age. Gabriel et al. (2003b) also reported more beneficial microbiota and lower count of coliform bacteria at 22 d of age in broilers fed whole wheat-based diets compared to broilers fed a ground wheat-based diet. Differences observed are possibly due to limited concentration of whole corn inclusion and differences in sampling age (21 d vs 42 d). Likely at 42 d of age, when samples were collected, ileal and cecal microbiota were already established after shifting from brooding to grow out composition (Ngunjiri et al., 2019). Whole grain inclusion is believed to promote changes in intestinal microbiota by decreasing gizzard pH through stimulation of gastric hydrochloric acid secretion and by altering transit time of digesta throughout the gastro-intestinal tract (Singh et al., 2014). Gabriel et al. (2003a) reported a significant reduction in gizzard pH when birds were fed a diet with 20% whole wheat instead of ground wheat. Santos et al. (2008) reported significant decrease in gizzard pH and cecal *Salmonella* population at 42 d of age in broilers fed diet with an average feed particle size of 1,700 μm compared to broilers fed diet with an average particle size of 560 μm. Based on performance data observed in this study, as much as 5% whole corn can be used to replace ground corn prior to pelleting in broiler diets between 1 to 42 d of age.

REFERENCES

Amerah, A. M., and V. Ravindran. 2008. Influence of method of whole-wheat feeding on the performance, digestive tract development and carcass traits of broiler chickens. Anim. Feed Sci. Technol. 147:326-339.

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007. Feed particle size: implications on the digestion and performance of poultry. World's Poult. Sci. J. 63: 439-455.

Association of Official Analytical Chemists. 2006. Official Methods of Analysis of AOAC International. 18th ed.

Bortoluzzi, C., A. A. Pedroso, J. J. Mallo, M. Puyalto, W. K. Kim, and T. J. Applegate. 2017. Sodium butyrate improved performance while modulating the cecal microbiota and regulating the expression of intestinal immune-related genes of broiler chickens. Poult. Sci. 96:3981-3993.

Bortoluzzi C., M. J. Rothrock, B. S. Vieira, J. J. Mallo, M. Puyalto, C. Hofacre and T. J. Applegate. 2018. Supplementation of protected sodium butyrate alone or in combination with essential oils modulated the cecal microbiota of broiler chickens challenged with coccidia and Clostridium perfringens. Front. Sustain. Food Syst. 2-72.

Clark, P. M., K. C. Behnke, and A. C. Fahrenholz. 2009. Effects of feeding cracked corn and concentrate protein pellets on broiler growth performance. J. Appl. Poult. Res. 18:259-268.

Deaton, J. W., B. D. Lott and J. D. Simmons. 1989. Hammer mill versus roller mill grinding of corn for commercial egg layer. Poult. Sci. 68:1342-1344.

Dozier, W. A., K. Behnke, M. T. Kidd, and S. L. Branton. 2006. Effects of the addition of roller mill ground corn to pelleted feed on pelleting parameters, broiler performance and intestinal strength. Journal of J. Appl. Poult. Res. 15:236-244.

Ewing, W. R. 1951. Poultry Nutrition. 4th ed. South Pasadena, CA, USA. Page: 106-127.

Gabriel, I., S. Mallet., M. Leconte, A. Travel, and J. P. Lalles. 2008. Effects of whole wheat feeding on the development of coccidial infection in slow-growing broiler chickens. Archive Fur Geflugelkunde. 71:219-227.

Gabriel, I., S. Mallet., M. Leconte. 2003a. Differences in the digestive tract characteristics of broiler chickens fed on complete pelleted diet or on whole wheat added to pelleted protein concentrate. Br. Poult. Sci. 44:283-290.

Gabriel, I., S. Mallet., M. Leconte, G. Fort, and M. Naciri. 2003b. Effects of whole wheat feeding on the development of coccidial infection in broiler chickens. Poult. Sci. 82:1668-1676.

Gentle, M. J. 1979. Sensory control of feed intake. Page 259-273 in Food Intake Regulation in Poultry. K. N. Boorman ed. B. M. Freeman, Edinburg, Britain.

Goodband, R. D., M. D. Tokach, and J. L. Nelssen. 2002. The effects of diet particle size on animal performance. Manhattan, Kansas: Cooperative Extension Service, Kansas State University.

Koch, K. 1996. Hammermills and rollermills. MF-2048 Feed Manufacturing, Department of Grain Science and Industry, Kansas State University.

Karaoğlu, M., M. İ. Aksu, N. Esenbuga, M. Macit and H. Durdağ. 2006. pH and colour characteristics of carcasses of broilers fed with dietary probiotics and slaughtered at different ages. Asian Australasian J. Anim. Sci.19:605-609.

Küçüközet, A. O., and M. K. Uslu. 2018. Cooking loss, tenderness, and sensory evaluation of chicken meat roasted after wrapping with edible films. Food Sci. Technol. Intl. 24:576-584.

Lee, Y., C. Owens, and J. Meullenet. 2009. changes in tenderness, color, and water holding capacity of broiler breast meat during postdeboning aging. J. Food Sci.74:449-454.

Lott, B. D., E. J. Day, J. W. Deaton, and J. D. May. 1992. The effect of temperature, dietary energy level, and corn particle size on broiler performance. Poultry Science 71:618-624.

Ngunjiri, M. j., J. M. Taylor, M. C. Abundo, H. Jang, M. Elaish, K. C. Mahesh, A. Ghorbani, S. Wijeratne, B. P. Weber, T. J. Johnson, and C. W. Leea. 2019. Farm stage, bird age, and body site dominantly affect the quantity, taxonomic composition, and dynamics of respiratory and gut microbiota of commercial layer chickens. Microbial Ecology.85: e03137.

Nir, I., G. Shefet, and Y. Aaroni. 1994. Effect of particle size on performance. 1. Corn. Poult. Sci. 73:45-49.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th.rev. ed. Natl. Acad. Press, Washington, DC.

Parsons, A. S., N. P. Buchanan, K. P. Blemings, M. E. Wilson, J. S. Moritz. 2006. Effect of corn particle size and pellet texture on broiler performance in the growing phase. J. Appl. Poult. Res. 15:245-255.

Plavnik, I., B. Macovsky, and D. Sklan. 2002. Effect of feeding whole wheat on performance of broiler chickens. Anim. Feed Sci. Technol. 96:229-236.

Portella, F. J., L. J. Caston, and S. Leeson. 1988. Apparent feed particle size preference by laying hens. Canadian Journal of Animal Science 68:915-922.

Santos, F. B. O., B. W. Sheldon, A. A. Santos, and P. R. Ferket. 2008. Influence of housing system, grain type, and particle size on Salmonella colonization and shedding of broilers fed triticale or corn-soybean meal diets. Poult. Sci. 87:405-420.

Schiffman, H. R. 1968 Texture preference in the domestic chick. J Comp. Phys. Psych. 66: 540-541.

Silva. G. A. 1997. The effect of the physical properties of particles of bulk-blended fertilizers on segregation by coning. Braz. j. Chem. Eng. 14:00-05.

Silva-Buzanello R. A., A. F. Schuch, A. W. Gasparin, A. S. Torquato, F. R. Scremin, C. Canan, and A. L. Soares. 2019. Quality parameters of chicken breast meat affected by carcass scalding conditions. Asian-Australasian J. Anim. Sci. 32:1186-1194.

Singh, Y., V. Ravindran, T. Wester, A. Molan, and G. Ravindran. 2014. Influence of prepelleting inclusion of whole corn on performance, nutrient utilization, digestive tract measurements, and cecal microbiota of young broilers. Poult. Sci. 93:3073-3082.

Singh, Y., A. L. Molan, and V. Ravindran. 2019. Influence of the method of whole wheat inclusion on performance and caecal microbiota profile of broiler chickens. Journal of Applied Animal Nutrition, 7:1-7.

Singh, Y., and V. Ravindran. 2019. Influence of feeding whole maize, differing in endosperm hardness, on the performance, nutrient utilisation and digestive tract development of broiler starters. J. Appl. Anim. Nutrition. 7:27-37.

Küçüközet, A. O., and M. K. Uslu. 2018. Cooking loss, tenderness, and sensory evaluation of chicken meat roasted after wrapping with edible films. Food Sci. and Technol. Intl. 24:576-584.

Svihus, B. 2011. The gizzard: function, influence of diet structure and effects on nutrient availability. World's Poult. Sci. J. 67:207-224.

Svihus, B., and H. Hetland, 2001. Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. Br. Poult. Sci. 42:633-637.

Svihus, B., H. Hetland, M. Choct, and F. Sundby. 2002. Passage rate through the anterior digestive tract of broiler chickens fed on diets with ground and whole wheat. Br. Poult. Sci. 43:662-668.

Wang, H., X. Qin, X. Li, X. Wang, Y. Lei, and C. Zhang. 2020. Effect of chilling methods on the surface color and water retention of yellow-feathered chickens. Poult. Sci. 99:2246-2255.

Wondra, K. J., J. D. Hancock, K. C. Behnke, R. H. Hines, and C. R. Stark. 1995. Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. Journal of Animal Science 73:757-763.

Wu, Y. B., V. Ravindran, D. G. Thomas, M. J. Birtles, and W. H. Hendriks. 2004. Influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digestive tract measurements and gut morphology of broilers. Br. Poult. Sci. 45:385-394.

Wu, Y. B., and V. Ravindran. 2004. Influence of whole wheat inclusion and xylanase supplementation on the performance, digestive tract measurements and carcass characteristics of broiler chickens. Anim. Feed Sci. Technol. 116:129-139.

Xu, Y., C. R. Stark, P. R. Ferket, C. M. Williams, W. J. Pacheco, and J. T. Brake. 2015. Effect of dietary coarsely ground corn on broiler live performance, gastrointestinal tract development, apparent ileal digestibility of energy and nitrogen, and digesta particle size distribution and retention time. Poult. Sci. 94:53-60.

Zaefarian, F., M. Abdollahi, and V. Ravindran. 2016. Particle size and feed form in broiler diets: Impact on gastrointestinal tract development and gut health. World Poult. Sci. 72:277-290.

Zayas J. F. 1997. Water holding capacity of proteins. In: Functionality of Proteins in Food. Springer, Berlin, Heidelberg

TABLES

Table 3.1: Ingredient and nutrient composition of dietary treatments fed to YPM × Ross 708 male broilers from 1 to 42 d of age.

Ingredient, % "as-fed"	Starter	Grower	Finisher
Corn	51.96	58.83	61.88
Soybean Meal, 46 % Crude Protein	35.97	27.56	23.14
Distillers dried grains with solubles (DDGS)	5.00	7.00	9.00
Poultry Oil	3.40	3.33	3.45
Dicalcium phosphate, 18% P	1.27	0.97	0.48
Calcium carbonate	1.24	1.16	1.02
Sodium chloride	0.37	0.37	0.28
D-L Methionine	0.28	0.25	0.20
L-Lysine	0.12	0.17	0.18
Trace mineral premix[1]	0.10	0.10	0.10
Vitamin premix[2]	0.10	0.10	0.10
Choline Chloride	0.08	0.07	0.08
L-Threonine	0.04	0.06	0.06
Copper chloride[3]	0.02	0.02	0.02
Quantum phytase[4]	0.008	0.008	0.008
	100.00	100.00	100.00
Calculated analysis, % (unless otherwise noted)			
AMEn, kcal/kg	3,025	3,117	3,175
Crude Protein[5]	23.24	20.45	19.01
Digestible Lys	1.18	1.02	0.95
Digestible Thr	0.77	0.68	0.63
Digestible Trp	0.23	0.19	0.18
Digestible TSAA[6]	0.91	0.78	0.72
Calcium	1.00	0.88	0.76
Available phosphorus	0.48	0.42	0.38

[1]Mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediamine dihydriodide), 1.4 mg; and Se (sodium selenite), 0.3 mg.

[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,7390 IU; Vitamin D (cholecalciferol),6,614IU;Vitamin-E(DL-alpha tocopherol acetate),6 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.6 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22 mg; niacin (niacinamide), 88 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[3]Intellibond® C (Micronutrients, Indianapolis, IN).

[4]Quantum® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[5]Analyzed crude protein content in starter, grower and finisher diets were 21.11 and 22.32, 19.17 and 20.31, and 17.63 and 18.74%, respectively (AOAC-2006)

[6]TSAA = Total sulfur amino acids.

Table 3.2: Growth performance of YPM × Ross 708 male broiler fed with 4 different levels of whole corn inclusion from 1 to 42 d.

Item[1] Whole corn, %	**BW[2] (gm/bird)**				**FI[3] (gm/bird)**			**FCR[4] (gm/gm)**			**PFCR[5] (gm/gm)**		
	---Days of Age---												
	0	**14**	**28**	**42**	**14**	**28**	**42**	**1-14**	**1-28**	**1-42**	**0-13**	**14-27**	**28-42**
0.0	39.04	462	1,660	3,312	525	2,250	5,524	1.24	1.39	1.70	1.24	1.44	2.01[A]
2.5	38.90	466	1,649	3,346	525	2,254	5,528	1.23	1.40	1.67	1.23	1.46	1.93[B]
5.0	39.00	460	1,642	3,307	522	2,227	5,466	1.24	1.39	1.67	1.24	1.45	1.95[B]
7.5	38.72	459	1,636	3,299	522	2,218	5,434	1.24	1.39	1.66	1.24	1.44	1.94[B]
SEM[6]	0.25	4	15	21	3	16	39	0.008	0.007	0.009	0.008	0.008	0.015
P value	0.80	0.71	0.75	0.43	0.88	0.32	0.29	0.61	0.57	0.07	0.61	0.29	0.006

[1]Dietary treatments consisted of 0, 2.5, 5.0, and 7.5% whole corn inclusion. Diet was provided in crumble form from 0 to 14 d, before shifting to pellets with 4 mm in diameter.
[a,b] Means column with different superscript differ significantly (P<0.05)
[2]BW= Body weight
[3]FI= Average feed intake
[4]FCR= Feed conversion ratio
[5]PFCR= Period feed conversion ratio
[6]SEM= Standard Error of Mean for 10 replicates. (n=10)

Table 3.3: Carcass and processing yield of YPM × Ross 708 male broiler fed with 4 different levels of whole corn inclusion.

Item1 Whole Corn, %	**Live Weight[2]** **(gm)**	**Carcass Weight[3]** **(gm)**	**Carcass Yield[4]** **(%)**	**Breast Meat Yield[5]** **(%)**	**Tender Yield[6]** **(%)**
0.0	3,311	2,541	76.74	29.11[ab]	5.72
2.5	3,388	2,593	76.53	29.03[ab]	5.71
5.0	3,367	2,583	76.71	29.24[a]	5.63
7.5	3,378	2,580	76.34	28.40[b]	5.60
[7]SEM	27	22	1.89	0.23	0.09
P value	0.14	0.29	0.37	0.04	0.76

[1]Dietary treatments consisted of 0, 2.5, 5.0, and 7.5% whole corn inclusion. Diet was provided in crumble form from 0 to 14 d, before shifting to pellets with 4 mm in diameter.
[a,b] Means column with different superscript differ significantly ($P<0.05$)
[2]Finished weight= Weight of live bird just before processing, measured in gram.
[3]Carcass weight= Wight of processed carcass after 4 hrs chilling in slush cold water, measured in gram.
[4]Carcass yield= Ratio of finished chilled carcass weight and finished weight expressed as percent.
[5]Breast meat yield= Weight of breast meat/Pectoral major is divided by chilled carcass weight and expressed as percent.
[6]Tender yield= Weight of tender/pectoral minor is divided by chilled carcass weight and expressed as percent.
[7]SEM= Standard Error of Mean of 10 replicates (n=10).

Table 3.4: Cooking loss of breast fillets of YPM × Ross 708 male broiler fed with 4 different levels of whole corn inclusion.

Item[1] Whole corn, %	**Initial Weight[2]** (gm)	**Final Weight[3]** (gm)	**Cooking Loss[4]** (%)	**Breast meat color***		
				L*[5]	**A*[6]**	**B*[7]**
0.0	376	255	31.97	59.97	4.96	8.38
2.5	380	261	31.55	60.29	5.02	9.47
5.0	379	260	31.26	59.89	4.94	9.13
7.5	374	261	30.05	59.43	5.09	8.96
SEM[8]	43.54	38.59	0.07	2.76	2.06	2.32
P value	0.84	0.37	0.11	0.15	0.78	0.25

[1]Dietary treatments consisted of 0, 2.5, 5.0, and 7.5% whole corn inclusion. Diet was provided in crumble form from 1 to 14 d, before shifting to pellet form with 4mm diameters.
[2]Initial Weight= The weight of breast fillet after deboning.
[3]Final Weight= The weight of breast fillet after cooking.
[4]Cooking loss%= Portion weight that a breast fillet loss during cooking, is expressed as a percentage of initial weight. The difference between initial and final weight is divided by initial weight and multiplied by 100 to get the cooking loss%.
Breast meat color* was evaluated after a 48 hour of water chilling following collection.
[5]L*= Measure of Lightness of the cooked meat.
[6]A*= Measure of redness (A*>0) of the cooked meat.
[7]B*= Measure of yellowness (B*>0) of the cooked meat.
[8]SEM= Standard Error of Mean for 10 replicates. (n=72)

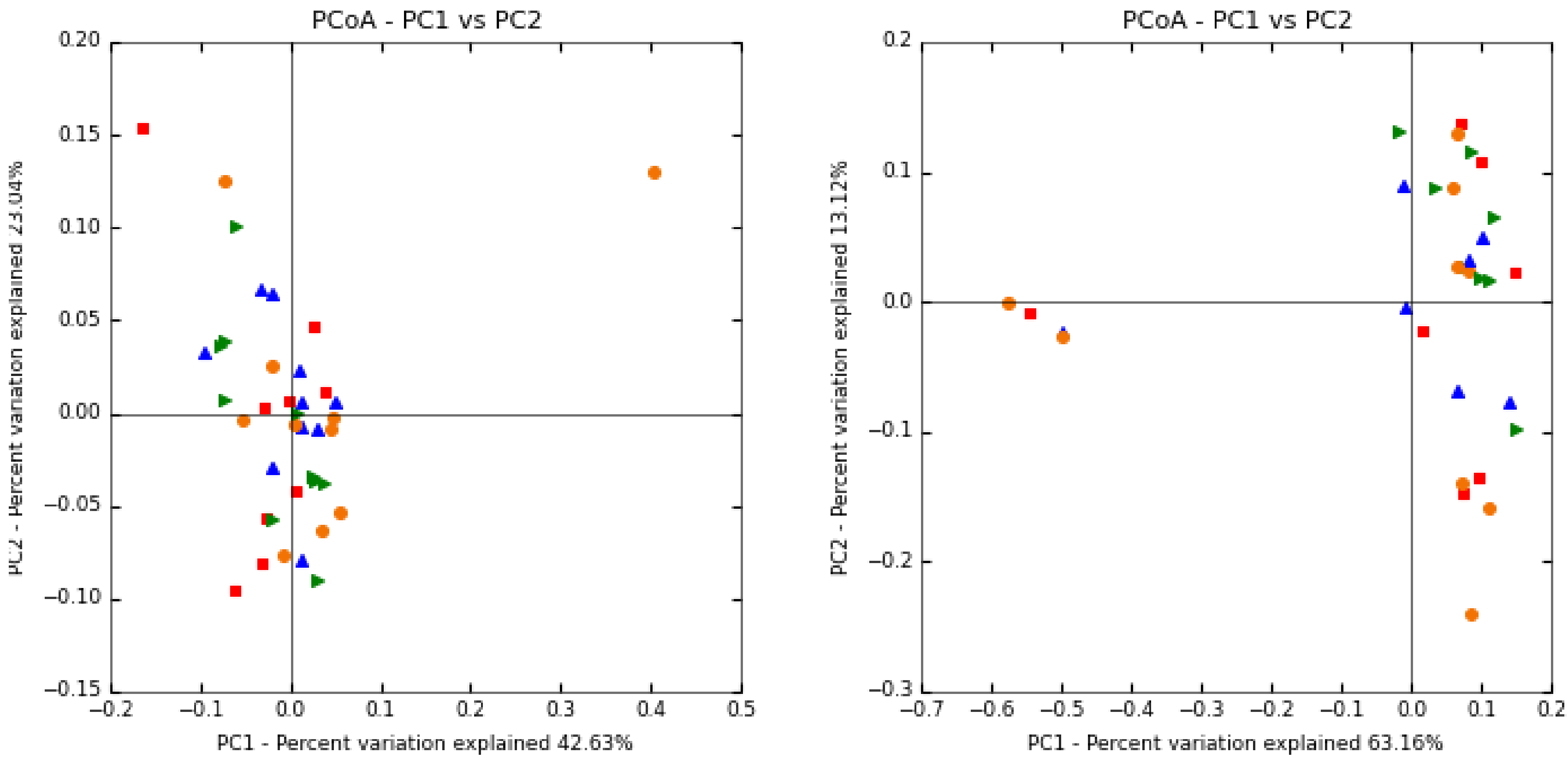
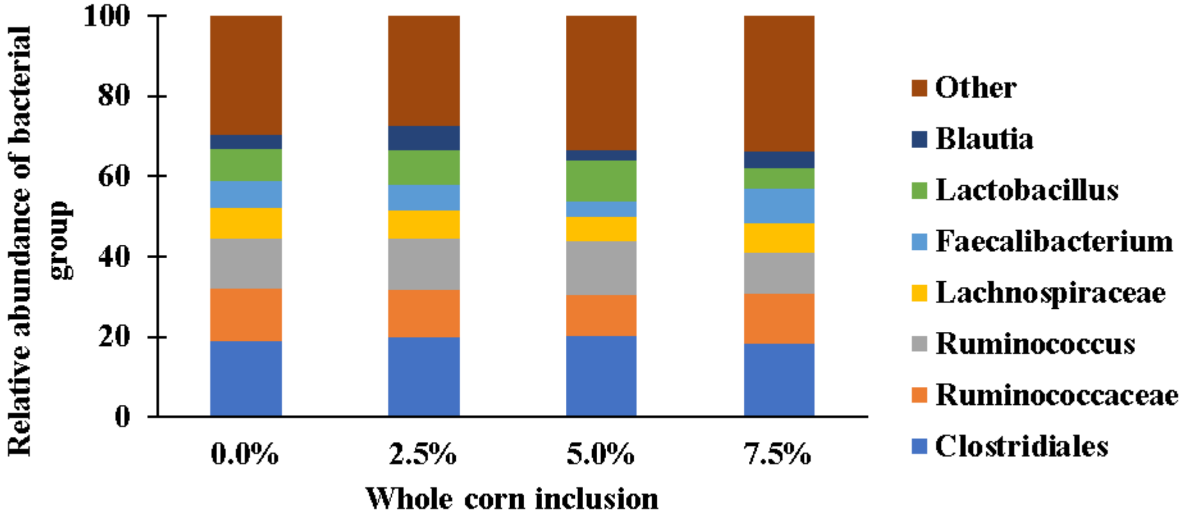
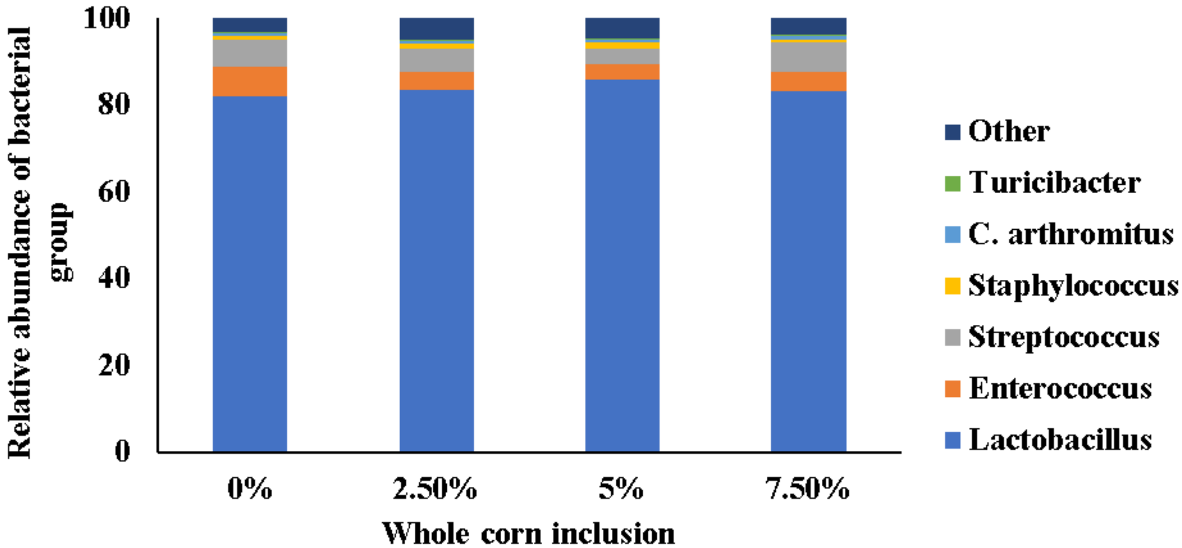


Figure 3.1: UniFrac PCoA plots of the ileal (A) and cecal (B) microbiota of broiler chickens at 42 d according to the treatments: Red: 0% WC; Blue: 2.5% WC; Orange: 5% WC; Green: 7.5% WC. No differences visible among experimental groups for alpha and beta diversity indices.



(A)



(B)

Figure 3.2: Relative abundance (%) of main bacterial groups present in ileal (A) and cecal (B) microbiota of broilers at 42 d according to each experimental treatment. Feeding 7.5% WC inclusion tended to increase *Faecalibacterium* (P = 0.07) and decrease *Lactobacillus* (P = 0.08) in cecal microbiota compared to 5% WC inclusion.

EFFECTS OF PRE-PELLETING WHOLE CORN INCLUSION ON FEED PARTICLE SIZE, PELLET QUALITY, GROWTH PERFORMANCE, CARCASS YIELD, AND DIGESTIVE ORGAN DEVELOPMENT AND INTESTINAL MICROBIOME OF BROILERS BETWEEN 14 TO 42 DAY OF AGE.

ABSTRACT

This study was conducted to evaluate the effects of pre-pelleting whole corn inclusion on body weight (BW), feed intake, feed conversion ratio (FCR), processing yield, organ development, and gut microbiome of broilers from 14 to 42 d of age. One-thousand male YPM × Ross 708 broilers were randomly distributed among four treatments with 10 replicate pens per treatment and 25 broilers per pen (0.12 m^2/chick). A common starter was fed from 1 to 14 d of age. At 14 d, four experimental treatments containing 0, 3, 6 and 9% whole corn pre-pelleting were provided. Feed consumption and BW were determined at 14, 28, and 42 d of age and FCR was calculated including the weights of the mortality. On d 28, two birds per pen were selected to evaluate the ileal and cecal microbiota. At 43 d, 10 birds/pen were processed for yield determination. Data were statistically evaluated by ANOVA test using GLM procedure of SAS and means were separated by Tukey HSD test. Inclusion of whole corn did not influence growth performance from 14 to 42 d of age ($P > 0.05$), but 9% whole corn increased carcass yield ($P < 0.05$). The inclusion of whole corn did not influence carcass traits ($P > 0.05$). Inclusion of 9% whole corn reduced the relative proventriculus weight ($P < 0.05$). *Romboutsia* spp. and ileal anaerobes increased when the level of WC exceeded 6% ($P < 0.05$). These data demonstrate that as much as 9% WC can be incorporated in broiler diets prior to pelleting from 14 to 42 d without negative effects on broiler performance and processing yield.

INTRODUCTION

Grinding is one of the most expensive cost-centers during feed manufacturing (Deaton et al., 1989) after pelleting. Nevertheless, grinding improves mixability, reduces feed segregation (Koch, 1996) and increases surface area for digestive enzyme interactions (Goodband et al., 2002). However, excessive grinding reduces grinding capacity and increases energy consumption in feed mill (Reece et al., 1986; Amerah et al., 2007). The degree of grinding is important, as chickens prefer coarser feed particles at all ages (Schiffman 1968; Gentle et al., 1979; Portella et al., 1988). Furthermore, chickens have naturally evolved a mechanical grinder "gizzard" in their digestive system, which reduces feed particle size, regulates feed-flow throughout the gastrointestinal tract, and contributes to physical and chemical degradation of nutrients (Svihus, 2011). Coarse particles facilitate gizzard development (Svihus, 2011) and their absence or limited inclusion can hamper its development and natural functionality (Zaefarian et al., 2016).

Previous researchers have reported that large corn particle size increases relative gizzard weight and improves performance and feed efficiency in pelleted broiler diets (Amerah and Ravindran 2008; Xu et al., 2015a; Xu et al., 2017), mash broiler diets (Xu et al., 2015b), and mash layer diets (Bozkurt et al., 2019). However, coarse particles (>1500 µm) offered during pre-starter and starter phases can reduce feed intake and BW, leading to an increased feed conversion ratio (FCR) by diverting a large portion of the dietary energy to gizzard contraction in young broilers (Lott et al., 1992). Lu et al. (2011) fed geese with a diet containing 64% whole corn from 8 to 28 d and 61.5% whole corn from 29 to 70 d and reported greater gizzard weight, without a significant effect on FCR compared to geese feed diets with ground corn. Particle size can also influence pellet quality, as particle size decreases, there is greater heat and moisture penetration during conditioning and higher surface for particles adhesion during pelleting. Several authors have reported improved pellet durability when particle size of corn was decreased (Wondra et al., 1995; Chewning et al., 2012; Abadi et al., 2019).

Therefore, an optimum balance between grinding by equipment and grinding in the gizzard is necessary to ensure maximum broiler performance and feed efficiency. Furthermore, additional particle size reduction during pelleting and crumbling should be considered when determining the optimum particle size of corn in poultry diets (Zaefarian et al., 2016).

Heretofore, coarsely ground corn (Amerah et al., 2007; Xu et al., 2015a), cracked corn (Dozier et al., 2006; Clark et al., 2009) and whole grain inclusion (Svihus and Hetland, 2001; Singh et al., 2014b; Singh et al., 2019; Singh and Ravindran, 2019) has been used to increase particle size of broiler feeds. Whole wheat has been included in poultry diets for more than 65 years (Ewing ,1951) and previous researchers have reported higher apparent metabolizable energy (McIntosh et al., 1962; Svihus and Hetland, 2001) and higher apparent ileal digestibility of dry matter (Svihus and Hetland, 2001; Svihus, 2011) in whole wheat containing diets. Whole corn inclusion may have similar benefits when included in broiler diets, but its kernel’s size is typically considered too big to be included in poultry diets without grinding. However, inclusion of whole corn prior to pelleting can be an alternative to overcome this challenge (Singh et al., 2014b). Grinding effect that occurs as feed is compressed between rolls and the die of pellet mill can be utilized to break the corn kernels and incorporate them inside the pellets.

Addition of whole corn prior to pelleting can also influence intestinal microbiota. Singh et al. (2014b) reported an increase of *Lactobacillus* in ceca with increased levels of whole corn inclusion prior to pelleting. *Lactobacillus* spp. are beneficial bacteria that produces lactic acid which lowers gut pH and releases bacteriocin-like compounds, that have a bacteriostatic effect against pathogenic bacteria (Chateau et al., 1993). In a more recent study, Singh et al. (2019) reported a decrease in *Clostridium* and *Campylobacter* at 21 d of age with the inclusion of 20% whole wheat. Clostridium spp. are often pathogenic to chicken (Achakzai et al. 2019) whereas campylobacter is a public health concern for their zoonotic potential (World Health Organization, 2009). Therefore, this experiment was conducted to determine the effects of pre-pelleting whole corn inclusion on feed quality broiler performance, carcass yield, organ development, and microbiome modulation from 14 to 42 d of age.

MATERIAL AND METHODS

All procedures involving live bird were approved by Auburn University Institutional Animal Care and Use Committee (PRN 2017–3018).

Husbandry Practices

A total of 1000 YPM × Ross 708 male broiler chicks were obtained from a commercial hatchery (Aviagen North America, Huntsville, AL) at 1 d of age. Upon arrival, broilers were weighed and randomly distributed among 40 floor pens (25 birds per pen, 0.12 m^2/chick) in a solid-sided house with negative pressure ventilation system. The house was equipped with exhaust fans, forced-air heaters, cooling pads, and electronic controllers to manage temperature and ventilation. Each pen was 150 cm in width, 200 cm in length, and 60 cm in height and equipped with five nipple drinkers and one tube feeder that granted birds *ad-libitum* access to feed and water throughout the experimental period. Photoperiod was set at 23L:1D from 1 to 7 d, 21L:3D from 8 to 20 d and 16L:8D from 21 to 42 d. Room temperature was 33°C during placement, 31.3°C from 2 to 5 d, 29.4°C from 6 to 14 d, and 28.3°C from 15 to 23 d, 26.7ºC from 24 to 28 d and 23.8ºC from 29 to 42 d. Birds were inspected at least twice daily and room temperature, bird condition, mortality and availability of feed and water were carefully checked during each inspection.

Feed Formulation, Manufacture, and Experimental Design

Diets was formulated to meet or exceed the NRC suggested minimum nutrient requirement of broilers (NRC, 1994) (Table 1). Each pen was randomly assigned to 1 of 4 treatments (0, 3, 6 or 9% whole corn) and each treatment had 10 replicate pens with 25 birds in each pen. Each treatment had identical ingredient and nutrient composition apart from the level of whole corn inclusion. Starter feed was provided in crumble form, while grower and finisher diets were provided as whole pellets of 4.0 mm diameter.

Corn was ground to a particle size of 696 µm for grower feed and 624 µm for finisher feed using a hammer mill (Model 11.5 × 38, Roskamp Champion, Waterloo, IA) equipped with 3.175 mm screen size. Particle size of the soybean meal used in the study was 832 and 796 in the grower and finisher diets. Ground corn, soybean meal, other ingredients were added to a twin shaft ribbon mixer (Model 726, Scott Equipment Co., New Prague, MN). Subsequently, depending on the treatment being mixed either 0, 3, 6 and 9% whole corn was added to the mix as a percentage of total diet respective to each treatment. All the ingredients were mixed for 150 seconds (30 second dry and 120 second wet) in the mixer to produce meal feeds. Feed was conditioned at 82°C for 45 seconds and then pelleted through a ring die of 4.0

mm diameter using a pellet mill (Model 1112-4, California Pellet Mill Co., Crawfordsville, IN). As the conditioned mash was compressed through the pellet die and rolls, whole corn was broken into pieces and incorporated into the pellets. Hot and moist pellets leaving the pellet mill, were dried and cooled in a counterflow pellet cooler (Model CC0909, California Pellet Mill Co., Crawfordsville, IN) using ambient air. Starter feed was crumbled in a crumbler (Model 624SS, California Pellet Mill Co., Crawfordsville,IN) with manual roll adjustment.

Initially, two feed samples of 500 g collected from each treatment were homogenized before nutrient analysis. Duplicate samples of 250 mg dried feed from each treatment were placed into aluminum foil and analyzed for crude protein (N x 6.25) by combustion method (AOAC 990.03) using Leco equipment (Leco Corporation, St. Joseph, MI).

The particle size of the ground corn, soybean meal, and pre-pelleting mash feed was analyzed using a Ro-tap shaker (Model RX-30 W.S. Tyler's Ro-Tap®, Mentor, OH) to sift 100 ± 5 g samples for 10 min using 13 sieves stack with US sieve (ASTM-E11) numbers 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, 270 and pan. Geometric mean diameter of particles by mass (D_{gw}) for the different treatments was determined using the quantity of material retained on each sieve according to American Society of Agricultural and Biological Engineers (ASABE) method S319.4 (ASABE standard, 2009).

In addition, pellet durability index (PDI) was measured according to the procedures described in ASABE Standard S269.5 (ASABE Standards, 2012). Initially, fines were removed using a US No 6 sieve with an opening of 3,350 µm. Samples were tumbled at 50 rpm for 10 min using a tumbler (Seedburo pellet durability tester) with a dimension of 12.7 × 30.5 × 30.5 cm containing a 5.1 × 22.9 cm plate affixed diagonally along 2.54 cm of the 30.5 × 30.5 cm sides. After tumbled samples were sifted again using a No. 6 sieve and weighed, PDI was calculated by dividing the weight of the pellets after tumbled by the weight of the pellets before tumbling and then multiplying it by 100.

Measurements

Broilers and feed were weighed at the beginning and at 14, 28, and 42 d to determine BW gain, feed intake, and FCR. Birds were observed twice a day, mortalities were removed daily and their BW was considered to adjust FCR. On 42 d, 10 birds from each pen were randomly selected and processed to determine carcass and parts yield. Feed was removed 10 hours prior to processing, but birds had access to water during the feed removal period. On the processing day, selected birds were loaded into coops and transferred to Auburn University Pilot Processing Plant. Broilers were electrically stunned, exsanguinated, scalded, picked and eviscerated mechanically, and placed on ice. Carcasses were chilled for 4 h in ice and then the excess water was drained for around 5 minutes before chilled carcass weights were determined. Chilled carcasses were deboned the following day by experienced personnel utilizing stationary cones. During deboning, breast fillets (*pectoralis major*), tenders (*pectoralis minor*), wings, and drum and thighs were weighed to determine parts weights and yield. Carcass yield was calculated in relation to live BW of the birds, whereas parts yield was calculated as a percentage of chilled carcass weight. Moreover, on d 42, two birds from each pen were randomly selected and their digestive organs (crop, proventriculus, gizzard, and ceca) were collected to determine their total and relative weight.

Sample collection and preparation for investigation of intestinal microbiota

On d 28, two birds per pen were randomly selected, euthanized by cervical dislocation, and the entire gastro-intestinal tract was collected into a ziploc bag, placed in ice, and taken to the laboratory. Ileal and cecal contents from the two birds were frozen at -20^{o}C for further analysis of ileal and cecal microbiota. Sample preparation was done in accordance with Bortoluzzi et al. (2017) with slight modifications. Cecal content and ileal digesta were diluted 1:10 in sterile microbiology grade water and 5 mL of mixed ileal solution and 1mL of the mixed cecal solution were centrifuged individually for 3 minutes at 3,270 g. Supernatant was discarded and 200 µg of the content were used as the source of metagenomic DNA. DNA was extracted using PowerSoil DNA extraction kit according to the manufacturer's instruction and the DNA was diluted to 1:5 by adding 20µl of molecular biology grade water to 5µl of purified DNA at 0.1ng/µl concentration. Then 2 µl of the diluted DNA solution were mixed with 25 µl of universal TaqMan reaction mix without uracil-N-glycoslyase and 0.1µl of both PCR primers at 100 µM concentration (Illumina -V4-515F-RJ: TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG GTG CCA GCM GCC GCG GTAA and Illumina -V4-806R-RJ: GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACH VGG GTW TCT AAT) and 23.8µl of molecular biology grade water to produce a 50µl solution which was used in polymerase chain reaction (PCR). Solution was subjected to initial denaturation for 10 minutes at 95°C followed by amplification by 35 cycles of denaturation + annealing + extension at 95°C for 15 sec + 55°C for 30 sec + 72C for 2 min. Aamplified reactions were purified using Ampure XP Magnetic Beads (Beckman Coulter A63881) as per manufacturer's instructions. Then 2 µl of each amplicon pool was indexed in a second PCR reaction using the same conditions as before with 5 µl of Illumina XT Index Primers (Illumina XT v2.0 #FC-131-2001-2004) and 25 µL 2× Kapa HiFi Hot Start Ready mix (Anachem, Dublin, Ireland) for 15 cycles. Indexed amplicons were then pooled and purified with AmPure XP Magnetic Beads (Labplan, Dublin, Ireland). Indexed and pooled amplicons were quantitated using the Kapa Illumina Library Quantification Kit (KAPA #KK4835) as per manufacturer's instructions. Finally, purified, quantitated, indexed, pools were loaded on the Illumina MiSeq at a final concentration of 9pM along with 10% Illumina PhiX (Illumina FC-110-3001) for sequencing.

Bioinformatics

Bioinformatic analysis was done according to Bortoluzzi et al., (2018). Briefly, sequences were paired-end and quality trimmed using Geneious (Newark, NJ). Operational taxonomic units (OTUs) were assigned at a 97% identity using SILVA database. Alpha (Chao 1, observed species, phylogenetic diversity of the whole tree, and Shannon indexes) and beta diversity indexes were calculated using QIIME v1.9.1. Principal Coordinates Analysis (PCoA) was used to visualize the data.

Statistical Analysis

Experiment was conducted as a randomized complete block design with pen location as the blocking factor. Each treatment had 10 replicate pens and each individual pen was considered as an experimental unit. Mortality data were subjected to arcsine transformation before analysis. Data of particle size, pellet quality, growth performance, carcass yield, and relative organ weight were analyzed using one-way ANOVA in GLM procedure of JMP software with the following model.

$$Yij=\mu+Ti+\varepsilon ij$$

Where, Yij =observed response in birds of each pen; μ = is the overall mean; Ti = fixed effect of feed form treatment; and εij=residual error.

Mean values among the four dietary treatments were compared using the Tukey HSD procedure with statistical significance considered at $P \leq 0.05$ unless otherwise indicated.

For microbiome analysis, the frequency of the main bacterial groups observed in the ileum and ceca was submitted to a non-parametric one-way ANOVA (Kruskal-Wallis test) and in case of significant difference, means were separated by Dunn's test.

RESULTS AND DISCUSSION

Particle size and pellet durability index

Feed particle size and PDI are presented in table 2. The particle size of the pre-pelleting mash increased ($P < 0.05$) in grower feed as the inclusion of whole corn increased from 0 and 3% to 9% and was nearly significant ($P=0.07$) in the finisher feeds. Pellet durability index was significantly higher in finisher feed when 9% whole corn was included in diet instead of 0% whole corn ($P<0.05$), and this effect was marginally significant ($P=0.07$) in grower feed. These results were similar to Silva et al. (2018) who reported higher PDI in diets with 850 µm corn particle size compared to diets with 650 µm of corn particle size. Abadi et al. (2019) reported that the combination of coarse particles with 3% pellet binder resulted in greater pellet hardness. Singh et al. (2014b) included 0, 15, 30, 45, 60% whole corn in broiler diets and reported a positive linear correlation between PDI and whole corn inclusion. Typically, as the particle size increases there is a reduction in PDI (Angulo et al., 1996) mainly because coarse particles have smaller surface area (Behnke 2001), and are more resistant to steam penetration during the conditioning process, which results in a poor starch gelatinization, protein gelation (Lund and Lorenz 1984) and lower pellet durability. The improvement of PDI associated with higher inclusion of whole corn observed in this experiment might have resulted from pieces of whole corn retained in the sieve after the tumbling process and thus counted as whole pellets during the PDI analysis.

Growth performance

Whole corn inclusion did not influence BW, feed intake, and FCR ($P > 0.05$; Table 3) at 28 and 42 d. Singh and Ravindran (2019) evaluated the effect corn kernel hardness (hard, semi-hard and soft) and whole corn inclusion (0 and 11.5%) in broilers from 1 to 21 d and found no effect of whole corn inclusion on BW gain or FCR. Singh et al. (2014b) used higher concentration of pre-pelleting whole corn (0, 15, 30, 45 and 60%) in 3 mm pelleted diets from 1 to 21 d and reported a linear decrease in feed intake and BW gain as the inclusion of whole corn increased. In this trial, 4 mm pellets were used and pre-pelleting whole corn inclusion did not exceed 9% and the treatment diet was provided from 14 to 42 d, allowing sufficient time for gizzard development before whole corn was included in feed.

Carcass and parts yield

Whole corn inclusion did not influence chilled carcass weight, breast meat yield, tender yield, wing yield, drum and thigh yield ($P > 0.05$; Table 4). However, there was an increase in carcass yield of broilers ($P < 0.05$) fed diet with 9% whole corn compared to broilers fed diet without whole corn. Similar results were reported by Plavnik et al. (2002) who reported an increase in carcass yield and abdominal fat when 5 and 15% whole wheat was included from 1 to 21 d and 21 to 45 days, respectively. The authors hypothesized that stimulated gizzard development associated with whole wheat inclusion, improved the overall nutrient utilization resulting in higher carcass yield and fat deposition.

Relative weight of digestive organs

The inclusion of whole corn did not influence relative weight of crop, gizzard, liver and ceca (P > 0.05; Table 5). Previous studies reported an increase in relative gizzard weight with inclusion of whole wheat (Amerah and Ravindran, 2008; Biggs and Parsons, 2009; Singh et al., 2019) and coarse corn (Amerah et al., 2008; Sing et al., 2014a; Xu et al., 2015a). Singh et al. (2014b) indicated a linear increase in gizzard weight at 21 d in broilers raised on battery cages, when the inclusion of whole corn increased from 0, to 15, 30, 45 and 60%. The lack of statistical differences obtained in this trial might have derived from a lower inclusion of whole corn fed during this experiment, as well as longer experimental period. Furthermore, as reported previously by Xu et al. (2017), the access to fresh pine wood shavings in the litter during this trial could have also reduced the differences in gizzard development between treatments. However, relative proventriculus weight decreased as the inclusion of whole corn increased, and broiler fed diets with 9% whole corn had significantly lower relative proventriculus weight (P < 0.05) compared to broiler fed diets without whole corn. Similar results were reported by Gracia et al. (2016) and Truong et al. (2017) where relative proventriculus weight decreased with increasing levels of whole wheat inclusion. Since the proventriculus is the site where protein digestion is initiated, a smaller proventriculus might increase the interaction between the digesta and pepsin and hydrochloric acid and promote better N retention.

Intestinal microbiome

In the intestinal microbiota analysis, 152 different bacterial species were identified, and the diversity in microbial population of the ileum showed no significant changes (P > 0.05) within the groups of experimental treatments for alpha and beta diversity indices (Figure 1). These diversity indices are predicated on relative abundance changes that are grouped by subcategories, common species between groups and contrasted by groups individual to singular community.

However, in the ileum, the relative abundance of *Romboutsia* spp. decreased with 3% whole corn inclusion compared to diet without whole corn inclusion, but later increased in higher concentration (6 and 9%) of whole corn diets (Figure 2). *Romboutsia* is a genus that is related to Clostridia and has been detected in a number of vertebrate hosts, including chickens (Maki et al., 2020; Jurburg et al., 2019; Chen et al., 2020). Its relevance is unclear, but it has been suggested that they play a role in maintaining the health status of the host (Chen et al., 2020; Ricaboni et al., 2016; Mangifesta et al., 2018). In the ileum, the relative abundance of obligatory anaerobes decreased when 3% whole corn was included, followed by a significant increase in diets containing 6 and 9% whole corn inclusion ($P < 0.05$; Figure 3). The inclusion of whole corn did not influence the population of *Lactobacillus* spp.in ileal content ($P > 0.05$; Figure 4). In a previous study, Singh et al. (2014b) reported a linear increase in *Lactobacillus* spp on cecal microbiota counts as the inclusion of pre-pelleting whole corn of the inclusion increased from 0 to 60% and the authors attributed the increase in *Lactobacillus* spp to lower gizzard pH and competitive exclusion. There were some *Escherichia coli* and *Clostridium* spp., sparsely distributed in random samples, but they were not associated with any specific dietary treatment. It was predicted that the significant biosecurity at the trial site and maintenance of unused bedding material restricted these bacteria from accumulating in the environment and initiating a circular pattern of inoculation.

The data from this trial demonstrate that up to 9% whole corn can be incorporated in broiler diets prior to pelleting from 14 to 42 d of age without negative effects on broiler performance.

REFERENCES

Abadi, M. G. M. H., H. Moravej, M. Shivazad, M. A. K. Torshizi, and W. K. Kim. 2019. Effects of feed form and particle size, and pellet binder on performance, digestive tract parameters, intestinal morphology, and cecal microflora populations in broilers. Poult. Sci. 98:1432-1440.

Achakzai, R., M. K. Taj, F. Abbas, I. Taj, S. A. Ali, Ahsanullah, A. Hussain, S. Azam, Z. Samreen, S. Khan, L. Bibi, B. Sazain, and S. Jogezai. 2019. Clostridium perfringens as a pathogenic organism in poultry. Intl. J. Biosci. 15:55-65.

Angulo, E., J. Brufau, and E. Esteve-Garcia. 1996. Effect of a sepiolite product on pellet durability in pig diets differing in particle size and in broiler starter and finisher diets. Anim. Feed Sci. and Technol. 63:25-34.

Amerah, A. M., V. Ravindran, R. Lentle, and D. Thomas. 2007. Feed particle size: Implications on the digestion and performance of poultry. World's Poult Sci. J. 63:439-455.

Amerah, A. M., V. Ravindran, R. Lentle, and D. Thomas. 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. Poult. Sci. 87:2320-2328.

Amerah, A. M., and V. Ravindran. 2008. Influence of method of whole-wheat feeding on the performance, digestive tract development and carcass traits of broiler chickens. Anim. Feed Sci. Technol. 147:326-339.

Association of Official Analytical Chemists. 2006. Official Methods of Analysis of AOAC International. 18th ed.

American Society of Agricultural and Biological Engineers standards. 2009. Method of determining and expressing fineness of feed materials by sieving. Am. Soc. Agric. Eng., St Joseph, MI S319.4.

American Society of Agricultural and Biological Engineers standards. 2012. Densified products for bulk handling-Definitions and method. Am. Soc. Agric. Eng., St Joseph, MI S269.5.

American Society for Testing and Materials. 2020. E11-20.

Behnke, K. C. 2001. Factors influencing pellet quality. Feed Technol. 5:19-22.

Biggs, P., and C. Parsons. 2009. The effects of whole grains on nutrient digestibilities, growth performance, and cecal short-chain fatty acid concentrations in young chicks fed ground corn-soybean meal diets. Poult. Sci. 88:1893-1905.

Bortoluzzi, C., A. A. Pedroso, J. J. Mallo, M. Puyalto, W. K. Kim, and T. J. Applegate. 2017. Sodium butyrate improved performance while modulating the cecal microbiota and regulating the expression of intestinal immune-related genes of broiler chickens. Poult. Sci. 96:3981-3993.

Bortoluzzi C., M. J. Rothrock, B. S. Vieira, J. J. Mallo, M. Puyalto, C. Hofacre, and T. J. Applegate. 2018. Supplementation of protected sodium butyrate alone or in combination with essential oils modulated the cecal microbiota of broiler chickens challenged with coccidia and Clostridium perfringens. Front. Sustain. Food Syst. 2-72.

Bozkurt, M., B. Koçer, G. Ege, A. E. Tüzün, H. H. Biyik, and E. Poyrazoğlu. 2019. Influence of the particle size and form of feed on growth performance, digestive tract traits and nutrient digestibility of white egg-laying pullets from 1 to 112 D of age. Poult. Sci. 98:4016-4029.

Chateau N., I. Castellanos, A. M. Deschamps. 1993. Distribution of pathogen inhibition in the Lactobacillus isolates of a commercial probiotic consortium. J Appl. Bacteriol. 74:36-40.

Chen H. L., X. Y. Zhao, G. X. Zhao, H. B. Huang, H. R. Li, C. W. Shi, W. T. Yang, Y. L. Jiang, J. Z. Wang, L. P. Ye, Q. Zhao, C. F. Wang, and G. L. Yang. 2020. Dissection of the cecal microbial community in chickens after Eimeria tenella infection. Parasit. Vectors. 13: 56-71.

Chewning, C. G., C. R. Stark, and J. Brake. 2012. Effects of particle size and feed form on broiler performance. J. Appl. Poult. Res. 21:830-837.

Clark, P. M., K. C. Behnke, and A. C. Fahrenholz. 2009. Effects of feeding cracked corn and concentrate protein pellets on broiler growth performance. J. Appl. Poult. Res. 18:259-268.

Deaton, J. W., B. D. Lott, and J. D. Simmons. 1989. Hammer mill versus roller mill grinding of corn for commercial egg layer. Poult. Sci. 68:1342-1344.

Dozier, W. A., K. Behnke, M. T. Kidd, and S. L. Branton. 2006. Effects of the addition of roller mill ground corn to pelleted feed on pelleting parameters, broiler performance and intestinal strength. J. Appl. Poult. Res. 15:236-244.

Ewing, W. R. 1951. Poultry Nutrition. 4th ed. South Pasadena, CA, USA.

Gentle, M. J. 1979. Sensory control of feed intake. Page 259-273 in Food Intake Regulation in Poultry. K. N. Boorman ed. B. M. Freeman, Edinburg, Britain.

Goodband, R. D., M. D. Tokach, and J. L. Nelssen. 2002. The effects of diet particle size on animal performance. Manhattan, Kansas: Cooperative Extension Service, Kansas State University.

Gracia, M. I., J. Sánchez, C. Millán, Ó. Casabuena, P. Vesseur, Á. Martín, and P. Medel. 2016. Effect of feed form and whole grain feeding on gastrointestinal weight and the prevalence of campylobacter jejuni in broilers orally infected. Plos One. 11(8): e0160858.

Jurburg, S. D., M. S. M. Brouwer, D. Ceccarelli, J. Van-der Goot, A. J. M. Jansman, and A. Bossers. 2019. Patterns of community assembly in the developing chicken microbiome reveal rapid primary succession. Microbiology open. 8(9):e00821.

Koch, K. 1996. Hammermills and rollermills. MF-2048 Feed Manufacturing, Department of Grain Science and Industry, Kansas State University.

Lott, B. D., E. J. Day, J. W. Deaton, and J. D. May. 1992. The effect of temperature, dietary energy level, and corn particle size on broiler performance. Poultry Science 71:618-624.

Lu, J., X. L. Kong, Z. Y. Wang, H. M. Yang, K. N. Zhang, and J. M. Zou. 2011. Influence of whole corn feeding on the performance of digestive tract development and nutrient retention of geese. Poult. Sci. 90:587-594.

Lund, D., and K. J. Lorenz.1984. Influence of time, temperature, moisture, ingredients, and processing conditions on starch gelatinization. Critical Reviews in Food Science and Nutrition 20:249-273.

Maki, J. J., D. W. Nielsen, and T. Looft. 2020. Complete Genome Sequence and Annotation for Romboutsia sp. Strain CE17. Microbiol Resour Announc. 9(23):e00382-20.

Mangifesta, M., L. Mancabelli, C. Milani, F. Gaiani, N. de'Angelis, G. L. de'Angelis, D. van-Sinderen, M. Ventura, and F. Turroni. 2018. Mucosal microbiota of intestinal polyps reveals putative biomarkers of colorectal cancer. Sci. Rep. 8:13974.

McIntosh, J. L., S. J. Singer, L. R. Sibbald, and G. C. Ashton. 1962. Factors affecting the metabolizable energy content of poultry feeds 7. the effects of grinding, pelleting and grit feeding on the availability of the energy of wheat, corn, oats and barley 8. A study on the effects of dietary balance. Poult. Sci. 41:445-456.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th.rev. ed. Natl. Acad. Press, Washington, DC.

Parsons, A., N. Buchanan, K. Blemings, M. Wilson, and J. Moritz. 2006. Effect of corn particle size and pellet texture on broiler performance in the growing phase. J. Appl. Poult. Res. 15:245-255.

Plavnik, I., B. Macovsky, and D. Sklan. 2002. Effect of feeding whole wheat on performance of broiler chickens. Anim. Feed Sci. and Technol. 96:229-236.

Portella, F. J., L. J. Caston, and S. Leeson. 1988. Apparent feed particle size preference by laying hens. Canadian Journal of Animal Science 68:915-922.

Reece, F. N., B. D. Lott, and J. W. Deaton.1986. The effects of hammer mill screen size on ground corn particle size, pellet durability, and broiler performance. Poult. Sci. 65:1257-1261.

Ricaboni, D., M. Mailhe, S. Khelaifia, D. Raoult, and M. Million. 2016. Romboutsia timonensis, a new species isolated from human gut. New Microbes New Infect. 12:6-7.

Schiffman, H. R. 1968. Texture preference in the domestic chick. J Comp. Phys. Psych. 66:540-541.

Silva, P. G. D., L. M. S. Oliveira, N. R. D. Oliveira, F. A. D. Moura Júnior, M. R. S. Silva, D. A. Cordeiro, and F. R. D. Santos. 2018. Effects of processing, particle size and moisturizing of sorghum-based feeds on pellet quality and broiler production. Asian-Australasian J. of Anim. Sci. 31:98-105.

Singh, Y., and V. Ravindran. 2019. Influence of feeding whole maize, differing in endosperm hardness, on the performance, nutrient utilisation and digestive tract development of broiler starters. J. Appl. Anim. Nutr. 7:27-37.

Singh, Y., A. L. Molan, and V. Ravindran. 2019. Influence of the method of whole wheat inclusion on performance and caecal microbiota profile of broiler chickens. J of Appl. Anim. Nutr. 7:1-7.

Singh, Y., V. Ravindran, T. Wester, A. Molan, and G. Ravindran. 2014a. Influence of feeding coarse corn on performance, nutrient utilization, digestive tract measurements, carcass characteristics, and cecal microflora counts of broilers. Poult. Sci. 93:607-616.

Singh, Y., V. Ravindran, T. Wester, A. Molan, and G. Ravindran. 2014b. Influence of pre-pelleting inclusion of whole corn on performance, nutrient utilization, digestive tract measurements, and cecal microbiota of young broilers. Poult. Sci. 93:3073-3082.

Svihus, B. 2011. The gizzard: function, influence of diet structure and effects on nutrient availability. Worlds Poult. Sci. J. 67:207-224.

Svihus, B., and H. Hetland. 2001. Ileal starch digestibility in growing broiler chickens fed on a wheat-based diet is improved by mash feeding, dilution with cellulose or whole wheat inclusion. Br. Poult. Sci. 42:633-637.

Truong, H. H., A. F. Moss, S. Y. Liu, and P. H. Selle. 2017. Pre- and post-pellet whole grain inclusions enhance feed conversion efficiency, energy utilisation and gut integrity in broiler chickens offered wheat-based diets. Anim. Feed Sci. and Technol. 224: 115-123.

World Health Organization. 2009. risk assessment of campylobacter spp. in broiler chickens. Technical report. Microbiological Risk Assessment Series No 12. Geneva. 132pp.

Wondra, K. J., J. D. Hancock, K. C. Behnke, R. H. Hines, and C. R. Stark. 1995. Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73:757-763.

Xu, Y., Y. Lin, C. R. Stark, P. R. Ferket, C. M. Williams, and J. T. Brake. 2017. Effects of dietary coarsely ground corn and 3 bedding floor types on broiler live performance, litter characteristics, gizzard and proventriculus weight, and nutrient digestibility. Poult. Sci. 96:2110-2119.

Xu, Y., C. R. Stark, P. R. Ferket, C. M. Williams, W. J. Pacheco, and J. T. Brake. 2015a. Effect of dietary coarsely ground corn on broiler live performance, gastrointestinal tract development, apparent ileal digestibility of energy and nitrogen, and digesta particle size distribution and retention time. Poult. Sci. 94:53-60.

Xu, Y., C. R. Stark, P. R. Ferket, C. M. Williams, and J. T. Brake. 2015b. Effects of feed form and dietary coarse ground corn on broiler live performance, body weight uniformity, relative gizzard weight, excreta nitrogen, and particle size preference behaviors. Poult. Sci. 94:1549-1556.

Zaefarian, F., M. Abdollahi, and V. Ravindran. 2016. Particle size and feed form in broiler diets: impact on gastrointestinal tract development and gut health. Worlds Poult. Sci. 72:277-290.

TABLES

Table 4.1: Ingredient and nutrient composition of dietary treatments fed to YPM × Ross 708 male broilers from 1 to 42 d of age

Ingredient, % "as-fed"	Starter	Grower	Finisher
Corn	52.00	59.00	59.82
Soybean meal	36.00	26.48	23.47
Distillers dried grains with solubles (DDGS)	5.00	7.00	9.00
Poultry Oil	3.40	4.26	4.96*
Dicalcium phosphate, 18% P	1.27	0.92	0.48
Calcium carbonate	1.24	1.11	1.02
Sodium chloride	0.37	0.34	0.28
D-L Methionine	0.31	0.24	0.20
L-Lysine	0.12	0.25	0.18
Trace mineral premix[1]	0.10	0.10	0.10
Vitamin premix[2]	0.10	0.08	0.10
Choline chloride	0.08	0.10	0.08
L-Threonine	0.04	0.08	0.06
Copper chloride[3]	0.02	0.03	0.02
Quantum phytase[4]	0.01	0.01	0.008
	100.00	100.00	100.00
Calculated analysis, % (unless otherwise noted)			
AME_n, kcal/kg	3,025	3,120	3,175
Crude Protein[5]	23.24	19.59	19.01
Digestible Lys	1.18	1.02	0.95
Digestible Thr	0.77	0.68	0.64
Digestible Trp	0.23	0.22	0.20
Digestible TSAA[6]	0.91	0.78	0.72
Calcium	1.00	0.88	0.76
Available phosphorus	0.48	0.42	0.38

[1]Mineral premix include per kg of diet: Mn (manganese sulfate), 120 mg; Zn (zinc sulfate), 100 mg; Fe (iron sulfate monohydrate), 30 mg; Cu (tri-basic copper chloride), 8 mg; I (ethylenediamine dihydriodide), 1.4 mg; and Se (sodium selenite), 0.3 mg.

[2]Vitamin premix includes per kg of diet: Vitamin A (Vitamin A acetate), 18,7390 IU; Vitamin D (cholecalciferol), 6,614 IU; Vitamin E (DL-alpha tocopherol acetate), 66 IU; menadione (menadione sodium bisulfate complex), 4 mg; Vitamin B12 (cyanocobalamin), 0.03 mg; folacin (folic acid), 2.6 mg: D-pantothenic acid (calcium pantothenate), 31 mg; riboflavin (riboflavin), 22 mg; niacin (niacinamide), 88 mg; thiamin (thiamin mononitrate), 5.5 mg; D-biotin (biotin), 0.18 mg; and pyridoxine (pyridoxine hydrochloride), 7.7 mg.

[3]Intellibond® C (Micronutrients, Indianapolis, IN).

[4]Quantum® Blue 5G (AB Vista Feed Ingredients, Marlborough, UK) provides per kg of diet: 500 FTU/kg of phytase activity.

[5]Analyzed crude protein content in starter, grower and finisher diets were 21.11 and 22.32, 19.17 and 20.31, and 17.63 and 18.74%, respectively (AOAC-2006)

[6]TSAA = Total sulfur amino acids.

*Poultry oil in finisher diet was incorporated as 1% in wet mixing and the remaining 3.96% was added as post pellet liquid application (PPLA).

Table 4.2: Particle size and pellet quality of feed manufactured with 4 different levels of pre-pelleting whole corn inclusion.

Item[1]	Particle size of pre-pelleting mash[2], µm		PDI[3], %	
Whole corn, %	Grower	Finisher	Grower	Finisher
0	762[bc]	665	52.21	52.01[b]
3	754[c]	794	53.58	59.29[ab]
6	930[ab]	831	54.51	54.55[ab]
9	1000[a]	837	55.88	61.12[a]
SEM	49	53	1.08	1.65
P-value	0.02	0.08	0.07	0.02

[a,bc] Means column with different superscript differ significantly ($P < 0.05$)

[1]Dietary treatments consisted 0, 3, 6 and 9% whole corn inclusion. A common starter diet was provided in crumble form from 0-14 day and the experimental treatments were provided as 4.0 mm pellets from 14 to 42 d of age.

[2]Particle size = Particle size of mixed mash feed prior to conditioning and pelleting was measured according to ASABE standard S319.4 (ASABE standards 2009).

[3]PDI= Pellet durability index measured according to ASABE Standard S269.5 (ASAE Standards, 2012) using a Seedburo pellet durability tester.

Table 4.3: Growth performance of YPM × Ross 708 male broilers fed with 4 different levels of whole corn inclusion from 14 to 42 d.

Item[1]	**BW, g/bird[2]**				**FI, g/bird[3]**			**FCR, g/g[4]**		
Whole corn, %	Days of Age									
	0	**14**	**28**	**42**	**14**	**28**	**42**	**1-14**	**1-28**	**1-42**
0.0	39	489	1,712	3,249	516	2,216	5,259	1.15	1.33	1.65
3.0	39	488	1,738	3,289	512	2,243	5,299	1.14	1.33	1.63
6.0	39	488	1,741	3,298	509	2,235	5,313	1.14	1.32	1.64
9.0	39	487	1,729	3,277	510	2,228	5,294	1.14	1.33	1.64
SEM[4]	1	6	18	26	6	21	45	0.011	0.005	0.013
P value	0.99	0.99	0.68	0.58	0.83	0.83	0.86	0.95	0.77	0.94

[1]Treatments consisted 0, 3, 6, and 9% whole corn inclusion. A common starter diet was provided in crumble form from 0-14 day and the experimental treatments were provided as 4.0 mm pellets from 14 to 42 d of age.
[2]BW= Average body weight of birds.
[3]FI= Average feed intake of birds.
[4]FCR= Feed conversion ratio.
[5]SEM= Standard error of mean for 10 replicates (n=10).

Table 4.4: Carcass and processing yield of YPM × Ross 708 male broilers fed with 4 different levels of whole corn inclusion.

Item[1] Whole corn, %	Carcass Weight[2]	Carcass Yield[3]	Breast Meat Yield[4]	Tender Yield[5]	Wing Yield[6]	Drum and Thigh Yield[7]
	(g)	------------------------------	------------------------------	(%)	------------------------------	------------------------------
0.0	2,627	77.32[b]	29.48	5.39	9.69	39.63
3.0	2,633	77.67[ab]	29.61	5.48	9.62	39.55
6.0	2,623	77.41[ab]	29.64	5.39	9.64	39.58
9.0	2,619	77.86[a]	29.46	5.40	9.61	39.55
SEM[7]	20	0.14	0.18	0.06	0.05	0.20
P-value	0.97	0.03	0.86	0.60	0.65	0.99

[a,b] Means column with different superscript differ significantly (P < 0.05)

[1]Dietary treatments consisted of 0, 3, 6, and 9% whole corn inclusion. A common starter diet was provided in crumble form from 0-14 day and the experimental treatments were provided as 4.0 mm pellets from 14 to 42 d of age.

[2]Carcass weight= Weight of processed carcass after 4 hrs chilling in slush cold water and measured in gram.

[3]Carcass yield= Chilled carcass weight is divided by the live weight of the bird and expressed as percent.

[4]Breast meat yield= Weight of breast meat/Pectoral major is divided by chilled carcass weight and expressed as percent.

[5]Tender yield= Weight of tender or Pectoral minors is divided by chilled carcass weight and expressed as percent.

[6]Wing yield= Weight of wing is divided by chilled carcass weight and expressed as percent.

[7]Drum and thigh yield= Weight of hind quarter (drum and thigh combined) is divided by chilled carcass weight and expressed as percent.

[8]SEM= Standard error of mean for 10 replicates (n=10).

Table 4.5: Relative weight of digestive organs of YPM × Ross 708 male broilers fed with 4 different levels of whole corn inclusion.

Item[1] Whole corn, %	Body weight[2]	Crop R[3]	Proventriculus R[4]	Gizzard R[5]	Liver R[6]	Ceca R[7]
	(g)	---------- (%) ----------				
0.0	3,611	0.29	0.35[a]	1.03	2.63	0.34
3.0	3,635	0.30	0.31[ab]	0.95	2.48	0.35
6.0	3,741	0.24	0.31[ab]	0.99	2.56	0.32
9.0	3,696	0.27	0.28[b]	1.02	2.46	0.32
SEM[8]	65	0.02	0.01	0.03	0.06	0.02
P value	0.500	0.120	0.004	0.28	0.13	0.63

[a,b] Means column with different superscript differ significantly ($P < 0.05$)

[1]Dietary treatments consisted of 0, 3, 6, and 9% whole corn inclusion. A common starter diet was provided in crumble form from 0-14 day and the experimental treatments were provided as 4.0 mm pellets from 14 to 42 d of age.

[2]Body weight= average weight of live bird before euthanasia. Two birds from every representative pens of each treatments were randomly selected and weighted to get the average live body weight for the treatments.

[3]Crop R= Relative crop weight is the ratio of average empty crop weight and average live weight expressed as percent.

[4]Proventriculus R= Relative proventriculus weight is the ratio of average empty proventriculus weight and average live weight expressed as percent.

[5]Gizzard R= Relative gizzard weight is the ratio of average empty gizzard weight and average live weight expressed as percent.

[6]Liver R= Relative liver weight is the ratio of average liver weight and average live weight expressed as percent.

[7]Ceca R= Relative ceca weight is the ratio of average empty ceca weight and average live weight expressed as percent.

[8]SEM= Standard error of mean for 10 replicates (n=10).

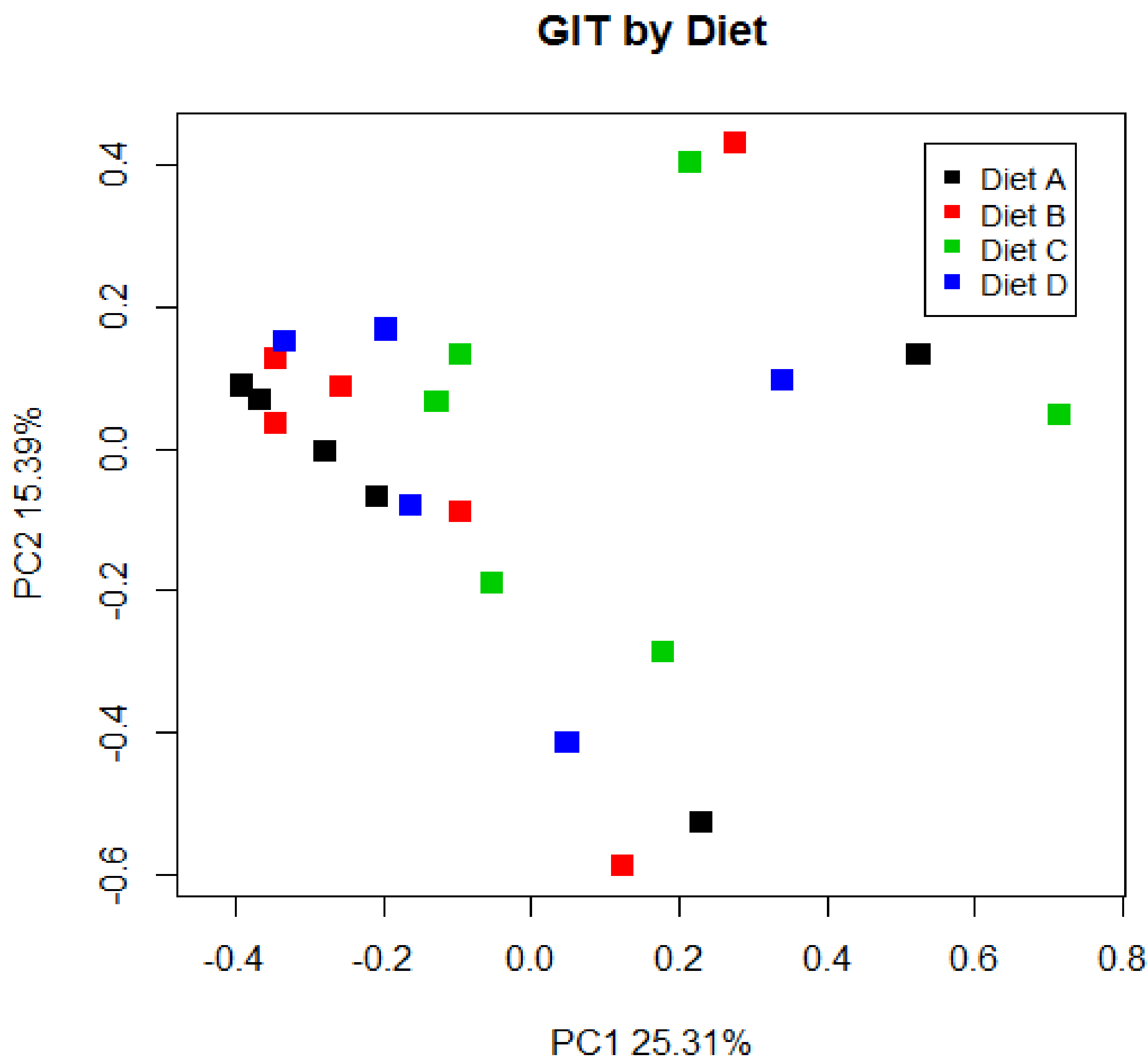


Figure 4.1: UniFrac PCoA plots of intestinal microbiota of broilers at 28 d in response to different concentration whole corn inclusion: Black: Diet A= 0% whole corn; Red: Diet B= 3% whole corn; Green: Diet C= 6% whole corn; and Blue: Diet D= 9% whole corn. A total of 80 birds were sampled (20 birds per treatment or 2 birds per pen). There were no differences among the experimental groups for the alpha and beta diversity indices.

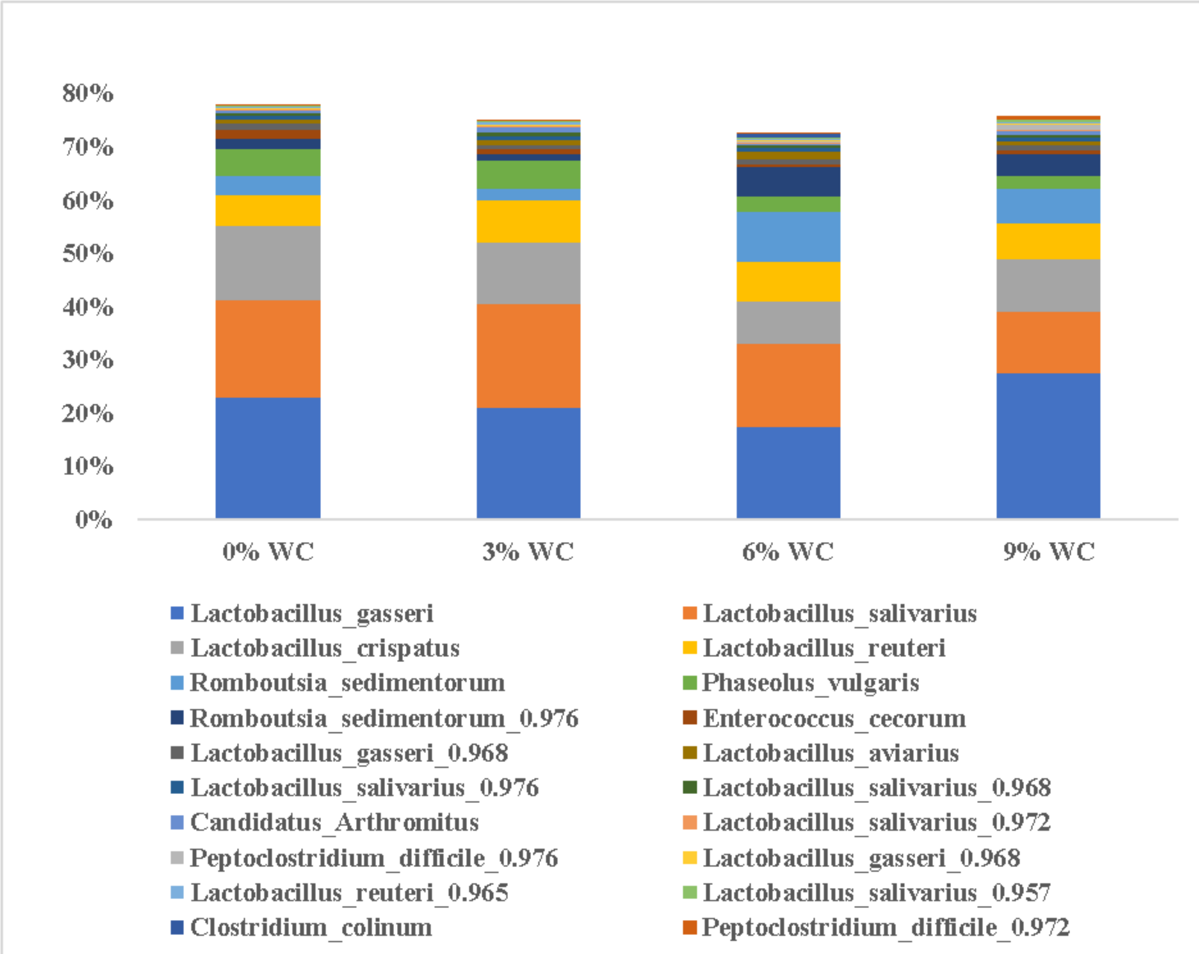


Figure 4.2: Relative abundance (%) of the main bacterial group present in ileal microbiota of broilers at 28 d in response to different concentration whole corn (**WC**) inclusion. A total of 80 birds were sampled (20 birds per treatment or 2 birds per pen). *Romboutsia* spp. and ileal anaerobes increased when the level of WC exceeded 6% ($P < 0.05$

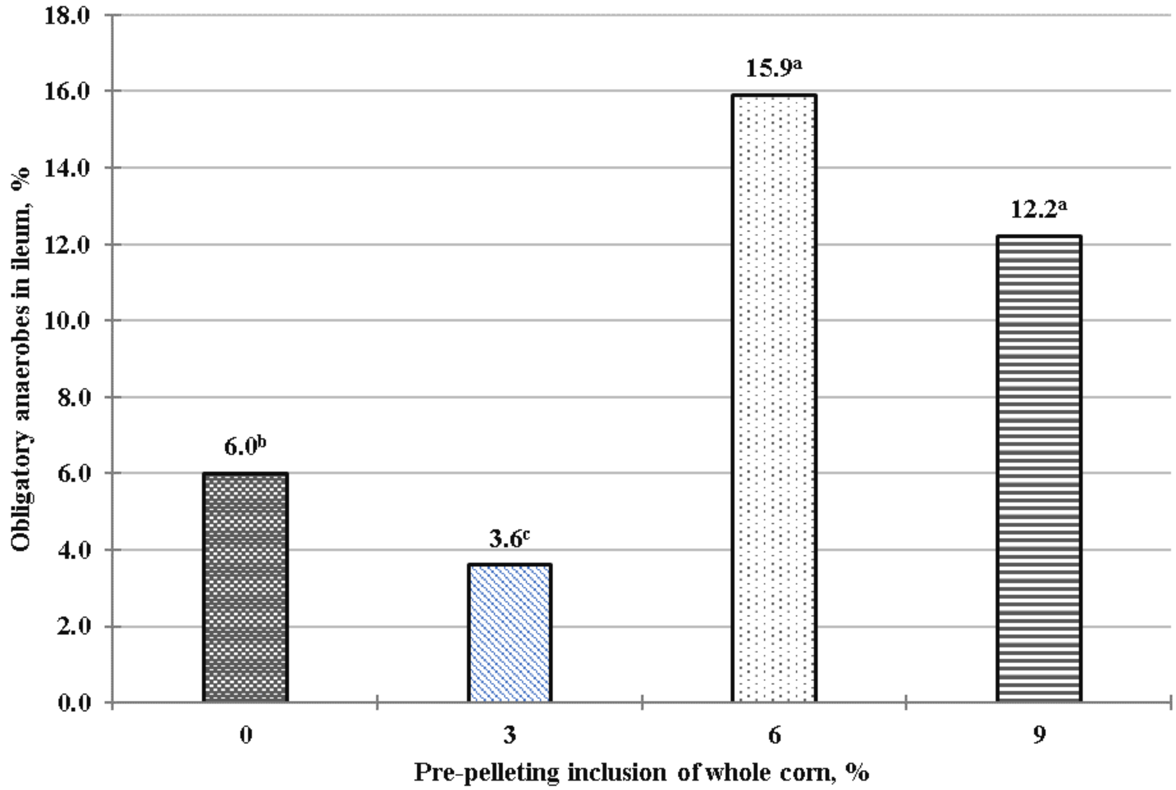


Figure 4.3: Relative abundance of obligate anaerobes in ileal microbiome at 28 d in response to different concentration whole corn inclusion. A total of 80 birds were sampled (20 birds per treatment or 2 birds per pen). Bars with different superscripts differs significantly ($P<0.05$). In the ileum, the relative abundance of obligatory anaerobes decreased when 3% pre-pelleting whole corn was included, followed by a significant increase in diets containing 6 and 9% pre-pelleting whole corn inclusion.

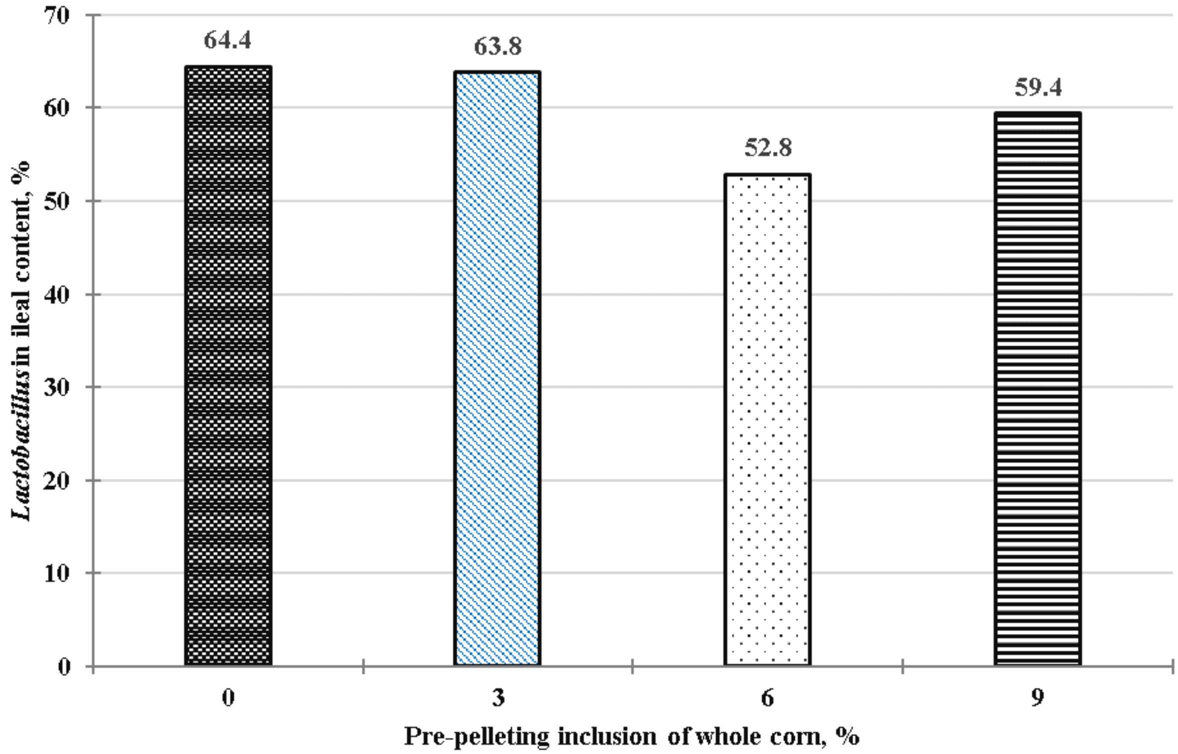


Figure 4.4: Relative abundance *Lactobacillus spp* in cecal microbiome of broilers at 28 d in response to different concentration whole corn inclusion. A total of 80 birds were sampled (20 birds per treatment or 2 birds per pen). Pre-pelleting inclusion of whole corn did not influence the population of *Lactobacillus* spp.in ileal content.

SUMMARY AND CONCLUSION

Pre-pelleting whole corn inclusion could be an effective alternative to reduce grinding cost by utilizing the additional grinding effect of pellet mill. Furthermore, whole corn inclusion at certain concentration have some potential benefit to growth performance and processing yield of broilers.

The first experiment was conducted to evaluate the effects of pre-pelleting whole corn inclusion (0, 2.5, 5 and 7.5%) on growth performance, processing yield, breast meat quality and intestinal microbiome of broilers from 1 to 42 d. Whole corn inclusion up to 7.5% did not interfere with feed intake or BW of broilers ($P>0.05$), which challenges the established notion that corn kernel is too big for pre-pelleting inclusion. However, breast meat yield was reduced for feeding diet containing 7.5% whole corn inclusion compared to diet with 0 and 5% whole corn. Furthermore, there was a marginal increase of *Faecalibacterium* ($P = 0.07$) and marginal decrease *Lactobacillus* ($P = 0.08$) in cecal microbiota at 42 d, as the concentration of whole corn was increased from 5 to 7.5%. Cooking loss and coloration of breast meat fillet was unaffected by whole corn concentration in diet ($P>0.05$). Based on these data it can be inferred that, as much as 5% pre-pelleting whole corn can be included in broiler diets from 1 to 42 d without negative effects on broiler growth performance or processing yield.

The objective of the second experiment was to challenge broilers with higher concentration of pre-pelleting whole corn (0, 3, 6 and 9%) from 14 to 42 d of age and to evaluate its effects on pellet durability, particle size, growth performance, processing yield, intestinal microbiome and relative weight of digestive organs. There was no influence of whole corn inclusion on feed intake, BW and FCR which implies that after two weeks of age, broilers diets can contain up to 9% whole corn pre-pelleting without compromising their growth performance.

Processing at 42 d revealed improved carcass yield ($P<0.05$) with 9% whole corn containing diet compared to 0% whole corn diet, without affecting breast meat or tender yield. Intestinal microbiome analysis at 28 d found that obligatory anaerobes population in ileum decreased ($P<0.05$) when 3% pre-pelleting whole corn was included, followed by a significant increase ($P<0.05$) in diets containing 6 and 9% pre-pelleting whole corn inclusion. This variation in anaerobic population indicate key shifts of ileal micro-environment between aerobic and anaerobic condition in response to different concentration of whole corn inclusion. Particle size of pre-pelleting mash of grower feed and pellet durability index of finisher feed increased ($P>0.05$) with 9% whole corn inclusion compared to 0% whole corn inclusion. These data demonstrate that as much as 9% whole corn can safely be included in broiler diet from 14 to 42 d.