EFFECTS OF FEED MANUFACTURING ON NUTRIENT METABOLISM, NUTRIENT RETENTION, AND GROWTH PERFORMANCE OF BROILER CHICKENS

by

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B.S., Kansas State University, 2005
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

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Abstract

Broiler chicken feed is processed. Cereal grains are ground to reduce particle size and the feed usually is pelleted. When pelleted, broiler diets are steam conditioned and forced through a die causing varying levels of starch gelatinization. Cereal grain particle size and starch gelatinization can be controlled during feed manufacturing. Earlier research has shown that starch gelatinization negatively affects growth performance of 0 to 21 d of age. An experiment was conducted to determine the effects of corn particle size and starch gelatinization on growth performance, dressing percentage, and gizzard size when fed to 22 to 42 d of age broilers. Increasing particle size from 470 to 1240 µm increased body weight gain, dressing percentage, and relative gizzard size. Starch gelatinization increased relative gizzard size. No interaction effects were detected. To expand on previous experiments, a trial was conducted to investigate the effect of starch gelatinization on broiler chick gastrointestinal pH, glucose absorption, and glucoregulation. Starch gelatinization level affected jejunum pH, with a higher pH reported at 20% starch gelatinization. Increases in starch gelatinization decreased blood glucose and increased glucagon level. Highest measured glucagon level was reported in broiler chicks fed the diet with 20% starch gelatinization level diet after 6 hours of starvation. A third experiment was conducted to determine the effect of starch gelatinization on metabolizable energy and amino acid digestibility. Increasing starch gelatinization from 0 to 100% increased true metabolizable energy and fecal output in roosters. No effect was found on apparent metabolizable energy or amino acid digestibility, with an increase in starch gelatinization from 0 to 20%. Increasing particle size from 470 to 1240 µm had a positive effect on 22 to 42 d growth performance. A starch gelatinization level of 20% or lower had no effect on metabolizable energy or amino acid digestion. Older broilers with larger gastrointestinal tracts are unaffected by 20% gelatinized starch; whereas, 20% gelatinized starch reduced blood glucose and increased glucagon levels of young broilers. Lower blood glucose and increased glucagon are indicative of lower glucose storage, and could cause reduced young broiler growth performance when fed diets with gelatinized starch.

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Dedication

I would like to dedicate this dissertation to all those who helped me with achieving my degree, and aiding me in surviving graduate school.

Chapter 1 - Literature Review

Introduction

Commercial meat-type *Galliforms*, broiler chickens and turkeys, are domesticated descendants of birds that naturally consumes a diet based on seeds and supplemented with insects and small animals. Broiler chicken and turkey behavior, nutrient requirements, and gastrointestinal tract characteristics provide evidence of ancestral diets. If *Galliforms* consumed exclusively seeds, their protein requirements would be lower, because seeds are high in energy but are low in protein. Poultry's scratching, hunting, and pecking behavior displays a need to uncover potential seeds and find small animals and insects. While all birds lack teeth, granivores, omnivores, insectivores, and herbivores have a small proventriculus and large ventriculus or gizzard (Denbow, 2000). A two-stomach design allows poultry to grind feed, reducing feed particle size and increasing surface area. In a commercial poultry barn, broiler chickens and turkeys are fed diets that are ground and sometimes heat processed. Commercial poultry feed is provided in a feed pan; therefore, broiler chickens and turkeys do not need to scratch and peck to obtain feed. Processing feed and feeding poultry in a feed pan does not prevent broiler chickens and turkeys from displaying feeding behaviors or cause broiler chickens and turkeys to change to a one-stomach gastrointestinal tract. Therefore, broiler chicken and turkey innate behaviors and gastrointestinal tract design must be taken into consideration to maximize economic returns. This review will focus on how basic traits of broilers and turkeys can be used to increase nutrient utilization and promote economic growth.

Behavior and Physiology of Feeding

Behavior Evolution

The ancestor to modern chickens, the Jungle fowl will engage in group feeding. Broilers retained this behavior and will engage in allelomimetric (group) feeding, as indicated by satiated broilers consuming feed if the entire group is feeding (Hughes, 1971). Commercial poultry display ancestral behaviors, but time spent doing each behavior has altered with increased growth rates. Chickens growing at a rapid rate consumed more feed, increased the number of

meals, and increased meal times (Barbato et al., 1980). Research comparing feeding strategies of Red Jungle Fowl, a non-commercial breed of chicken, and Hy-Line commercial egg layers revealed that commercial egg layers will consume more feed from a standard free-food system versus a system of food mixed with wood shavings (Schu□ltz and Jensen, 2001). In contrast, non-commercial strains of chicken will consume more feed from a mixed food than from a free food source, indicating intensive breeding has altered behavior. Decreased feed availability will increase broiler aggressive and eviction behaviors at the feeders (Murphy and Preston, 1988). Eviction behaviors were defined as pulling on feathers, hopping over other birds, and going under other birds in order to gain access to feeders. Broilers were calmer with full feeders versus feeders with low levels of feed. Chickens have a social hierarchy, and evicted broilers will evict broilers that are lower on the social hierarchy.

Ground hunting behavior is well documented in Jungle Fowl, but breeding broilers for high body weight gains (BWG) and efficient growth has altered behavior. Broilers' spend more time inactive and less time engaging in a ground foraging behavior than Jungle Fowl (Lindqvist et al., 2006). Weeks et al. (2000) observed that 5 wk old broilers spend around 76 to 80% of their time resting, 5.3 to 5.5% of their time eating, and 3.4% of their time drinking. Broiler leg quality differences were stated as the reason for the large variation in standing time, accounting for disparity in time spent resting. Bokkers and Koene (2003a) reported that fast growing broilers spend more time feeding and resting than slow growing broilers, which caused discrepancies in growth rates. Time spent feeding and resting alters broiler BWG and feed conversion (FC), which affects profitability. Male broilers have been documented to spend more time consuming feed during 4 to 5 wks of age than male layer chicks (Bokkers and Koene, 2003b). When comparing four different genetic strains, the fastest growing broilers consume more feed per trip and made fewer number of trips to the feeder (Howie et al., 2009). Genetic changes to broiler chicken weight gains and feed efficiency have resulted in increased feed consumption per trip and reduced the amount of energy spent traveling to feeders.

Sensory Effects on Feed Consumption

Commercial poultry are sight-based feeders, choosing feed based on color and size. Chagneau et al. (2006) observed broilers prefer larger and lighter colored feed. Broiler chicks

between 8 to 16 d of age prefer feed particles between 1.18 mm and 2.36 mm in diameter, and broiler chicks over 16 d of age prefer feed particles over 2.36 mm in diameter (Portella et al., 1988b). Birds adjust their preference to larger particles during growth, reducing energy exerted to obtain feed. Laying hens have shown a similar preference for larger feed particles (Portella et al., 1988a). Other researched observed that laying hens consumed particles larger than 1,180 μm when fed mash, leaving a higher proportion of smaller particles in feeders, 60.2%, than was observed in the hopper, 46.8% (Tang et al., 2006).

Sight is the most important sense for broiler feed consumption, but smell and chemical signals have been shown to alter feed consumption. Porter et al. (2002) blocked nasal passages of 2 d old broiler chicks with acrylic cement. Broiler chicks with both nasal passages blocked had decreased BWG when compared to control or broiler chicks with only one nasal passage blocked. A decreased ability to breathe may have been partially responsible for decreased gain, but decreased response to olfactory stimuli (mint oil) demonstrated that reduced olfactory sensation was responsible. Fatty acid profile can effect bird feed consumption, with male white Leghorn chicks preferring diets containing long-chain fatty acids over diets containing medium chain fatty acids (Furuse et al., 1993). At 400 minutes after broilers were fed, feed intake for the diet containing long-chain fatty acids was over double of diets containing C-8 or C-10 fatty acids.

Self-Selection

Commercial birds are fed a complete feed that is formulated to provide all the nutrients required for optimal growth. Research has been conducted to test the commercial fowl's ability to self-regulate nutrients for optimal growth, which would eliminate the need for mixing a complete feed. Four wk old commercial strain turkeys fed a split-diet will consume a diet that is higher in protein, 36 % CP, than control birds fed a standard diet with 28.4% CP (Leeson and Summers, 1978). The split-diet was defined as giving the turkeys a choice between a high protein feed or a high energy feed. Split-diet fed turkeys also had decreased body weights during first 8 wks of age. At 19 wks of age split-diet fed females were similar to control fed females, and males fed split-diets had larger body weights when compared to control fed males. Feed conversion was similar for both sexes over the entire experimental period. This indicates that

turkeys can select diets that provide required nutrients for growth, but will select a high protein diet not conducive to economic growth. It should be noted that the high energy diet was primarily cereal grains and high protein diet was primarily soybean meal.

Broiler chicks allowed to self-regulate dietary protein intake by selecting between high and low protein diets are lower in BWG compared to broiler chicks fed a standard, 23 % protein control diet (Elkin et al., 1985). Follow up research involving feeding broiler chicks a standard diet, or feeding a choice between a high protein-low energy diet and a low protein-high energy diet, found similar results with decreased BWG with choice feeding (Siegel et al., 1997). Feed conversion was poorer in choice fed birds when compared to standard diet fed birds. When given a choice between a pelleted or mash protein concentrate and whole, cracked or ground corn, broilers will consume more of a protein concentrate when it is pelleted (Yo et al., 1997). Broilers spent less time consuming the pelleted protein concentrate versus the mash protein concentrate. This difference in feed consumption time led to increased crude protein consumption with a pelleted protein concentrate. Whole corn feeding resulted in less time spent consuming corn and less total corn consumption compared to ground or cracked corn. Broilers will consume more corn in the first hour when given a choice between corn and a pelleted soybean meal and fish meal protein concentrate (Yo et al., 1998). After the first hour, broilers will consume more of the protein concentrate. In comparison with control diet fed broilers, broilers fed choice diets had lower 42 d old body weights. Sequential feeding broilers a high energy and a high protein diet or a standard diet over a 48 hr period resulted in similar 10 to 29 d of age BWG as the control fed broilers (Bouvarel et al., 2008). No differences were noted in carcass characteristics between sequentially fed or control fed broilers. Overall FC was poorer for the sequentially fed broilers treatments when compared to the control.

Broilers given a choice between a protein supplement and cereal grain source consume similar amounts of energy but lower amounts of lysine than control fed broilers (Sinurat and Balnave, 1986). Low lysine consumption resulted in poorer BWG and FC at 21 d of age. This effect was not seen in 44 d of age broilers, indicating that broilers will correct for lower lysine consumption. Young poultry will adjust their feed intake if there is an excess of an ingredient and an example is a synthetic amino acid. When added to a 23% CP diet at 4% above

requirement, crystalline amino acids reduced broiler chick growth (Edmonds and Baker, 1987). Commercial broilers reduced overall protein intake when given a choice between a high protein and a high energy feed if there is an excess of an amino acid (Robel, 1973). Broilers reduced intake to reduce energy expenditure associated with catabolism of excess amino acids.

Reacting to nutrient levels has allowed *Galliforms* to choose feed for maximal chances of survival and reproduction. Broiler chicks will alter nutrient consumption when given a choice of adequate or deficient lysine diets; however, broiler chicks do not consume sufficient quantities of lysine adequate diets to have similar body weights as control chicks (Newman and Sands, 1983). Broiler chicks offered a deficient diet and access to crystalline L-lysine were observed to have body weights 58-62% of control body weights, indicating importance of providing feed in a manner that prevents segregation.

Effects of Feed Form on Behavior

Differences in feeding time have been reported when comparing a mash diet to a pelleted diet, with less time devoted to feeding when commercial broilers were fed pelleted diets versus mash diets (Savory, 1974). Reduced feeding time resulted in significantly larger birds at 40 d of age with broilers fed pelleted diets compared to mash fed broilers. Laying hens fed a pelleted diet have been observed to spend less time feeding than birds fed a mash diet (Vilariño et al., 1996). Nir et al. (1994) observed that broilers fed pellets spent more time resting and less time consuming feed. Broilers consuming pelleted feed spent less time eating, and more time resting and drinking than broilers fed mash feed (Skinner-Noble et al., 2005). Broilers fed pellets had the best BWG and FC from 33 to 44 d of age.

Changes in feed form can affect bird feed consumption. For instance turkeys consumed less feed for 20 minutes after switching from a cumbled feed to a pelleted feed when compared to turkeys that were not switched diets (Lecuelle et al., 2010). Behavior suggested that turkeys were spending time investigating the feed via exploratory behaviors such as feeder scratching, dropping feed, and scattering feed. Switching feed form from mash to pellets reduced the number of broiler chick pecks per second, and even different forms of pellets demonstrated an effect (Martaresche et al., 2000). Cylindrical pellets had a lower broiler chick pecking rate than

pellets that were semiovoid shaped, and this only occurred when feed form changed. After adaption to pelleted diets, no difference was observed in broiler chick pecking rate. Feeding is an autonomic behavior and a sensory responsive behavior indicated by broiler and turkey responses to changes in feed form.

Feedback Control of Intake

It has been observed that injecting concentrated plasma via intracerebroventricular (ICV) injection from free feeding broilers into starved cockerels decreased feeding behavior, indicating satiety was detected by the experimental cockerels (Skewe et al., 1986a). Follow up work involved separating the plasma harvested from *ad libitum* fed cockerels by gel filtration into fractions differing in molecular weight, varying from <1500 molecular weight to >5000 molecular weight (Skewes et al., 1986b). There was a reported reduction in feed consumption with the injection of <1500 molecular weight plasma, but not with the larger molecular weight plasma. This suggests that the results from the previous trial were due to the plasma components with low molecular weight not the plasma components with a high molecular weight.

A possible explanation could be nutrients in the plasma; however, intrajugular and intrahepatic injections of free amino acids have been shown to have no effect on short-term feed consumption of Single Comb White Leghorns (SCWL) or broilers (Lacy et al., 1986a). Intrahepatic injections of lipids into SCWL reduced feed intake at the 3 hr measurement, and a negative linear effect of intrahepatic injection on feed intake was observed. No differences were observed in broilers, indicating genetic selection for growth has altered satiety receptors in fast-growing broilers. When fatty acids are oxidized for energy it can result in ketone bodies, and when β-hydroxybutyrate (β-HB), a ketone body, was injected via ICV at a level of 1.5 g/kg body weight, broiler chick feed intake was reduced 30 minutes after injection (Sashihara et al., 2001). Similar results were observed when β-HB was injected at a level of 1.00 mg/μl, with reduced broiler chick feed intake at 30 and 60 min post injection. When a fatty acid oxidation inhibitor, mercaptoacetate (MA) was injected via ICV, broiler chick feed intake was reduced and high injection levels of MA resulted in mortalities. These results indicate that ketone bodies inhibit feed intake and not fatty acid oxidation. Fatty acid oxidation may have no direct effect on broiler chick feed intake. Propionic acid has been shown to decrease broiler chick nutrient intake to 80

% of control intake when intubated at a level of 600 mg/kg body weight (Pinchasov and Jensen, 1989).

Commercial poultry behavior has changed because of intensive genetic selection. Broilers prefer feeding from feeders to foraging from the litter, and will become aggressive if there is feed restriction. A preference for larger particles was observed in broilers and broiler particle size preference increases with age. When given a choice, turkeys will choose a diet with higher protein, while broilers prefer a diet with higher energy. Reasons for energy selection could be due to a higher fat content. Broilers select feed containing longer chain fatty acids over those containing shorter chain fatty acids. Standard diet fed broilers outperformed choice fed broilers, demonstrating a need to prevent broilers from sifting and sorting through feed. Pelleting feed can prevent sorting and will reduce time spent feeding. Reducing the feeding time of broilers will reduce energy expended, because of increased resting time of broilers. Reduced feeding time decreases feedback inhibition from feed, and broilers will increase their protein and energy intakes. Increases in feed intake will result in larger broilers, and reduced energy expenditures will lead to more efficient growth and better economic returns.

Broiler Gastrointestinal Growth and Development

Avian embryos develop in an external egg, without the benefit of a continuous maternal nutrient supply. Eggs contain all of the nutrients an embryo requires for development, hatching, and first hours of life. Avian neonates are split into two groups, altricial and precocial. Chickens and turkeys are precocial and are able to obtain, consume, and digest an adult diet consisting of cereal grains and legume proteins. Altricial young are dependent on the parents for nutrients, and song birds are a common example. Young chicks or poults can digest and utilize nutrients from an adult diet post-hatch, but their gastrointestinal tract is not mature. This next section will discuss how the gastrointestinal tract of young poultry grows, develops, and matures.

Gastrointestinal Growth

Body weight gain of broiler chicks is supported by a growing and maturing gastrointestinal tract. Research has been conducted to determine when the gastrointestinal (GI) tract reaches a size, relative to the rest of the body, which allows for proper growth and

development. Early research reported that the gastrointestinal tract relative size of broiler chicks peaks at around 8 d of age (Dror et al., 1977). In comparison with other GI organs, the small intestine was reported to have the highest relative weight at 3 d of age. Intensive breeding and selection has changed broiler chicken genetics, and later research observed that broiler chick pancreatic and small intestinal relative growth maximizing at 8 d of age, with liver mazimizing at 12 d of age (Nitsan et al., 1991). Nir et al. (1993) reported that broiler chick small intestinal relative weights peaked at 5 d of age post-hatch and decreased; whereas, broiler chick pancreas weights maximized at 5 d of age and plateaued instead of decreasing. In accordance with previous research, liver relative weights maximized at 10 d of age and decreased with broiler chick growth. In confirmation with previous work, Dunnington and Segel (1995) reported that broiler chick GI tract relative weights peaked at approximately 8 d of age.

In conflict with previous work, Iji et al., (2001a) reported broiler chick liver relative size remains constant, while gizzard and pancreas relative weights are largest at hatch and decreased as the chick grows. However, the small intestine reached maximum relative size around 7 d of age, in agreement with previous work. Broiler chick intestinal length increased with age, and duodenal length increased to 1.2 cm during first 7 d, but only increased in length to 1.3 cm by 21 d of age. The jejunum length increased to 1.6 cm and the ileum length to 1.5 cm during first 7 d of life, however, the jejunum and ileum will only increase to 1.7 cm in length by 21 d of age. Feeding diets that are diluted with wheat bran, to reduce protein and metabolizable energy, to broiler chicks resulted in decreased proventriculus, gizzard, liver, pancreas, and intestinal weights at 6 d of age (Picard et al., 1999). This was associated with decreased weight gain and no relative organ weights were reported. Similar results in decreased broiler chick live weight, gizzard weight, and liver weight were observed at 11 d of age, indicating protein and metabolizable energy levels affects chick organ weights and body weight.

Research with turkeys showed similar patterns, with small intestinal and pancreatic relative weights increasing until 6 d of age (Sell et al., 1991). Unlike the intestine and pancreas, poult gizzard relative weight maximized at 4 d of age and proventriculus weight increased until end of experimental period, 8 d of age. Pinchasov and Noy (1994) found that turkey poults relative GI tract weights maximized at 4 d post-hatch. Later, poult research reported that relative

intestinal weight peaked at 7 d of age, gizzard relative weight continually increased, and pancreatic relative weight remained constant (Uni et al., 1998). Corless and Sell (1999) agreed with previous work, observing maximum relative GI weights at 4 to 5 d of age.

Intestinal Development

As the small intestine increases in size, it matures by increasing surface area. Increased surface area increases nutrient absorptive area and is an indicator of a healthy small intestine. Small intestinal surface area increases by intestinal villi growth. Research of intestinal villi growth reported an increase in broiler chick villus volume in all sections of the small intestine from 4 to 10 d of age (Uni et al., 1995). Interestingly, enterocyte density did not display the same trend with duodenum density decreasing with age and jejunum density increasing. Broiler chick duodenum villi were the largest at all measurement ages, but had lower enterocyte density when compared to the jejunum. In agreement with previous research, broiler chick villus height was observed to double by 7 d of age, with villus width and perimeter following a similar pattern of doubling over the first 7 d post-hatch (Uni et al., 1996). Likewise, broiler chick intestinal villus perimeter and crypt depth doubled by 7 d post-hatch. An increase in intestinal villi perimeter increases nutrient absorptive area, permitting efficient access to nutrients. Uni et al. (1998a) observed that broiler chick villus height and volume increased in all three intestinal sections, with the duodenum and jejunum having a higher rate of increase when compared to the ileum. Follow up research reported increases in broiler chick intestinal villus volume for all sections, similar to previous trials (Uni et al., 1998b). Broiler chick intestinal crypt depth was reported to increase from 0 to 15 d post-hatch and only jejunum enterocyte density increased from 0 to 15 d of age.

Iji et al. (2001a) continued research into broiler chick intestinal development and documented duodenum and jejunum crypt depths being larger at 21 d of age versus 1 d of age. There were no reported differences in crypt depth for the ileum between 1 and 21 d of age. Villus height increased from 1 to 21 d of age for all sections of the small intestine, with duodenum and jejunum villus height being larger than ileum villus height. Villus surface area had a similar pattern as villus height. Geyra et al. (2001) investigated 48 h post-hatch broiler chick intestinal development, and observed that crypts per villus and number of cells in the

crypts increase rapidly during first 48 h post-hatch. Enterocytes matured quickly during the first 48 h with rapid increases in enterocyte length and intestinal brush-border membrane development. While enterocyte and crypt maturation are similar across the broiler chick small intestine, villus surface area increased most rapidly in the duodenum and did not plateau until after the jejunum or ileum surface area. Poult villus development is similar with higher rate of surface area increase for the duodenum and the jejunum when compared to the ileum (Noy et al., 2001). In contradiction to previous research, cell proliferation in the villi was observed to increase through the end of the experimental period, 9 d of age. Literature reports the rapid development and adjustment of the small intestine to an external diet during the first hours and days post-hatch.

Maturation of Enzymatic Secretions

Pancreatic Enzymes

Pancreatic and intestinal enzyme levels fluctuate as broilers and poults grow and develop. Pancreatic enzyme secretions will change to accommodate an external diet that is high in non-structural carbohydrates. Early research reported an increase in amylase and protease levels during broiler embryonic development with a rapid increase days before hatching (Marchaim and Kulka, 1967). The rate of increase slowed after hatch, but enzymatic levels were still increasing at the end of the study period, 3 d post-hatch. Later research found similar results with broiler chick amylase and lipase peaking activity relative to body weight at 8 d of age with trypsin and chymotrypsin relative activity peaking at 10 d of age (Nitsan et al., 1991). Turkey poults had a continual increase in relative pancreatic amylase and trypsin activity until the end of the experimental period, 8 d of age; however, pancreatic lipase relative activity peaked at 6 day of age (Sell et al., 1991). Nir et al. (1993) reported that broiler chick trypsin and chymotrypsin relative activity levels continually increased as birds grew. Amylase relative activity peaked at 5 d of age and trypsin relative activity peaked at 12 d of age. Similar to pancreatic amylase relative activity, intestinal amylase relative activity peaked at 5 d of age. Small intestinal lipase relative activity peaked at 8 d of age, similar to other enzymes.

Pinchasov and Noy (1994) reported that poult amylase activity level was highest at 1 to 2 d post-hatch, decreased, and then increased to reach post-hatch levels at 10 d of age. Starch

digestion rates in the gizzard and lower small intestine maximized at 2 d of age. Poult crop digestion rates increased throughout the experimental period and ceca digestion rates decreased. Since upper small intestine rates were not measured, a conclusion of increased digestion and absorption rates in upper small intestine was reached based on decreased cecal and lower small intestine starch digestion. Uni et al. (1995) found no age differences in broiler chick amylase secretion per g of feed, but trypsin secretion per g of feed decreased after 4 d of age, and lipase secretion was lower at 14 d of age than at 4 d of age. Protein digestibility increased with age, but starch digestibility was high at 4 d of age, between 90 and 95%, and remained constant. In contrast to previous work, Dunnington and Siegel (1995) observed that broiler chick enzymatic relative activity levels for pancreatic amylase, trypsin, chymotrypsin, and lipase increase from 6 to 15 d of age.

Brush-Border Enzymes

Broiler chick intestinal sucrase and maltase relative secretions were observed to peak at 2 d post-hatch; whereas, alkaline phosphatase (AP) relative secretions peaked at 1 d post-hatch in the duodenum and at 4 d post-hatch in the jejunum (Uni et al., 1998a). Further work reported that broiler chick duodenal brush border enzymes increase first few days post-hatch and then plateau at d 3 or 4 post-hatch (Uni *et al.*, 1998b). Iji *et al.*, (2001b) reported that broiler chick brush border enzymes, maltase, sucrase, aminopeptidase N (APN), and AP decrease in concentration (μ mol product/mg protein/min) from hatch to 21 d of age, in all three small intestine sections. While brush border enzyme concentrations decreased, total activities (μ mol product/min) increased in all intestinal sections. When enzyme activity was measured per unit area ($Ab/\mu m^2$) at 1 d and 21 d of age, α-glucosidase, APN, and AP activities were similar in all sections with the exception of AP decreasing in the jejunum.

Absorption

Research investigating changes in broiler chick absorption rates reported that L-tryptophan absorption rate decreased with chick age (Iji *et al*, 2001c). Similar results were observed in ileum absorption rates, but absorption rates were higher in the ileum than the jejunum. Rate of absorption decreased but total absorptive capacity increased with age, allowing

for the support of growth and a larger body weight. Broiler chick glucose absorption has been documented to increase from less than 50% to over 80% of consumed glucose, supporting high starch digestibilities previously reported (Noy and Sklan, 2001).

Broiler chicks and turkey poults adjust rapidly to commercial poultry feed. Gastrointestinal tracts and accessory organs increase rapidly in relative size during the first week to two weeks of life and then plateau. Rapid increases in size allow chicks and poults to digest and absorb feed nutrients. As the small intestine increases in length and mass, intestinal villi grow and mature. Intestinal villi growth increases the absorptive area of the intestine, which increases absorptive capacity of growing broiler chicks and poults. Growth of the intestine and the intestinal villi is supported by increasing digestive enzymatic secretions. Increasing enzymatic secretions by the pancreas and the intestine allows chicks and poults to efficiently break down non-structural carbohydrates, lipids, and proteins. Efficient enzymatic degradation of diets enables commercial poultry to have rapid and efficient body weight gains.

Starch Formation, Gelatinization, Digestion, and Glucose Absorption and Regulation

Cereal grains comprise more than half of poultry rations and are the major energy source in diets. Most of the energy stored in cereal grains are carbohydrates, specifically starches, with some stored as protein and lipids. Cereal grains convert CO_2 and H_2O into glucose utilizing solar energy, through a process called photosynthesis. What is important to poultry growers is what happens to the glucose after formation, because it can be stored as cellular wall material, cellulose, or as energy storage, starch (Banks and Muir, 1980). Monogastric animals, such as poultry, cannot digest cellulose but they do have the ability to digest starch. The basic difference between cellulose and starch is the type of bonds between glucose molecules. Cellulose has β-bonds and starch has α-bonds. Starch is comprised of two types of α-bonds, α-1,4 a linear bond, and α-1,6 a branching bond. Alpha-bond ratios determine the type of starch polymer, with amylose being almost entirely linear bonds, and amylopectin has high numbers of both bonds, creating a highly branched structure (Banks and Muir, 1980; Moran Jr., 1982; Smith, 2001; Carre, 2003; Tester et al., 2004a; Tester et al., 2004b). Major properties of amylose and amylopectin are shown in Table 1.1. Amylose has the ability to form a double-helix (Moran Jr.,

1982) and can form complexes with lipids (Tester et al., 2004a). Whereas, amylopectin forms a cluster-like arrangement, and combining these two structures allows for the formation of starch granules (Smith, 2001).

Starch

Glucose Polymer Formation

The two different glucose polymers start from the same source, sucrose (Smith, 2001; Tester et al., 2004a). Sucrose is broken down into uridine diphosphate glucose (UDP-glucose) and fructose via sucrose synthase (Ball, 1995; Buléon et al., 1998a; Denyer et al., 2001; Emes et al., 2003; Smith et al., 1997; Smith, 2001; Tester and Karkalas, 2002). Conversion of UDP-glucose to glucose-1-phosphate is facilitated by UDP-glucose pyrophosphorylase (Smith, 2001). In cereal grains, glucose-1-phosphate is converted into ADP-glucose in the cytosol, and then transported via specific transporters into the amyloplast, where it is converted into starch (Denyer et al., 1996; Shannon et al., 1998; Ballicora et al., 2000; Beckles et al., 2001). In the amyloplast starch polymer formation is controlled by enzyme types and levels.

The two basic classes of starch synthesis enzymes are starch synthase and starch branching enzyme, and different enzyme isoforms determine type and shape of the starch polymer formed (Kosssman et al., 1999). Four starch synthase isoforms have been discovered; granule bound starch synthase (GBSS), SSI, SSII, and SSIII, and two isoforms of starch branching enzymes, SBE-A and SBE-B (Boyer and Priess, 1978; Burton et al., 1995; Abel et al., 1996; Craig et al., 1998; Gao et al., 1998; Harn et al., 1998; Knight et al., 1998; Edwards et al., 1999; Jobling et al., 1999; Smith, 2001). For amylopectin synthesis, the important starch synthase enzymes are SSII and SSIII, which work in combination with starch branching enzymes SBE-A and SBE-B. Starch synthases, SSII and SSIII, form branch chains working in conjunction with starch branching enzymes, SBEA and SBEB, to form a pre-amylopectin polymer. This form of amylopectin is not sufficiently organized to form a granule and is trimmed by starch debranching enzymes or isoamylase, allowing amylopectin polymer formation. Amylose synthesis relies on GBSSI, and the lack of this enzyme causes a “waxy” (almost pure amylopectin) starch formation. Most cereal starch granules contain both polymers, unless it has been genetically selected for waxy starches or high amylose starches.

Granule Formation

Cereal grains store starch in the form of a granule. Granules are composed of the polymers of starch, amylose and amylopectin, which comprise 98-99% of the dry weight (Tester *et al.*, 2004a). Differences in amylopectin branch chain length, distribution, and clusters, form a semicrystalline matrix (Smith, 2001). Adjacent glucose chains form double helices within the clusters, forming the crystalline lamellae between branching points of the amylopectin. Alternating between amorphous and crystalline lamellae creates "growth rings" (Figure 1-1).

Amylose and amylopectin polymers interact to form a starch granule's crystalline structure (Tester et al., 2004a). Cereal amylose, has an A-type crystalline structure; whereas, tuber amylose has an B-type crystalline structure (Banks and Muir, 1980; Moran Jr., 1982; Banks and Greenwood, 1975; Sarko and Wu, 1978; French, 1984; Veregin et al., 1986; Blanshard, 1987; Gidley and Bulpin, 1987; Zobel, 1988a,b, 1992; Gernat et al., 1990; Imberty et al., 1991; Cairns et al., 1997; Biliaderis, 1998; Buléon et al., 1998a,b; McPherson and Jane, 1999; Sevenou et al., 2002; Tester et al., 2004a, 2004b) . Starch in typical poultry diets is cereal, or A type, and will be the focus of this review. The A-type structure has a uniform distribution of water, affecting granule swelling properties (Figure 1-2). Typical corn starch granules contain between 20-35% amylose, with the remainder consisting of amylopectin (Cluskey et al., 1980; Tester et al., 2004a). Corn starch granules are usually between 2-30 µm (Tester et al., 2004a) in diameter, with Cluskey et al. (1980) reporting an average of 11 µm. In cereal starch, lipids can bind to the amylose but only compose ~1% at the greatest level (Tester et al., 2004a). Crystallinity is determined by the amylose interacting with amylopectin chains (Tester et al., 2004a) forming the lamellae (Figure 1-1). This crystalline structure enables plants to store energy in a compact form, allowing for large amounts of energy to be stored in a limited space.

Gelatinization

Starch granules are designed to store energy until released by enteric amylases. If heat and moisture are applied to starch granules, the granules will swell and its crystalline structure will break down in a process called gelatinization. Water can penetrate the surface structure of starch granules, but heat is required for water to fully penetrate the crystalline lamellae (Moran,

1982). Gelatinization can only fully occur when sufficient heat and water are present and is defined as the swelling of starch granules with the addition of heat and water (Banks and Muir, 1980; Tester et al., 2004b). As the temperature increases, starch granules lose their organized form and continued heating prevents reversal. Gelatinization causes a disruption in the hydrogen bonds of the granule and the granule will lose its shape (Moran, 1982). In excess water, the onset of gelatinization occurs at ~45 ^{o}C and the process is complete at 75 ^{o}C, but onset and completion temperatures will be higher with limited moisture (Tester et al., 2004b). Limiting moisture level causes only some of the granules to gelatinize. Cereal starch granules swell during gelatinization, but typically do not burst (Banks and Muir, 1980). Figure 1-3 displays what happens to corn starch granules during the gelatinization process. During manufacturing of feed, some starch gelatinization will occur, altering poultry feed chemically and physically.

Starch Digestion and Glucose Absorption

Broilers and turkeys do not masticate; therefore, do not secrete any salivary enzymes to aid in digestion. Birds use their beaks for food apprehension, and quickly swallow. No enzymatic digestion will occur until the food reaches the small intestine, but in more mature birds small amounts of microbial digestion may occur while food is in the crop. Acid hydrolyzation of starch will occur in the proventriculus and the gizzard but is not considered a major method of starch degradation. Wild fowl have adapted their enzymatic output to dietary starch levels, with granivores excreting large amounts of amylase (Kohl et al., 2011). Osman (1982) observed chicken jejunum amylase levels approximately ten times higher than ileum or duodenum levels. Since chickens are granivores and consume high starch diets, intestinal and pancreatic amylase is secreted. Broiler chick jejunum tissue amylase levels are higher than the other intestinal sections, but when compared to intestinal content levels, duodenum levels are similar to jejunum (Osman, 1982). Riesenfeld et al. (1980) observed that the majority of glucose absorption occurred in the jejunum in both 3 wk and 7 wk old broilers. Older broilers absorbed more glucose in the ileum than younger broilers, but over 80 % of glucose was absorbed prior to the ileum. High amounts of amylase has led to measurements of total tract starch digestibility of 95 to 98% for the two commonly fed cereal grains in the U.S., corn and sorghum (Weurding et al., 2001). How broilers achieve high starch digestibility will be explored in the next section by discussing the actions of amylase, brush border saccharidases, and glucose transporters.

Amylase Type, Structure, Active Site, and Mode of Action

In poultry, the pancreas and small intestine secrete amylase, but are not identical in structure or optimal pH. Osman (1982) reported that the optimal pH for intestinal amylase was lower at 6.9, than pancreatic amylase at 7.5. Amylases differed on response to chloride (Cl), with Cl required for pancreatic amylase activity, and intestinal amylase does not require Cl but had improved activity when Cl was present. Pancreatic amylase activity improves with increased starch concentrations, and intestinal amylase has a higher relative activity level at lower starch concentrations. While there are differences in intestinal and pancreatic amylase, this review will concentrate on pancreatic amylase due to higher importance to starch digestion and the availability of literature.

While pancreatic amylase is referred to as a general enzyme, according to Lehrner and Malacinski (1975) there are several different α-amylase phenotypes but all have an approximate molecular weight of 55,000 kDa. A study of the location of pancreatic α-amylase on the chicken chromosomes revealed two distinct loci locations, suggesting evolution of pancreatic amylase (Benkel et al., 2005). While different amylases do exist, the active site of porcine α-amylase is similar to chicken pancreatic α-amylase. Early research into the activity of pancreatic amylase revealed a dependency on Cl (Julian et al., 1968; Wakim et al., 1969; Osman, 1982). Research revealed the active site of mammalian α-amylase contains four Cl^- and one calcium (Ca^{2+}) ions, which alter active site shape and aid in substrate recognition (Ishikawa et al., 1993; Qian et al., 1993, 2001, 2005; Bompard-Gilles et al., 1996; Machius et al., 1996; Numao et al., 2002; Payan and Qian, 2003; Ramasubbu et al., 2003; Maurus et al., 2005).

Pancreatic α-amylase hydrolyzes α-bonds within a starch granule from the reducing end, and produces several different lengths of saccharides (Robyt and French, 1970a, 1970b; Chan et al., 1984; Mazur and Natatani, 1993; Kandra et al., 1997). Alpha-amylase interacts with the reducing end of an amylose chain by binding to five glucose units, hydrolyzing the bond between the second and third glucose unit, and creating a di- or trisaccharide (Robyt and French, 1970a; Chan et al., 1984; Mazur and Natatani, 1993; Kandra et al., 1997). This reaction is not perfect and will result in saccharides of multiple chain lengths. Saccharides of different chain lengths

are relatively minor and α-amylase degradation usually results in maltose and alpha-limit dextrins.

Brush Border Enzymes

In order for starch glucose to be absorbed by enterocytes, products of α-amylase must be broken down into glucose by the brush border enzymes. A survey of chicken brush border enzymes revealed sucrase, isomaltase, and maltase, with minor amounts of lactase being attributed to microorganisms (Siddons, 1969; Siddons and Coates, 1972). The optimal pH of disaccharidases is 6, with isomaltase being the most sensitive to pH change (Mizuno et al., 1982). Sucrase is designed to cleave sucrose into fructose and glucose, but has been shown to hydrolyze maltose into two glucose units (Matsushita, 1983; Rodriguez et al., 1984; Heymann et al., 1995). Isomaltase is responsible for hydrolyzing α-1,6 bonds commonly found in amylopectin (Larner and Gillespie, 1956, 1957; Larner and Schliselfeld, 1956; Siddons, 1969; Siddons and Coates, 1972; Prakash et al., 1983; Rodriguez et al., 1984; Shinohara et al., 1993; Heymann et al., 1995). Maltase is responsible for cleaving maltose into two glucose units (Larner and Gillespie, 1956, 1957; Larner and Schliselfeld, 1956; Siddons, 1969; Siddons and Coates, 1972; Prakash et al., 1983; Biviano et al., 1993). Sucrase-isomaltase is anchored to the intestinal brush border via a hydrophobic amino acid sequence, which is anchored in the enterocyte luminal membrane (Frank et al., 1978; Brunner et al., 1979). Intestinal saccharidases are anchored to the brush border membrane, allowing rapid absorption of glucose released from starch or other saccharides in the intestinal lumen.

Glucose Absorption

After starch has been hydrolyzed into glucose, it is absorbed by enterocytes and transported into the blood stream for use as an energy source. Glucose is transported into enterocytes through two ways, active and passive transport, (Diamond et al., 1986; Levey and Cipollini, 1996; Fan et al., 1998) which will adjust to glucose level in diets (Karasov et al., 1996). Active transport is accomplished by SGLT1 (Hopfer et al., 1977; Eveloff et al., 1980; Hopfer and Groseclose, 1980; Peterlink et al., 1981; Kaunitz et al., 1982; Hoshi et al., 1986; Storelli et al., 1986; Wolffram et al., 1989; Stevens et al., 1990; Hirayama et al., 1991; Bennett and Kimmich, 1992, 1996; Shirazi-Beechey et al., 1994; Bindslev et al., 1997; Mizuma and Awazu, 1998; Mizuna et al., 1998; Garriga et al., 1999) and is dependent on dietary sodium (Na)

(Hopfer and Groseclose, 1980; Bindslev et al., 1997). Survey of SGLT1 genetics across species revealed a conservation of DNA coding, meaning similar SGLT1 structure across species (Pajor et al., 1992). Active transport by SGLT1 utilizes the Na and the electrochemical created by Na^+/K^+-ATPase, allowing for glucose absorption against the concentration gradient (Murer and Hopfer, 1974; Carter-Su and Kimmich, 1980; Eveloff et al., 1980; Storelli et al., 1986; Hoshi et al., 1986; Bennett and Kimmich, 1992, 1996). Glucose active transport is greater than passive transport (Amat et al., 1996; Fan et al., 1998). Levey and Cipollini (1996) found passive transport in Northern Bobwhite Quail allows for large amounts of glucose absorption. Passive transport has been shown to be facilitated by GLUT2 on the brush-border membrane, and concentrations increase with increasing glucose concentrations (Kellet and Helliwell, 2000).

Chicken intestines have the highest concentrations of SGLT1 in the jejunum, followed by the ileum and duodenum (Garriga et al., 1999; Mott et al., 2008). Glucose transport by SGLT1 allows for maximal glucose absorption, but does require energy in the form of Na^+/K^+-ATPase (Murer et al., 1974). The mechanism of SGLT1 is displayed in Figure 1-4, and SGLT1 requires 2 Na per glucose unit (Kaunitz et al., 1982). Research conducted on SGLT1 revealed adjustments in prevalence related to diet (Karasove et al., 1983; Shirazi-Beechey et al., 1994; Habold et al., 2005) and greater expression of SGLT1 and GLUT2 mRNA occurs with increased glucose content of diets (Miyamoto et al., 1993; Habold et al., 2005). Induced stress on chickens decreases SGLT1 mRNA expression, but increases GLUT2 mRNA expression; however, there is a limit to the increase and expression will decrease if there is enough stress (Li et al., 2009). Monensin is a common feed additive to poultry diets to control coccidiosis and has no effect on glucose transport (Riley et al., 1986). Yokota and Coates (1982) reported no difference in glucose transport in germ free or conventional chicks, and concluded normal microorganisms did not interfere with glucose absorption. Glucose is transported to the intestinal blood supply by the enterocyte using GLUT2 transporters, which also transport galactose and fructose (Maenz and Cheeseman, 1987; Miyamoto et al., 1993; Helliwell et al., 2000; Harold et al., 2005; Li et al., 2009).

Blood Glucose Regulation

Commercial poultry are granivores and regulate glucose differently than mammals. Broilers and turkeys have different levels of hormonal response, higher blood glucose levels, and different responses to hormones. Chicken blood glucose levels vary between 12 and 17 mmol/L depending on fed state (Rideau et al., 2008; Proszkoweic-Weglarz et al., 2009). As with mammals, the two glucose regulating hormones in chickens are insulin and glucagon. Insulin causes a reduction in blood glucose and glucagon causes an increase in blood glucose. Poultry level of response to glucose regulatory hormones is different than mammals. Simon et al., (1977) observed lower numbers of insulin and glucagon binding sites in chicken liver cells in comparison with rat liver cells. Poultry have high metabolic rates and this has led to glucagon being the main regulatory hormone instead of insulin (Hazelwood, 2000). Insulin and glucagon are released by the pancreas, with glucagon released by α-cells and insulin released by β-cells (Ruffier et al., 1998). Chickens can regulate blood glucose independent of pancreatic insulin, because blood glucose level of a depancreatized chicken will return to normal 5 d post operation (Colca and Hazelwood, 1976). Pancreatic remnants were observed to increase in size and could have aided in return of normal glucose levels; however, after 16 d the pancreas was still smaller than the original size. Later research reported a possible explanation, with chicken cells containing more of the insulin independent glucose transporter, GLUT8, than an insulin dependent glucose transporter, GLUT4 (Seki et al., 2003, 2005). Seki et al. (2005) continued the research and reported high expression of non-insulin dependent glucose transporters, GLUT1, GLUT2, GLUT3, and GLUT8 in broiler chicks. Even with low GLUT4, chickens will have a negative response to insulin removal with negative effects on glucagon secretion and regulatory pathways (Dupont et al., 2008)

After consuming a meal, intestinal glucose absorption increases blood glucose level, causing an increase in insulin level (King and Hazelwood, 1976; Hazelwood and Langslow, 1978; Simon and Rosselin, 1978; Smith and Hazelwood, 1981; Touchburn et al., 1981; McMurtry et al., 1983; Krestel-Rickert et al., 1986; Tokushima et al., 2003; Dupont et al., 2008; Rideau et al., 2008; Proszkoweic-Weglarz et al., 2009), and returning blood glucose to normal levels (Nir and Levy, 1973; Simon, 1980; Akiba et al., 1999). While insulin does decrease post-meal blood glucose level, avian β-cells are more sensitive to amino acids and glucagon than

glucose (Hazelwood, 2000). The small intestine will convert up to 38 % of absorbed glucose to lactate, aiding in glucose regulation by reducing the blood glucose spike associated with feeding (Riesenfeld et al., 1982). After fasting, blood glucose and insulin levels will decrease (McMurtry et al., 1983; Krestel-Rickert et al., 1986; Proszkoweic-Weglarz et al., 2009), and glucagon levels will increase causing a release of stored glucose (Hazelwood and Langslow, 1978; Ruff and Allen, 1982; Proszkoweic-Weglarz et al., 2009; Rideau et al., 2010). Richards and McMurtry (2008) found high amounts of glucagon receptors on the liver and abdominal fat, indicating increases in blood glucose are caused by the release of stored liver glucose (Dickson and Langslow, 1978; Tinker et al., 1984) and an increase in triglycerides is caused by abdominal fat (Kitabgi et al., 1976; Oscar, 1992, 1995). In poultry, glucose is stored as glycogen, a highly branched polysaccharide, which is easily converted back into glucose (Hazelwood and Barksdale, 1970; Ruff and Allen, 1982).

Insulin and glucagon interact to maintain a stable blood glucose level for use in metabolism and growth. Insulin causes a decrease in glucagon secretion (Sitbon and Miahle, 1978, 1979; Honey and Weir, 1979) and glucagon causes an increase in insulin secretion (King and Hazelwood, 1976; Hazelwood and Langslow, 1978; Honey et al., 1980). The liver is the primary glycogen storage organ, and liver glycogen levels increase in response to insulin and decrease in response to glucagon. Dupont et al. (1998) observed an increase in liver insulin receptors during fasting when compared to *ad libitum* fed chickens, indicating increased liver sensitivity to insulin when birds were re-fed. The liver removes glucose from blood by phosphorylation via glucokinase (GK), and experimentally induced increase in GK will decrease blood glucose, causing a decrease in insulin levels and an increase in glucagon levels (Rideau et al., 2010). Glucokinase increases in response to high amounts of glucose and insulin (Rideau et al., 2008). Edwards et al. (1999) observed that feed withdrawal causes increased glucagon and decreased liver glycogen. Liver gluconeogensis is increased by glucagon, and the glucose formed is released from hepatocytes to increase blood glucose level (Dickson and Langslow, 1978). The kidneys become an important source of glucose, via gluconeogenesis, when liver glycogen levels decrease after a multiple day fast (Tinker et al., 1984). Muscle glycogen levels were unaffected by fasting, signifying the liver is the major site of stored glucose during fasting.

Liver lipids are affected by glucose and hormonal levels, with increases in lipogenic enzyme genes with increasing blood glucose or insulin levels (Proszkoweic-Weglarz et al., 2009).

Glucose is the primary source of energy in poultry diets, and is supplied by cereal grain starches. Cereal grains form glucose via photosynthesis and glucose is stored as starch. Starch is formed to provide the seedling with energy for growth. Aves have developed the ability to access this energy using a combination of amylase and brush-border saccharidases. Amylase and brush-border saccharidases hydrolyze starch into glucose, allowing for intestinal absorption. Intestinal enterocytes absorb glucose by active and passive transport. Passive transport allows for rapid absorption when glucose is abundant and active transport enables the absorption of glucose against the concentration gradient. After transport across the luminal membrane, glucose can be catabolized for energy or converted to glycogen for energy storage. Two hormones regulate this process, insulin and glucagon. These hormones interact to maintain a constant supply of blood glucose. Poultry have the ability to regulate high levels of glucose by having a high number of insulin-independent glucose transporters. High blood glucose levels are indicative of poultry evolving to be on dependent glucose for energy, and evidence that cereal grains and seeds are an important part of a granivores diet.

Poultry Feed Processing

Commercial poultry feed for meat-type birds is processed to improve nutrient utilization and economics of growth performance. Meat-type poultry rations are comprised of cereal grains, vegetable or legume proteins, sometimes animal by-product protein, inorganic calcium and phosphorus, and vitamins and minerals. Cereal grains used in the U.S. are typically corn or sorghum, but in other countries wheat, rye, and barley are the primary cereal grains. Vegetable or legume proteins are typically by-products of plant oil industries and in the U.S. it is primarily soybean meal. Depending on price, soybean meal can be replaced with canola meal, peanut meal, sunflower meal, safflower meal, flax meal, and in certain cases lupin. Animal by-product meals are included at lower levels, usually poultry in origin, and price dictates product used. Animal proteins are unpopular with certain consumer groups and may be excluded from niche market poultry diets. Calcium and phosphorus sources are mostly mineral in origin and source is dependent on availability and price.

Because cereal grains are large, especially corn, they are ground to reduce particle size and increase surface area. Increased surface area enhances enzymatic digestion by increasing enzymatic access. After combining diet ingredients poultry feed is mixed for uniformity. Uniformity ensures poultry consume adequate nutrition and prevents problems of excesses or deficiencies. After mixing, diets are pelleted using a pellet mill. Pellet mills form pellets by mixing feed with steam and forcing the feed through a die, causing compression of the feed and pellet formation. This next section will discuss how grinding and pelleting affect broiler growth and development.

Grinding

In the U.S., feed for poultry is primarily ground in two ways, a hammer mill and a roller mill. Hammer mills are popular because of ease of maintenance, and operate by having a metal "hammer" hit a cereal grain at a high rate of speed, smashing the grain into smaller particles. Particle size is determined by screen size. Roller mills operate by having two or more large rollers that compress the cereal grain causing it to fracture and reduce particle size. After passing over two to three roller pairs a desired particle size can be achieved. By adjusting roller spacing, different particle sizes can be obtained. Hammer mills are the primary choice for grinding in the U.S. due to ease of maintenance. Poultry do not possess teeth for mastication but have a gizzard to grind their feed. A mature bird can easily digest a wild seed diet because of the ability to grind it to smaller sizes in the gizzard. Commercial broilers never reach maturity, but are still able to develop a gizzard capable of reducing the particle size of cereal grains.

Starter Growth Period

Because of the costs associated with grinding, several researchers have investigated if there is an optimal particle size. Smaller particle sizes require more electricity and decreases throughput of a feed processing mill. Broiler growth is typically separated into starter and grower periods because of different nutritional requirements, broiler body composition, and feed consumption levels. Because the chick is immature, there may be a relationship between particle size and function of the gizzard. Nir et al. (1990) ground sorghum to fine (540 to 580 µm), medium (670 to 740 µm), and coarse (870 to 910 µm) particle sizes, using a hammer or roller mill and diets were fed from 7 to 14 d of age. Broiler chicks fed finely ground sorghum diets

had the lowest BWG, but FC was similar to broiler chicks fed medium and coarse sorghum diets. Research into corn based diets reported that broiler chicks fed diets containing corn ground to a particle size of 900 µm had better BWG and FC during 1 to 21 d of age than diets containing corn ground to 2,010 µm (Nir et al., 1994a). From 7 to 21 d of age, broiler chicks fed medium grind diets had better growth performance when compared to broiler chicks consuming diets containing corn ground to 1,100 or 2,010 µm. Lott et al. (1992) reported that broiler chicks fed diets with 720 µm corn had improved 1 to 21 d of age growth performance versus broiler chicks fed diets with 1,200 µm corn. The corn was ground using a hammer mill and different particle sizes were achieved by using two hammer mill screen sizes. Corn, sorghum, and wheat were ground and sieved to create different particle sizes and fed to broiler chicks from 1 to 21 d of age (Nir et al., 1994b). Broiler chicks fed diets containing the smallest particle size (570 to 670 µm) had the poorest growth performance. Broiler chicks fed medium (1,130 to 1,230 µm) and coarse (2,010 to 2,100 µm) diets resulted in similar growth performance, in contradiction with previous research. Broiler chick growth performance may have been altered by the cereal grain, causing a diminished effect of particle size. Jacobs et al. (2010) fed broiler chicks diets containing corn ground to different particle sizes ranging from 560 to 1,400 µm, with no difference detected in broiler growth performance at 21 d of age. No effect of particle size on broiler chick growth performance was observed when broiler chicks were fed diets containing corn ground to 500 or 1,000 µm from 4 to 18 d of age (Kim et al., 2002). Broiler chicks will benefit from tactile stimulation of the gizzard by the feeding of larger particle sizes. Large active gizzards are thought to improve small intestinal stimulation and motility via hormonal and chemical signals; however, too large of a gizzard can have a negative effect on efficiency of poultry growth. A grind size that is too large increases retention time and energy expenditure to a level beyond what is beneficial for poultry growth performance. A large gizzard required to grind coarse cereal grains has a large maintenance energy requirement. The combination of maintenance and grinding energy diminishes the positive effect of increased gizzard size and stimulation.

Grower Growth Period

The starter phase rations are fed until 18 to 21 d of age, with broilers being fed a grower phase ration until 30 to 42 d of age. After 10 to 14 d of age, the digestive tract of broilers will

have reached a maximal relative size; thus, the gizzard of broilers will be larger and have an improved grinding capacity when they are consuming grower phase rations. Reece et al. (1986) conducted two experiments with the first experiment feeding broilers diets containing corn ground through a hammer mill screen of 4.76 or 6.35 mm, with no differences detected in growth performance at 21 or 42 d of age. Curiously, Experiment 2 reported that 42 d old broiler growth performance was improved when broilers were fed diets containing corn ground to 680 or 1,300 µm in comparison with broilers fed diets containing corn ground to 1,000 µm. There was no explanation as to why the medium particle size fed broilers had depressed growth performance. Lott et al. (1992) observed no particle size effects on 42 d old BWG or FC when broilers were fed diets containing corn ground to 720 or 1,200 µm. Broilers were either fed the same corn particle size for starter and grower diets, or opposite particle size for starter and grower diets. Nir et al. (1995) ground sorghum and wheat using a roller or a hammer mill, which produced a particle size range of 630 to 680 µm for the hammer mill and a particle size range of 1,400 to 2,200 µm for the roller mill. Male broilers fed diets with roller milled corn from 0 to 49 d of age had better growth performance than male broilers fed diets with hammer milled corn. In a second experiment, broilers fed diets with roller milled corn resulted in improved body weight at 28 d of age, and improved FC at 42 and 49 d of age. Two experiments conducted under two different rearing temperatures, to simulate normal and summer conditions, found no difference in broiler 49 d old body weight or FC amongst different particle sizes (Deaton *et al.*, 1995). Differences were detected between rearing temperatures, but no differences were detected between diets containing corn ground with a hammer mill to particle sizes of 680, 990, and 1290 µm.

Hamilton and Proudfoot (1995) fed broilers mash diets containing wheat or corn, ground to three particle sizes fine, coarse, and very coarse. Broilers fed diets with very coarse grain had the highest 42 d old body weights, and broilers fed diets with coarse grain had higher body weights than birds fed diets with fine grain. There were no differences detected in FC, and monetary return calculations indicated that broilers fed diets with coarse grain had the best returns. Complete diets were analyzed for particle size distribution and no cereal grain particle size analysis was reported. Broilers fed experimental diets from 21 to 42 d of age, have been shown to have similar BWG when fed a diet containing corn ground to particle size ranging from

780 to 2,250 μm (Parsons et al., 2006). Diets with the coarsest particle size (2,250 μm) resulted in the highest broiler feed intake and poorest FC. Reduced efficiency of growth may be due to energy expended to reduce dietary particle size in the gizzard. Diets containing corn ground to 1,100 μm had similar broiler growth performance as diets containing finer ground corn. Clark et al. (2009) demonstrated that corn cracked via a roller mill can replace 25 % of ground corn in a broiler diet with no effects on average daily gain or FC of 41 d old broilers. Adding up to 30 % screen rolled corn to a pelleted broiler diet fed from 18 to 56 d of age produced similar results of no differences in broiler growth performance (Dozier et al., 2009). Similar to younger broiler chicks, broilers fed grower phase rations have an upper limit to the benefits of increasing particle size; however, more mature gastrointestinal tracts are able to reduce particle sizes of larger grind sizes. If the grind size is too large increases in energy expenditure and retention time will negate positive effects of gizzard stimulation. However, gizzard stimulation has been shown to improve nutrient availability to poultry, and may be the reason for increases seen in growth performance.

Nutrient Availability and Mineral Retention

Particle size has been shown to alter nutrient availability, by changing gizzard size and feed retention time. Broiler chicks fed coarse or standard ground corn diets had no difference in digestibilities of starch or protein, and similar apparent metabolizable energy (AME) at 26 d of age (Rougière et al., 2009). Gizzard and pancreas weights were reported to be larger in coarse corn fed broiler chicks. Broiler chicks fed a diet containing finely or coarsely ground wheat affected nutrient digestibilities in 21 d old broiler chicks (Péron et al., 2005). Starch digestibility and AME were improved with finer grinding, but no difference in lipid or protein digestibility was detected. Amerah et al. (2008) reported that diets with wheat ground to 890 μm had improved AME when compared to diets with wheat ground to 290 μm. Flaxseed grind size has been shown to not affect broiler chick growth performance or fat digestibility when fed in a complete diet from 5 to 18 d of age (Jia and Slominski, 2010).

Increasing phosphorus availability is important to broiler and turkey producers, because phosphorus (P) is expensive and there are pollution concerns with high P in manure. Early research revealed coarse ingredients improved broiler chick P retention, measured using bone ash (Griffith, 1969). Research has revealed a decreased available P requirement in 1 to 21 d old

broiler chick when medium size limestone was fed (McNaughton, 1981). No differences in bone ash, BWG, and FC were detected between diets formulated with 0.25 and 0.30 % available P when broiler chicks were fed medium sized limestone. Broiler tibia ash at 21 d of age has been shown to improve when fed a calcium source of medium particle size versus fine or coarse (McNaughton et al., 1974). Guinotte et al. (1991) reported similar results with broiler chicks fed diets containing coarse particle size Ca sources decreasing Ca retention. Decreased Ca absorption resulted in decreased 28 d old broiler tibia bone quality. Medium sized limestone has been shown to stimulate the gizzard, while not being too large as to prevent proper acid solubilization of Ca.

Meat and bone meal particle size has not been shown to impact P availability when fed to turkey poults, but this could be due to high bioavailability of P in meat and bone meal (Sell and Jeffrey, 1996). Phosphorus and Ca utilization by 16 d old broiler chicks has been shown to improve when feeding a diet containing 900 μm particle size corn over broiler chicks fed diets containing corn ground to less than 580 μm (Kasim and Edwards, 2000). Follow up research showed that coarser ground corn (2,900 to 3,500 μm) improved 16 d old broiler chick bone ash, Ca retention, and phytate P retention (Kilburn and Edwards, 2001). Phytate P has a low availability to broilers or turkeys because of the P being bound by phytic acid. The majority of the dietary phytate P is secreted in the feces, and will increase fecal P levels. Coarse soybean meal enhanced 16 d old broiler chick phytate P utilization, bone ash, and plasma P level (Kilburn and Edwards, 2004). Experimental broiler chick diets contained either coarse (1,239 μm) soybean meal or fine (891 μm) soybean meal. Turkey poults had a linear increase in 28 d of age tibia ash and phytate P retention when dietary corn particle size increases from 600 to 1,100 μm (Charbeneau and Roberson, 2004). A second experiment revealed that turkey poult phytate P retention improves linearly with increasing soybean meal particle size. Increases in dietary particle size have been shown to improve mineral retention. Improvements in gizzard size, and activity have been linked to retention time, and it is thought this improve acid solubilization of phytate P, P, and Ca.

Cereal grain particle size can be easily controlled by altering roller mill spacing or changing a hammer mill screen size. Because of differences in the ability of growing poultry to

efficiently grind feed in gizzards particle size has different effects on starter and grower age broilers. Soybean meal particle size can be altered, and larger particle size soybean meal has been shown to increase nutrient retention. The optimal grind sizes of cereal grains for starter poultry have been shown to maximize around 900 μm and grower birds can efficiently gain at larger particles sizes. If a poultry company can dedicate hammer mills or feed mills to different ages of poultry, optimization of particle size can be achieved. If a poultry producer is unable to dedicate a feed mill or a hammer mill to different growth phases, then maximizing early bird growth is ideal and a grind size of around 900 μm is recommended. This particle size should increase throughput and increase nutrient retention, specifically increase phytate P retention to reduce dietary costs and poultry fecal P levels.

Pelleting

After grinding, the components of a commercial poultry feed are weighed, combined, and then blended in a mixer. This is designed to create uniformity and to ensure that each bite or peck a production animal takes will have a consistent nutrient content. Commercial layer chicken feed is typically fed as a mash, but commercial broiler chicken and turkey feed is pelleted. In its simplest form, pelleting involves forcing a mash feed through a die and typically involves steam conditioning. Pelleting is widely known to improve meat bird growth performance, and this next section will explore how pelleting affects broiler growth, efficiency of growth, and metabolism.

Pellet Quality

Pellet quality is the ability for a pelleted feed to withstand normal handling, shipping, and feeding practices. Numerous factors can influence pellet quality including dietary ingredients, conditioner temperature, and pellet mill production rate. Corn has been bred to contain different starch profiles and oil contents. Corn having waxy starch and low oil content has been shown to produce the higher quality pellets, than corn having higher oil content and normal type starch (Zarate et al., 2004). These higher quality pellets did not result in any differences in 49 d old broiler growth performance. Crumbles are pellets that are broken into smaller particles using a roller mill and are fed to starter phase poultry because of their smaller size. When crumbles were fed during the entire growth period, lower broiler body weights were observed at 42 d of age than pellet fed broilers (Arce-Menocal et al., 2009). Broiler chicks fed diets that were crumbled

or pelleted using a 1.59 or 3.17 mm die had similar growth performance from 0 to 13 d of age and growth performance was improved over mash fed broiler chicks (Cerrate et al., 2009). At 13 d of age, all broiler chicks were switched to a pelleted diet, and broilers fed mash diets from 0 to 13 d of age had similar 0 to 41 d of age growth performance as broilers fed pelleted or crumbled diets from 0 to 13 d of age. No differences between pelleted and mash fed broilers were most likely due to the majority of BWG occurring from 13 to 41 d of age. Adding moisture increases pellet quality and had no effect on 0 to 21 d of age broiler chick growth performance, but moisture addition did improve broiler FC when fed from 21 to 42 d of age (Moritz et al., 2001). Broilers fed lower quality pellets still had improved broiler growth performance compared to broilers fed mash diets. Increasing pellet durability from 37 to 74 % by adding water resulted in increased broiler BWG, when fed to broilers from 21 to 42 d of age (Moritz et al., 2002). Greenwood et al. (2004) investigated increasing levels of dietary pellet fines on 14 to 30 d of age broiler growth, and reported a decrease in BWG when fines increased from 20 to 30%. Broilers fed diets with 30% fines had higher BWG than broilers fed 60% fines. Demonstrating poor pellet quality can decrease the benefits of pelleting broiler feed.

Increasing proportions of mash in broiler diets to simulate poor pellet quality resulted in decreased broiler BWG and poorer broiler FC (Quentin et al., 2004). Broilers fed diets containing different ratios of pellets to fines from 38 to 45 d of age resulted improved BWG when broilers were fed 100% pellets versus broilers fed 20% pellets (McKinney and Teeter, 2004). Feed conversion was increased when broilers were fed 100% pellets, in comparison with broilers fed only fines. When analyzing pellet consumption, broilers consumed a higher percentage of pellets than were present in the diet; thus, broilers were selecting pellets over fines. Calculations on the caloric value of pellets revealed decreasing the percentage of pellets from 80 to 60% reduced caloric value by 56 ME_n/kg, and decreasing from 70 and 60% pellets resulted in a lowering of 33 ME_n/kg in caloric value. The authors surmised that this decrease was due to increased activity of broilers fed lower quality pellets. Dozier et al. (2010) reported that feeding broilers high quality pellets from 15 to 42 d of age resulted in increased BWG when compared to broilers fed low quality pellets. Feed conversion was similar and increased broiler BWG was attributed to increased feed intake. Broilers fed high quality pellets with poor quality protein had similar 14 to 35 d of age BWG as broilers fed poor pelleted diets with higher quality protein

(Lemme et al., 2006). Pellet quality did not affect FC but FC was negatively affected by poorer quality protein. High quality pellets allowed the broilers to consume higher amounts of feed to compensate for poor protein quality. Cutlip et al. (2008) increased pellet durability from 90 to 94 % using higher steam temperatures resulting in improved FC of 29 to 35 d old broilers. Research using an expander to create higher quality pellets did not result in improved broiler 0 to 21 d of age growth performance or 21 to 42 d of age broiler growth performance (Cramer et al., 2003). Contrary to most research, Oliver and Junker (1997) reported broilers fed a pelleted diet from 21 to 56 d of age did not have any broiler growth performance differences when compared to a mash diet. Research into the relationship of pellet quality and broiler growth performance has been inconsistent. Higher quality pellets have been shown to improve growth performance, but how much increased pellet durability will translate to BWG and FC is unknown. Research using pellet fines has shown that fines in the feed pan are of higher importance than measured pellet quality. However, measured pellet quality using pellet durability can aid in determining the amount of pellets that will reach the feed pans of commercial broilers.

Pelleting Effects on Broiler Health

Increases in growth performance associated with feeding broilers pelleted feed can have negative effects on broiler skeletal quality and increase stress on the cardiovascular system. One drawback to improved BWG is increased incidences of tibial dyschondroplasia (TD), a leg malformation, and sudden death syndrome (SDS) when older broilers were fed pelleted versus those fed mash (Hulan and Proudfoot, 1987). Proudfoot et al. (1982) found similar results of increased incidence of SDS in crumble fed versus mash fed broiler chicks. Sudden death syndrome is attributed to high stress on the aorta coinciding with high growth rates. Rapid increases in stress can induce SDS and exposes a weak cardiovascular system caused by high rates of BWG. Investigation of pelleting and leg quality revealed poorer gait scores and lower bone ash content when broilers were fed pellets instead of mash (Brickett et al., 2007). Pellet fed broilers have been shown to have lower relative pancreas weights, higher ileum numbers of coliform bacteria and enterococci, and increased cecal bacterial fermentation (Enberg et al., 2002). Increases in cecal fermentation could be due to decreased digestion, or increased intestinal secretions because of consuming a pelleted feed. Reduced digestion of dietary

nutrients increases nutrients available for bacterial growth, and could increase levels of disease causing bacteria.

Pelleting Effects on Nutrient Availability and Metabolism

During the pelleting of poultry feed, the feed is exposed to high heat, friction, and additional moisture. A combination of feed chemical and physical changes and broiler feed consumption rates can alter the digestive process and subsequent nutrient availability. Pesti et al. (1983) found different responses to increase in dietary density of broilers fed crumbled diets compared to mash fed birds. Regression analysis of the data revealed a lower response to dietary density by crumble fed broiler chicks in comparison with mash fed broiler chicks. Plavnik et al. (1997) observed similar lower responses to increased dietary fat by pellet fed broilers and turkeys than those fed mash. Increased feed consumption rates of broiler fed pellets allows for broilers to adjust their energy intake, minimizing the effects of rations with lower caloric density. Broilers fed mash rations had a lower 16 to 30 d of age lysine requirement than broilers fed a pelleted ration (Greenwood et al., 2005). Broilers fed the mash ration had a lysine requirement for BWG of 0.87 % and pellet fed broilers had a lysine requirement for BWG is 1.00 %. The higher broiler growth rates of pellet fed birds increases the lysine requirement, and needs to be considered when formulating a pelleted broiler ration.

Research has shown that pelleting increases growth performance and AME (Preston et al., 2000). Pelleting or crumbling a diet containing flaxseed improved broiler AME of flaxseed, in comparison with a mash diet (Gonzalez-Esquerra and Leeson, 2000). When compared to mash feed, pelleted feed improved broiler growth performance during both starter and grower phases (Zhang et al., 2009). Pelleted diets had higher AME, which was indicated as a reason for increase in growth performance. Preston et al. (2000) reported a higher dietary AME with pelleted diets when fed to 42 d old broiler. Pelleting feed has been shown to increase dietary AME for 21 to 24 d old broilers (Svihus et al., 2004). Other research has shown reduced AME with feeding pellets versus mash to 21 d old broiler chicks (Amerah et al., 2007). Yang et al. (2010) observed no difference in digestibility between mash and crumbled diets. Steam conditioning temperature has been shown to not affect starch digestibility or AME in corn based diets (Abdollahi et al., 2010). Increases in dietary AME when feeding broilers a pelleted diet

would allow a poultry grower to have a lower ME level in the feed and would decreases dietary costs. Feed consumption rates are known to increase with the feeding of pelleted diets and may be responsible for increases in ME.

The Effects of Pelleting on Feed Consumption

Pelleting alters feed form of commercial poultry feed, and has been previously shown to alter feeding behavior of broilers and turkeys. Broilers switched from mash to pelleted feed at 42 d of age consumed more feed from 42 to 50 d of age than broilers kept on mash feed, resulting in increased BWG (Deaton et al., 1988). Laying hens fed a pelleted diet have been observed to expend less time feeding than laying hens fed a mash diet (Vilariño *et al.*, 1996). When broilers have restricted feed time, broilers fed a pelleted diet will have improved FC in comparison with broilers fed a mash diet (Proudfoot and Hulan, 1982). Broilers fed mash diets with high nutrient density have been shown to have lower body weights than broilers fed pelleted, low nutrient density diets (Brickett et al., 2007). Broiler FC improved with increasing nutrient density, and pellet fed broilers compensated for low nutrient density by consuming more feed. Skinner-Noble et al. (2005) observed that broilers fed pelleted diets spend more time resting and less time consuming feed than broilers fed mash diets. Nir et al. (1994) also observed similar results of broilers fed pellets spending more time resting and less time consuming feed.

Pelleting has been repeatedly reported to improve broiler growth performance but this can lead to negative effects on broiler health. Increased broiler growth rates can lead to increases in SDS and decreases in broiler leg quality. Pelleted feed has also been shown to increase AME, and has been linked to reduced feeding time. Broilers have been shown to decrease time spent consuming feed, which decreases energy expenditure. Decreased feeding energy expenditure is a major reason for increases in growth performance and is directly related to pellet quality. Poor pellet quality results in more fines caused by handling, which have been documented to reduce growth performance benefits of pelleting. Pellet fed broilers and turkeys can compensate for poor quality ingredients by consuming more feed, and give a broiler or turkey producer more options when determining ingredients in a diet. With a rise in corn and other ingredient prices, maximizing pellet quality increases in importance.

Influence of Feed Processing on the Digestive System

Changes in particle size and pelleting affect broiler and turkey feed chemically and physically. As a broiler grows, develops, and matures the digestive system will adjust to feed by altering digestive tract size, digestive tract length, and intestinal villi size. In comparison with *ad libitum* fed broilers, meal fed broilers have larger storage organs (crop and gizzard) but similar relative intestinal weights (Barash et al., 1993). Inclusion of barley in diets has been linked to increases in viscosity of digesta, and broilers fed barley based diets have lower growth performance and intestinal villus area than broilers fed corn based diets (Onderci et al., 2008). . Increased dietary corn particle size has been show to increase the relative gizzard size of 26 d old broilers (Rougière et al., 2009). Increased dietary particle size by including increasing amounts of roller cracked corn in broiler diets increases 41 d old broiler gizzard size (Clark et al., 2009). The increase in dietary particle resulted in more tactile stimulation of the gizzard and increased broiler gizzard size. Feeding broilers wheat ground using either a hammer mill or an older style attrition mill has been shown to have no effect on broiler intestinal relative length, relative weight, or pH (Afshamanesh et al., 2006). Péron et al. (2005) reported that feeding coarsely ground wheat increases 21 d old broiler relative gizzard size. Contrary to most research, Amerah et al. (2008) observed no differences in relative gizzard size between diets with corn or wheat ground to 2 different particle sizes.

Increasing dietary corn particle size from 380 μm to 800 μm has been observed to increase 8 d old turkey poult gizzard and liver relative size, even when included in a crumbled or micropelleted feed (Favaro et al., 2009). Poults fed crumbled feed had higher relative gizzard weights, which were attributed to increased feed intakes. Micropelleting could have reduced dietary particle size by forcing the diet through a small die, affecting relative gizzard size. At 39 d of age broiler relative gizzard weight has been show to increase with dietary wheat particle size, and relative gizzard weight decreased when feeding broilers a pelleted versus a mash diet (Enberg et al., 2002). Broiler pancreatic weights were decreased by feeding pelleted diets. Ileal viscosity was observed to decrease with feeding broilers pellets, but was not affected by dietary wheat particle size. Feed form affected gastrointestinal pH, with broilers fed pellets having a higher gizzard pH and a lower intestinal pH. Lower relative gizzard weights could have reduced gizzard retention time, and caused an increase in gizzard pH. Amerah et al. (2007) also observed

a decrease in broiler chick gizzard relative weight and small intestine relative length with feeding a pelleted diet.

Feeding broiler chicks a crumbled starter diet and a pelleted grower diet reduced the relative weights of total gastrointestinal tract and gizzard (Choi et al., 1986). Broiler chicks fed diets containing corn that was steam cooked had decreased average relative gizzard weights and increased average relative liver weights (González-Alvarado et al., 2008), when fed from 0 to 22 d of age. There were no gastrointestinal tract pH differences detected between broiler chicks fed cooked or raw corn diets. Brown layer pullets fed pelleted diets had reduced relative digestive tract and gizzard weights, in comparison to mash fed pullets (Frikha et al., 2009). Relative small intestine length was reduced and gizzard pH was increased in pullets fed pelleted diets. Similar to pullets, broilers fed pelleted diets had reduced relative gizzard weights and increased ileal viscosity, when compared to broilers fed a mash diet (Preston et al., 2000). Viscosity negatively affects broiler growth by decreasing mixing of digestive enzymes and feed, and increasing the water layer that inhibits nutrient transport. Relative gizzard weights of 30 d old broilers have been observed to decrease when fed pelleted diets (Svihus et al., 2004). Increasing the steam conditioning temperature of a pellet mill from 60 to 75 ^{o}C increased broiler small intestinal relative length (Abdollahi et al., 2010). Interestingly, pelleted feed that was steam conditioned at 75 ^{o}C resulted in higher broiler relative duodenal weights than broilers fed pellets steam conditioned at 60 or 90 ^{o} C. Gizzard contents were decreased with feeding of pelleted diets, and proventriculus contests were increased, indicating rapid gizzard emptying and less gizzard grinding activity. The results are similar to previous research, and are suggestive of decreases in gizzard function. Decreases in gizzard function would decrease gizzard retention time, which can decrease dietary macromineral retention. Reductions in relative gizzard size observed with the feeding of pelleted diets are similar to effects of decreasing dietary particle size on relative gizzard size. The effect of pelleting on relative gizzard size suggests that pelleting reduces dietary particle size, reducing stimulation and growth of the gizzard.

The villus surface area in the small intestine decreased when broilers were fed diets with pellet quality increasing from 80 to 90% pellet durability index (PDI) (Buchanan et al., 2010b). Broiler chicks fed diets with pellet quality of 90% PDI had a lower villus surface area at 21 d of

age, versus broiler chicks fed diets with pellet quality of 70 to 83% PDI. Interestingly, different rates of pellet production had an effect on 40 d old broiler intestinal surface area, with high rates of pellet production increasing small intestinal villus height and surface area. Within an experimental dietary formulation, fast production rates had lower pellet qualities than those reported for slow production rates. Zhang et al. (2009) documented pellet fed broiler chicks had increased villus height, which was believed to increase nutrient absorption. This increase in small intestinal villus surface area could be related to increases in feed consumption observed with the feeding of pelleted diets.

Altering cereal grain particle size and pelleting of diets alters the gastrointestinal tract by changing organ size, digesta pH, and absorptive area of the small intestine. Increasing the particle size of cereal grains increases gizzard sizes through tactile stimulation of the gizzard. Pelleting has been shown to have the same effect on gizzard size as reducing dietary particle size; thus, forming a pellet may reduce dietary particle size. Pelleting increased pancreatic and liver size and pelleting can reduce broiler intestinal tract relative weight and length. Reduced intestinal tract relative weight may be related to decreased gizzard function. Increases in feed consumption associated with consuming a pelleted feed increases nutrient intake and absorption, and could cause an increase in liver and pancreatic size. The pH of the broiler gizzard has been shown to increase when feeding pelleted rations, which may allow for more harmful bacterial growth in the gizzard. The villus surface area of the small intestine has been shown in increase with the feeding of pelleted diets. This increase in absorptive area may be caused by more feed consumption, similar to liver and pancreatic weights. For a poultry producer increasing pellet mill production rates may have a positive effect on intestinal villi, but if pellet quality is too poor this affect may be negated due to decreases in feed consumption.

Commercial poultry feed must be processed to maximize efficiency of broiler and turkey growth performance. Broilers are marketed at an immature age, and do not develop a gastrointestinal tract capable of efficiently digesting a whole grain based diet. Research using cracked corn has shown that older broilers have the ability to grind very large particle size corn, and when included in rations at low levels can economically gain weight. However, younger broilers have shown a limited ability to process larger particle size corn, but efficiently grow

when corn is ground to around 900 μm. Once broilers are 18 to 21 d of age, a larger corn particle size can be used and is recommended if a broiler producer can dedicate feed manufacturing resources to different broiler growth phases. Increasing dietary particle size also has beneficial effects on phytate P and Ca retention, and may be due to increased retention time in the gizzard. Increased gizzard retention time increases acid hydrolyzation and physical breakdown of feed. This combination will solubilize more Ca and phytate bound P, increasing intestinal absorption.

Broilers prefer to consume larger particles and will consume large particles in a disproportionate amount in comparison with fine particles. Pellets are large, and broilers will selectively consume the whole pellets over the fines. Decreased energy output by consuming higher quality pellets increased growth, and research revealed increases in relative caloric density with higher quality pellets. Particle size of grain in a commercial broiler operation where feed is pelleted may not be as important as pellet quality. Grind size should be optimal for throughput in a mill, and for efficiency of feed production. Once that has been achieved, most of the focus should be on pellet quality because it has been shown that increased fines in feed pans will result in decreased relative metabolizable energy.

Literature Review Summary

Broiler chickens have been selectively bred to consume more feed, grow faster, increase skeletal muscle yield, and consume feed in a more efficient manner. Breeding has changed feeding behavior from preferring to consume feed in a foraging setting to preferring to consume feed from feeder pans. While this change has occurred, commercial poultry have still maintained a social feeding behavior and will compete for feed when it is limited. Sight is the primary sense poultry use for finding and selecting feed. Because of this, poultry have been shown to prefer larger feed particles, and will selectively consume increasingly larger particles as poultry grow and develop. Allowing broiler chickens to sort and select feed particles can lead to poor nutrition, and affect growth. Broilers do not self-select a diet that promotes efficient growth, and will choose a diet that has an improper balance of energy and protein. Preventing broilers and turkeys from self-selecting and sorting feed is necessary for economic growth.

One of the reasons for increased growth rates in contemporary commercial broilers is the rapid development of the broiler digestive system. Broilers are precocious and are capable of digesting and absorbing a commercial diet within the first few days after hatching, unlike mammalian production animals. The gastrointestinal tract will reach maximum relative weights within the first week post-hatch, permitting rapid growth. Amylases, proteases and lipases rapidly increase in relative quantity and will be at adult levels within 5 to 8 d post-hatch. Increasing digestive enzymes will increase nutrients available for absorption, stimulating intestinal development and maturity. Enterocytes mature within first 48 hrs post-hatch, and increases in number of enterocytes will increase villus height, width, and surface area. Absorptive area increases with villi surface area; therefore, increasing nutrient utilization. Understanding how feed manufacturing can aid or hurt the development of the digestive system is important for maximizing health of broilers and economic returns for the producer.

Cereal grains are a major portion of broiler and turkey diets, and are the primary source of dietary energy. Cereal grains primarily store energy as starch, which is formed from sugars created during photosynthesis. Glucose units are combined via two α-linkages to form two types of starch, amylose and amylopectin. The form of starch granules is different in each plant, and this differs between cereal grains. Poultry digest starch granules into glucose units using pancreatic and intestinal amylase, and brush-border saccharidases. All particle size reduction occurs in the gizzard, while most starch digestion occurs in the small intestine. Amylase attacks the reducing end of starch, hydrolyzing α-1,4 bonds, and creating α-limit dextrins and maltose as its products. Products of amylase hydrolyzation are broken down by intestinal brush-border enzymes into glucose. Glucose is absorbed by active transport using SGLT-1 and passive transport using GLUT2. Glucose is the primary energy source for poultry cells, and blood glucose levels are higher than mammals. Due to high levels of glucose, glucagon has become the primary blood glucose regulatory hormone instead of insulin. Glucose is readily absorbed in poultry using non-insulin dependent transporters, and the liver will convert excess glucose into glycogen for storage. Glucagon will cause release of liver glycogen, increasing blood glucose level. When feed is processed and pelleted, moisture and heat are added. Starch encountering heat and moisture will gelatinize and lose its crystalline form. During feed processing starch

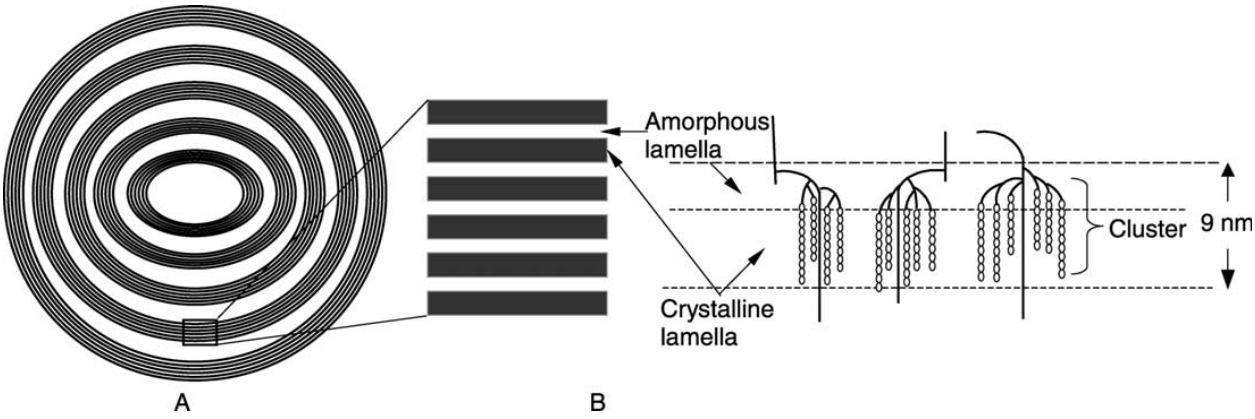
gelatinization occurs, especially during pelleting. The gelatinization of starch could be the cause of some of the negative effects observed when feeding a pelleted diet.

As broilers grow, larger cereal grain particle sizes can be fed and phytate P and Ca retention increases with larger dietary particle sizes. Larger cereal grain particle sizes require less grinding energy and will improve feed mill throughput. However, broiler feed is pelleted and pelleting limits throughput in a feed mill and is more costly than grinding. During pelleting steam and heat are added, causing starch gelatinization. Feeding pelleted diets has been shown to improve broiler weight gain and feed efficiency. An increase in broiler growth performance caused by feeding pelleted diets is linked to pellet quality. Pellet quality is measured as a pellet durability index, and increases in pellet quality is associated with increasing starch gelatinization (Table 1.2). Broiler feeding activity has been shown to decrease with high quality pellets, resulting in increased resting time. Reducing energy spent consuming feed will increase energy available for growth, improving feed efficiency. Consuming pelleted rations causes rapid broiler growth, increasing incidences of TD and SDS, which increases mortality. Pelleting broiler diets can also lead to decreases in broiler intestinal and gizzard size, increases in gizzard pH, increases in gut viscosity, and alters villi size. Decreases in gizzard size can alter nutrient availability, and can increase gizzard pH. The villi size of the small intestine will increase with pelleted feed, most likely due to increases in feed consumption. Increases in viscosity could be due to the loss of the granular form that occurs during the gelatinization of starch.

Commercial broilers have been genetically selected to grow quickly and efficiently. Because broilers are precocial, a diet based on cereal grains and legume proteins can readily be consumed, digested, and absorbed immediately after hatching. Starch is the primary energy source of these diets, and broilers are able to digest and absorb over 90% of consumed starch from an early age. Due to high starch digestion rates, broilers have high blood glucose levels and are not dependent on insulin for cellular glucose absorption. Broilers prefer consuming larger particles, and can grind these particles to smaller sizes in the gizzard. Other monogastric species will benefit from further grinding but additional particle reduction eventually reduces gizzard functionality, which causes reduced dietary macromineral retention. Increased gizzard function can improve intestinal health by altering the digesta pH. Proper digestive tract development and

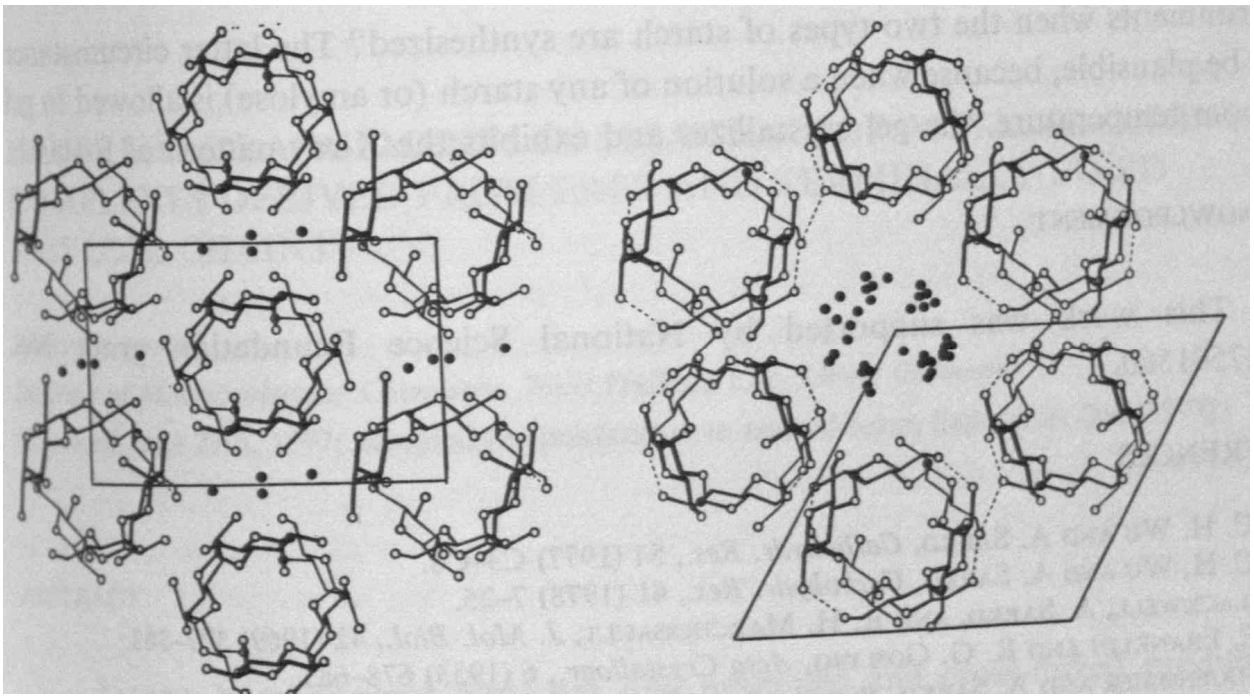
function is essential for maximizing broiler growth performance. Feed manufacturing can alter gizzard size and function, intestinal absorptive area, and health of the digestive tract. Pelleting of feed has been shown to improve broiler growth performance because of reduced feeding times and overall activity levels. However, improvements in growth performance may be hindered by pelleted feed reducing gizzard functionality, increasing gizzard pH, and increasing intestinal viscosity. This leaves questions about, what causes these changes and how can they be tested without the interference of feed form.

Figure 1-1 Starch Granule Formation and Structure



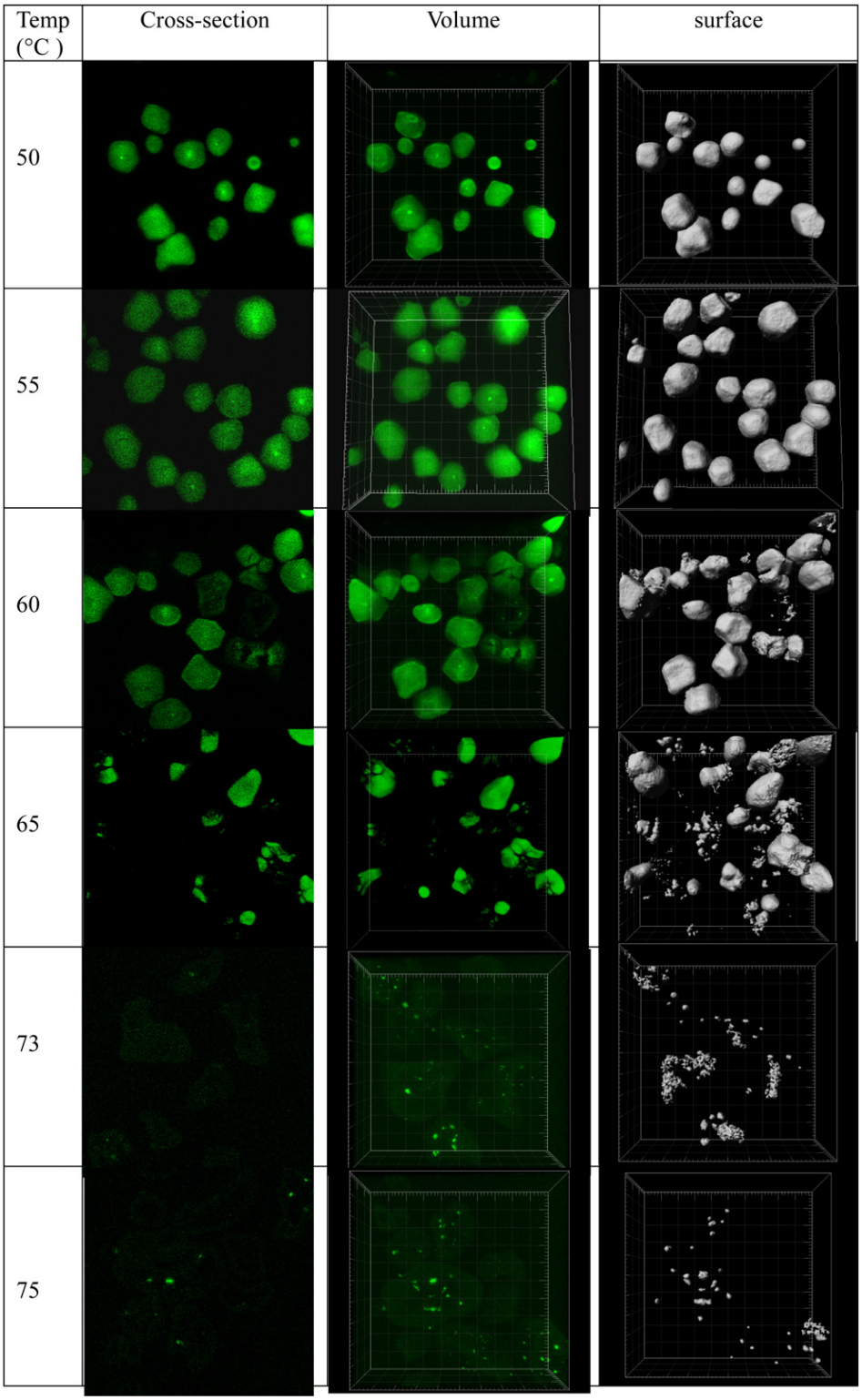
Diagrammatic representation of the lamellar structure of a starch granule, with (A) the stacks of microcrystalline lamellae separated by amorphous growth rings. (B) Magnified view of the amorphous and crystalline regions. (C) Double helical structures formed by adjacent chains of amylopectin give rise to crystalline lamellae. Branching points constitute the amorphous regions (Donald *et al.*, 1997).

Figure 1-2 Structure of Crystalline Amylose



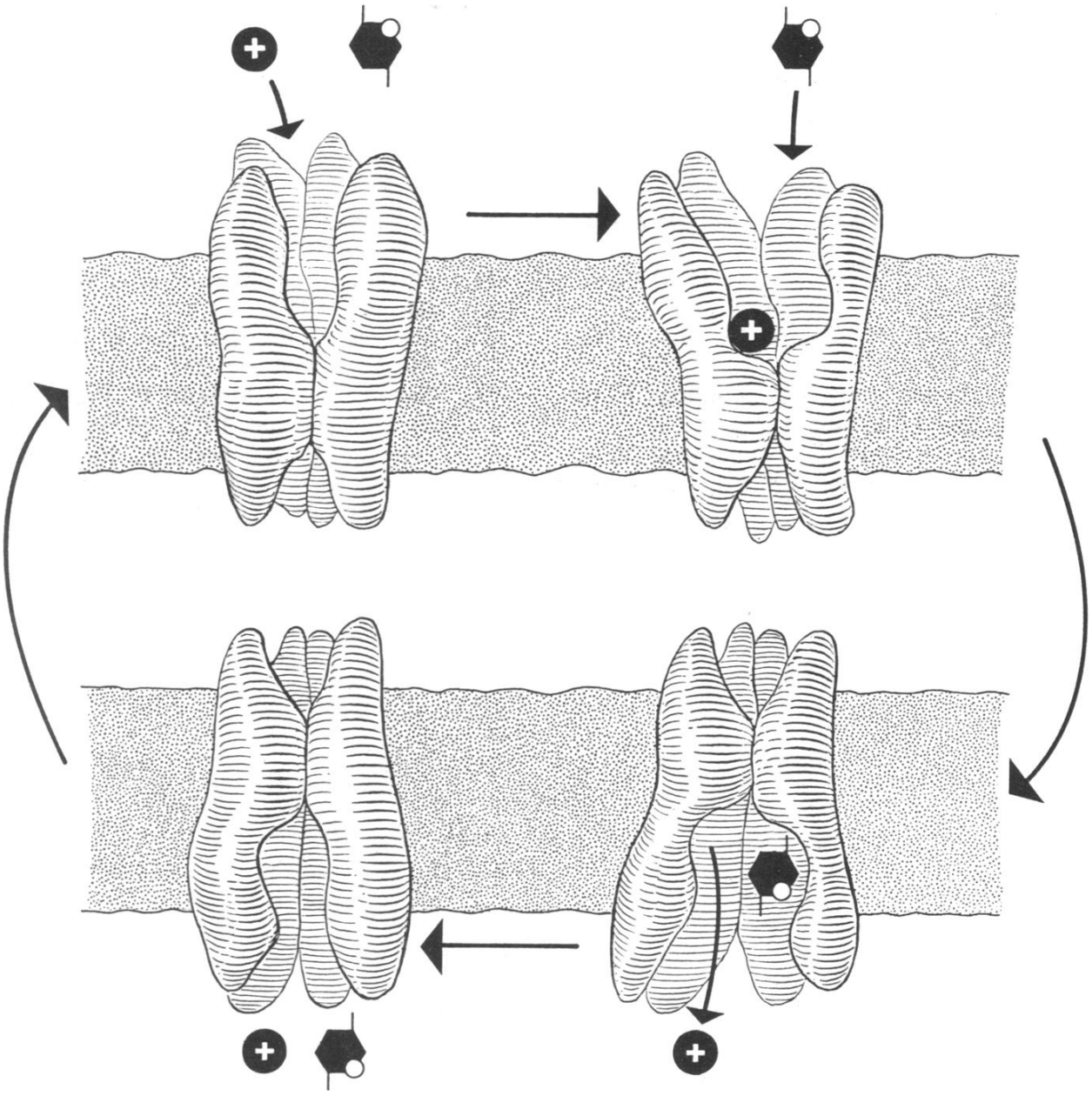
The structure on the left is the A-type crystalline amylose, where water is distributed evenly in the crystalline structure. This is the structure used by cereal starches. The structure on the right is the B-type crystalline amylose, where water is concentrated in the center of the crystalline structure. This structure is used by potato starches. (H.C.H Wu and A. Sarko, 1978)

Figure 1-3 Phase Transition of Corn Starch Granules during Heating Observed using Hot-Stage Confocal Laser Scanning Microscopy



Chen et al., 2011

Figure 1-4 Model of SGLT-1 Glucose Transport Mode of Action



Stevens et al., 1990

Table 1.1 Major Properties of the Starch Polymers

Major Properties	Amylose	Amylopectin
Molecular Configuration	Essentially Linear	Highly branched
Average Molecular Weight	10^6	10^8
X-Ray Diffraction	Crystalline	Amorphous
Complex Formation	Readily forms complexes with iodine and polar substances	Very limited complex formation
Stability in aqueous solution	Unstable, tends to retrograde	Stable

Shows the physical property differences between the two main polymers of glucose (W. Banks and D.D. Muir, 1980)

Table 1.2 Summary of Relationship between Pellet Quality and Starch Gelatinization Levels

Pellet Quality[1] (%)	Starch Gelatinization Level (%)
<60[2,4]	7 to 16
60 to 70[2,5]	11 to 18
70 to 80[2,3,5,6]	6 to 17
80 to 85[3,6,7]	1 to 25
>85[2-7]	14 to 32

Summary of literature data on relationship between pellet quality and gelatinization level
[1]Measured using Pellet Durability Index
[2]Moritz et al. (2001)
[3]Moritz et al. (2002)
[4]Cramer et al. (2003)
[5]Moritz et al. (2003)
[6]Svihus et al. (2004)
[7]Buchanan et al. (2010a)

Chapter 2 - The Effects of Corn Particle Size and Starch Gelatinization on Broiler Grower Growth Performance and Carcass characteristics

Abstract

This study evaluated the effects of starch gelatinization, corn particle size, and their respective interactions on 22-to 42-d old broiler growth performance, dressing percentage, and gizzard size. The treatments were arranged as a 3 x 3 factorial, with 3 different corn particle sizes (fine, medium, and coarse), and 3 different starch gelatinization levels (0, 10, and 20%). Analyzed corn particle sizes were 465, 877, and 1240 μm. Gelatinized starch levels were created by replacing 20% of dietary starch with conventional unmodified corn starch and pregelatinized corn starch blended at 3 different ratios (100:0, 50:50, and 0:100). Broiler chicks were fed a standard starter diet from 0 to 21 d of age, and fed the experimental diets from 22-to-42-d of age. At 42 d of age, 3 broilers were selected from each pen and processed for collection of carcass data. Particle size influenced body weight gain ($P<0.001$), indicating a linear increase in body weight gain with increasing particle size ($P<0.05$). Broiler growth performance was unaffected by gelatinization level ($P>0.05$). Interactions between gelatinization level and particle size did not affect body weight gain ($P>0.05$), and there were no treatment effects detected for feed consumption or feed:gain ($P>0.05$). Dressing percentage was unaffected by gelatinized starch in the diet ($P>0.05$), but gelatinization level increased gizzard relative weight ($P<0.05$). Increases in particle size resulted in increases in dressing percentage and relative gizzard size ($P<0.05$). There were no interactive effects detected ($P>0.05$) on dressing percentage or gizzard relative weight. The results indicate that coarser corn particles sizes will increase body weight gain and dressing percentage of 22-to-42-d of age broilers without negatively affecting feed conversion. Diets with gelatinized starch did not affect broiler growth performance, although gizzard relative weight increased.

Introduction

Broiler feed is manufactured in a feed mill and has several processes that aid in efficient broiler growth. The first step is to reduce cereal grain particle size, in order to aid in efficient

digestion and utilization. Due to their large size, cereal grains are ground to reduce particle size. Reducing particle size increases the surface area and allows enzymatic access, and is also required to maximize pellet quality. Broilers are also capable of reducing dietary particle size through grinding activity of the gizzard. Because of increased enzymatic access and gizzard activity, particle size has been shown to affect broiler growth performance (Nir et al., 1990, 1994a, 1994b, 1995; Lott et al., 1992). Most research on 0 to 21 d of age broiler chicks reported a decrease in performance for particle sizes larger than 1,000 µm (Lott et al., 1992; Nir et al., 1994a). Whereas, research on older broilers has revealed economic broiler growth with cereal grain particle sizes greater than 1,000 µm (Lott et al. 1992; Parsons et al., 2006; Clark et al., 2009).

The increased broiler growth performance associated with coarser particle sizes is thought to be a result of increased gizzard size and function (Clark et al., 2009; Favaro et al., 2009; Rougière et al., 2009). Increased performance due to gizzard function is supported by research reporting that feeding broilers diets with larger particle sizes of cereal grains or soybean meal improves Ca and phytate P retention (Griffith, 1969; Kasim and Edwards, 2000; Charbeneau and Roberson, 2004; Kilburn and Edwards, 2004). The improvements in mineral retention are thought to be related to increased retention time in the gizzard. Increased gizzard retention time increases phytate P and Ca exposure to gastric acid and gizzard grinding forces. Increases in gizzard function are also thought to improve motility in the gastrointestinal tract.

There have been investigations of the effects of particle size on carcass yield, but with mixed results (Clark et al., 2009; Dozier et al., 2009). Clark et al. (2009) reported decreasing carcass yield with increasing inclusion levels of cracked corn, thus increasing dietary particle size. Whereas, increasing additions of roller milled corn has been shown to have no effect on carcass yield (Dozier et al., 2009). Research demonstrated that larger particle sizes can be beneficial, but the range of particle sizes is inconsistent and not conducive to making conclusions. Most of the previous research focused on the broiler starter period or entire growth period, but few studies concentrated on the broiler grower period when feed intake is maximized.

After grinding the cereal grains and mixing dietary ingredients, broiler feed is commonly pelleted because of improvements broiler growth performance. Increases in growth performance have been linked to reduced feeding time, and are associated with increased pellet quality (Savory, 1974; Skinner-Noble et al., 2005). Because of this, improvements in pellet quality have been shown to have a positive effect on broiler body weight gain and feed conversion (Mortiz et al., 2001, 2002; Greenwood et al., 2004; McKinney and Teeter, 2004; Quentin et al., 2004; Lemme et al., 2006; Cutlip et al., 2008; Dozier et al., 2010). Post-mix grinding and reduction of particle size have been shown to improve pellet quality (McEllhiney, 1992; Dozier, 2005). However, in mash feed, larger particle size has been shown to improve broiler growth performance.

Steam conditioning and pellet die forces increase broiler feed temperature. Pelleting will gelatinize the starch in broiler diets, due to increases in feed temperature (Moritz et al., 2001, 2002, 2003; Cramer et al., 2003; Svihus et al., 2004; Buchanan et al., 2010a). Increases in pellet quality have been associated with increases in starch gelatinization level (Moritz et al., 2001, 2002, 2003; Cramer et al., 2003; Buchanan et al., 2010a). Increases in starch gelatinization have been shown to effect broiler growth performance. Moritz et al. (2005) reported positive effects of gelatinizing starch on broiler chick starter BWG, but no effects on feed conversion. Earlier research conducted in our lab showed decreasing broiler chick starter BWG with increasing levels of starch gelatinization (Rude, 2008).

Pelleting broiler diets has also been reported to affect gastrointestinal tract size. Feeding broilers pelleted diets resulted in reduced gizzard and intestinal size (Choi et al., 1986; Gonzalez-Esquerra and Leeson, 2000; Preston et al., 2000; Enberg et al., 2002; Svihus et al., 2004; Zhang et al., 2009). Broilers fed pelleted diets have reduced relative gizzard weights at 39 to 42 d of age (Preston et al., 2000; Enberg et al., 2002; Amerah et al., 2007). It is believed that heat processing can affect gizzard size, which is supported by cooked grains decreasing gizzard size (González-Alvarado et al., 2008). Grinding and pelleting are important factors in feed processing and are a large cost of feed production. Using coarse corn particles in feed requires less energy and can increase feed mill throughput. Increasing pellet quality is associated with increasing starch gelatinization, and may cause negative effects on gizzard size. It is unclear how this

increase in starch gelatinization affects grower phase broiler growth performance, and carcass characteristics. An experiment was conducted to evaluate effects of starch gelatinization and corn particle size on broiler grower period growth performance and carcass characteristics.

Materials and Methods

All animals were reared following protocols established by the Kansas State University Institution of Animal Care and Use Committee. Broilers were housed at the Thomas B. Avery Poultry Research Unit (Manhattan, KS). Male Cobb 500 broiler chicks were obtained from a commercial hatchery, and upon arrival at the research farm, 30 newly hatched broiler chicks were assigned to 72 floor pens, for a total of 2,160 birds. The broiler chicks were fed a starter diet formulated to meet or exceed NRC (1994) recommendations (Table 2.1). The chicks were kept under 24 h of light for the first 3 d, and 23L:1D for the duration of the experiment. The broilers were raised at 33 ^{o}C for first 3 d, and house temperature was reduced by 2.5 ^{o}C per 7 d for the duration of the experiment. Feed and water were provided *ad libitum*, and mortalities were removed and weighed daily. At 21 d of age, the broilers were weighed, reduced to 25 birds per pen, and assigned to the experimental treatments.

Corn Grinding and Treatment Diets

Corn was ground to three particle sizes (PS): fine particle size (FP), medium particle size (MP) and coarse particle size (CP), using a three-high roller mill (Model K, Roskamp Manufacturing, Cedar Rapids, IA) at the Kansas State University Grain Science Feed Mill (Manhattan, KS). Target particle sizes were set for 400 to 500 µm, 800 to 900 µm, and 1200 to 1300 µm. Particle size ranges were chosen to represent a grind finer than industry standard, equal to industry standard, and larger than industry standard. The samples were taken from roller mill and bagged corn to ensure the targeted particle size was achieved. All samples were analyzed individually at the mill, and screen weights were pooled to find the mean particle size of the corn. Particle size was determined using a Tyler Rotap (Mentor, OH), and U.S. standard size 6-270 screens.

Grower diets were formulated to meet or exceed NRC (1994) nutrient requirements (Table 2.1). To create different gelatinization levels, diets were designed to replace 20% of

dietary starch with unmodified corn starch or pregelatinized corn starch (product number 12030, Cargill Foods, Minneapolis, MN). Different gelatinization levels were created by blending conventional unmodified corn starch and pregelatinized corn starch at ratios of 1:0 for 0%, 1:1 for 10%, and 0:1 for 20% starch gelatinization. Particle sizes and gelatinization levels (GL) were combined to form a 3 x 3 factorial arrangement, for a total of 9 treatment diets. The experimental design was a complete randomized block design (CRBD), with broiler pens blocked according to building location, and treatments randomly assigned to pens within blocks. Pens were divided into 4 blocks, with 2 replications per block for a total of 8 replications per treatment.

The broilers were fed the experimental diets from 22 to 42 d of age, monitored daily, and all mortalities were collected and weighed for feed conversion calculations. At 42 d of age the broilers and feed were weighed to determine BWG, feed consumption (FC), and this data was used to calculate feed:gain (F:G) adjusted for mortality. For dressing percentage and gizzard size determination, 3 randomly selected birds per pen were removed from 6 pens per treatment. The broilers were weighed as a pen, euthanized via CO_2 asphyxiation then bled, scalded, plucked, eviscerated, and weighed without giblets. After the gizzards were collected from each carcass, the abdominal fat and koilen were removed and then were weighed.

Statistical Analysis

Experimental data were analyzed as a CRBD with pen as the experimental unit, and an alpha of 0.05. All data were analyzed using the MIXED procedure of SAS (Release 9.1 for Windows, SAS Institute, Cary, NC). When *F*-tests indicated significant treatment effects, least square means were separated using the Tukey-Kramer method. Contrasts were conducted to determine linear and quadratic effects of gelatinization level and particle size on measured variables.

Results

The targeted range of corn grind size was achieved, and distributions of the particles within each grind size are shown in Figure 2-1. The geometric mean particle size for FP, MP, and CP was 465 µm, 877 µm, and 1,240 µm respectively. Gelatinization of corn starch did not

affect 21 to 42 d of age broiler growth performance (Table 2.2). However, body weight gain was increased by feed made with larger corn particles, with both the CP and MP having greater BWG than FP (1.836 and 1.816 kg versus 1.696 kg). There was a quadratic effect of PS on BWG, with a greater increase in BWG between FP and MP, than MP and CP treatments. Feed conversion was unaffected by PS. There were no PS and GL interactions observed on broiler growth performance.

The effects of GL and PS on dressing percentage and relative gizzard weight are shown in Table 3.3. Starch gelatinization level did not influence carcass dressing percentage, and there was minimal variation amongst GL treatment carcass dressing percentage (69.9 to 70.1 %). Relative gizzard weight was significantly affected by GL with the 10 % GL treatment having a lower relative gizzard weight than the 20 % GL treatment (1.26 versus 1.32 g/100g of BW). Starch gelatinization level had a quadratic effect on relative gizzard size. Dressing percentage was significantly increased with coarse PS (69.7 to 70.5 %), with a linear increase in dressing percentage with increasing PS. Similar to dressing percentage, relative gizzard weight was linearly increased by an increase in PS (1.19 to 1.32 g/100g of BW). There were no GL and PS interactions observed on dressing percentage, or relative gizzard weight.

Discussion

Previous research has shown conflicting results of the effects of gelatinized starch on the performance of broiler chicks. Rude (2008) demonstrated that broiler growth performance during the starter phase was negatively affected by inclusion of gelatinized starch, and is in conflict with broiler grower phase growth performance. Batal and Parsons (2004) reported lower ME_n values with diets containing gelatinized corn starch versus corn starch based diets, and the reduction in ME_n is not supported with the growth performance data. Broiler growth performance results are supported by González-Alvarado et al. (2007), who found no effects on 21 d of age broiler growth performance when fed cooked rice or corn. However, gizzard size is contradictory with no effects of cooked rice or corn on gizzard size reported, and a higher GL increased relative gizzard size in this research. Moritz et al. (2001) observed no differences in 3 to 6 wk BWG with GL increasing from 16 to 22 %, and is supported by no effect of GL on BWG observed in this research. In contrast, feed efficiency was reported to be affected by GL, with

adjusted feed efficiency improving with higher GL, and is contradicted by this research. No differences in growth performance were observed by Cramer et al. (2003), with 3 to 6 wk BWG and feed efficiency unaffected by GL increasing from 16 to 31 %. Lack of differences in 3 to 6 wk broiler growth performance is supported with this research. Research is supported by Moritz et al. (2003) who decreased GL by adding moisture to diets, and resulted in no differences in 3 to 6 wk broiler growth performance. Svihus et al. (2004) supports conclusions of this research with 11 to 30 d old broiler growth performance unaffected by GL. This research and previous work supports the idea of more mature broiler gastrointestinal tracts being capable of digesting and absorbing nutrients in a wider variety of intestinal conditions when compared to young chicks. Because of larger and more mature gastrointestinal tracts, broiler 21 to 42 d old growth performance was unaffected by increasing GL from 0 to 20%.

Little research has been conducted on GL and carcass characteristics, and this study revealed no effects of GL on carcass characteristics. As stated, larger broilers, over 21 d of age, have the ability to digest the gelatinized starch without negatively affecting broiler growth and subsequent carcass dressing percentage. Gelatinization level affected relative gizzard weight, and effects of GL on relative gizzard weight are similar to effects reported when fiber is added to the diet. Higher relative gizzard weights have been reported with the addition of oat hulls to broiler diets, similar to results of this experiment (González-Alvarado et al., 2007; Jiménez-Moreno et al., 2009b). Other poultry have had similar responses to increased dietary fiber, with feeding pullets a diet containing oats as a fiber source increases relative gizzard weight (Scheideler et al., 1998). Gosling relative gizzard weights have been reported to increase when barley hulls or pectin were added to the diet (Lin et al., 2010). Wood shavings, a source of fiber, have been shown to increase relative gizzard size of 21 d old broilers, while another source of fiber, cellulose, did not affect gizzard size (Amerah et al., 2009). Gelatinized starch has a higher viscosity than uncooked starch (Yang and Rao, 1998; Mahasukhonthachat et al., 2010; Horn et al., 2011). Increased viscosity may result in similar gizzard conditions caused by some fibers. Increased gizzard size may be the reason for differences in effects of starch gelatinization observed between younger and older broiler growth performance. Gizzard size has been shown to be related to nutrient absorption, and changes in gizzard size are a way for broilers to adjust to

diets. With increased gizzard size, broilers fed diets with higher GL were able to adjust to higher feed viscosity.

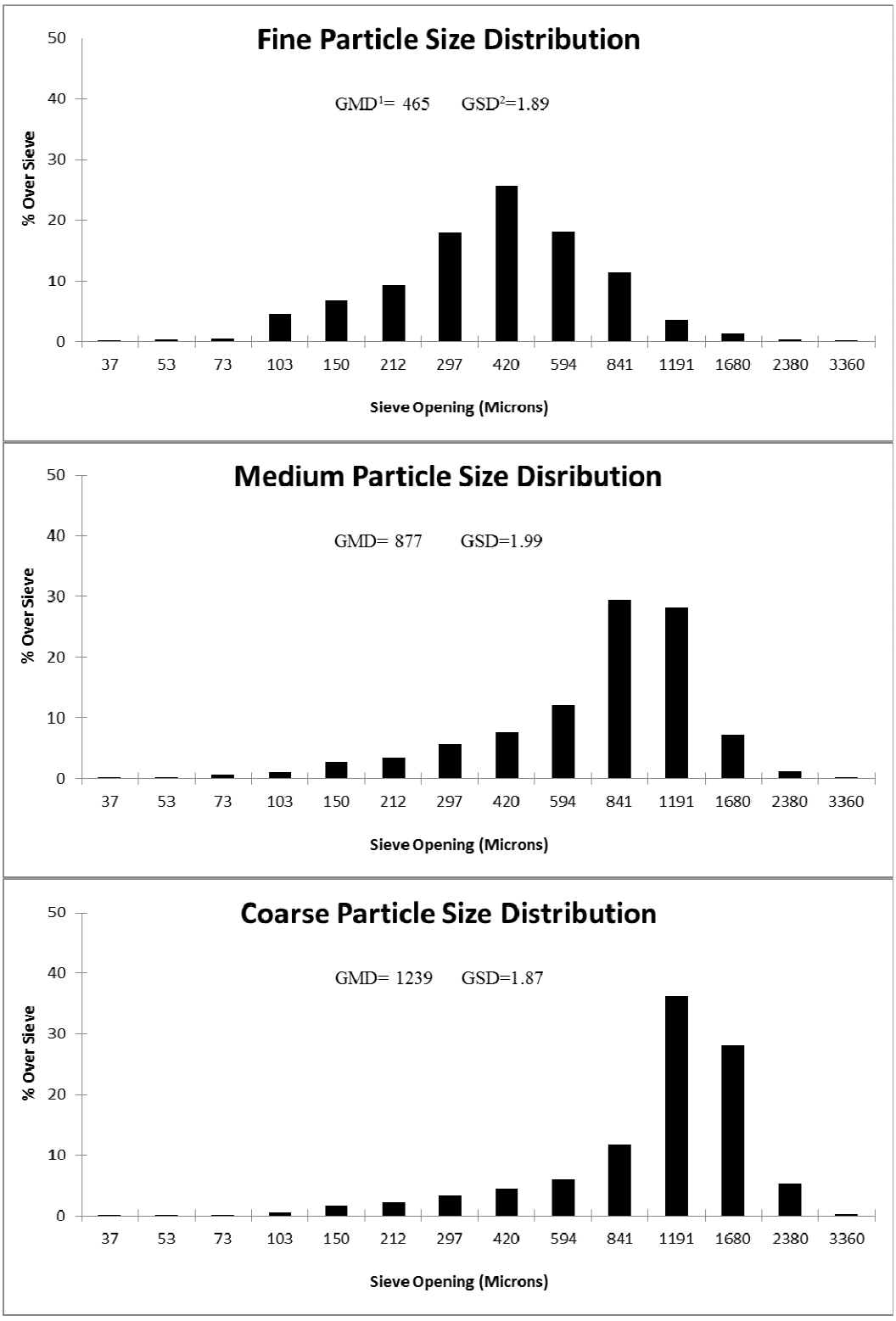
Increased growth performance due to larger particle size is in agreement with previous experiments. Broilers have been shown to have a preference for larger feed particles, and preferred PS increases with age (Portella et al., 1988b). Hamilton and Proudfoot (1995) observed similar increases in BWG with larger PS. Lott et al. (1992) observed no particle size effects on 42 d old BWG or FC, when broilers were fed diets containing 720 or 1,200 µm corn, contradictory to research. Those results may have been affected by starter particle size, and could have led to differences between experimental results. In support of research, most published literature reveals no differences in feed efficiency (Reece et al., 1986; Lott et al., 1992; Deaton et al., 1995; Hamilton and Proudfoot, 1995; Preston et al., 2006). Nir et al. (1995) observed improved BWG and feed conversion when feeding diets with corn PS of 1,400 to 2,200 µm, compared to broilers fed diets with corn PS of 630 to 680 µm. Improvements in BWG with PS greater than 1,240 µm, indicates 22 to 42 d of age broilers can efficiently grow with corn particle sizes larger than was used in this study. Parsons et al. (2006) fed broilers diets with corn PS ranging from 780 to 2,250 µm without affecting BWG. However, feed conversion was reduced with the 2,250 µm PS corn, but the PS similar to the CP used in this study, 1,100 µm, did not affect feed conversion. Reported research and the quadratic response of BWG with increasing PS shows that there could be a limit to how large corn PS could be for optimal broiler grower performance, but the upper limit PS is likely larger than reported in this study.

There is little work on effects of particle size on carcass dressing percentage. Contrary to this research, Clark et al. (2009) observed a linear decrease in carcass yield with increased addition of cracked corn, which increased dietary particle size. Dozier et al. (2009) observed no differences in carcass yield with addition of rolled corn to pelleted feed, in contrast with this study. Carcass yield or dressing percentage is related to efficiency of gain, and increased efficiency could have been caused by increased gizzard size and function. An increased gizzard size was expected, because of support in the literature. Clark et al. (2009) reported gizzard size was increased with increased cracked corn. Rougière et al. (2009) observed increases in dietary corn PS increased relative gizzard size of 26 d old broilers. Increasing dietary corn PS from 380

µm to 800 µm has been observed to increase 8 d old turkey poult gizzard relative weight (Favaro et al., 2009). At 39 d of age broiler relative gizzard weight has been shown to increase with dietary wheat particle size (Enberg et al., 2002). Péron et al. (2005) found that feeding coarsely ground wheat increased 21 d old broiler relative gizzard size. Literature and experimental results suggest gizzards will increase to grind the dietary PS, in order to reduce PS and increase surface area for enzymatic degradation. Because they do not possess teeth, poultry rely on the gizzard to decrease diet PS, and the gizzards will respond to dietary stimuli. This increase in gizzard size is thought to increase intestinal function, and improve broiler performance. Calcium and phosphorus availability have been reported to improve with use of larger ground grains or soybean meal (Kasim and Edwards, 2000; Kilburn and Edwards, 2001, 2004; Charbeneau and Roberson, 2004). Increased gizzard function was thought to be related to increased nutrient absorption. Improved nutrient availability from larger gizzards may have been responsible for improvements in broiler BWG, and subsequent carcass dressing percentage.

In conclusion, broiler 21 to 42 d of age growth performance was unaffected by increasing starch gelatinization level. Increased gizzard size allowed broilers to adjust to increasing gut viscosity associated with gelatinized starch, and efficiently metabolize nutrients in feed. Increasing corn particle size broilers improves BWG and dressing percentage, and may be due to increased gizzard function and nutrient retention. Increasing relative gizzard weight via stimulation from corn particle size will improve broiler body weight gain and carcass dressing percentage without negatively affecting feed conversion.

Figure 2-1 Particle distributions of corn ground using a three-high roller mill



[1]Geometric mean diameter
[2]Geometric standard deviation

Table 2.1 Standard starter diet and experimental grower diet formulation and nutrient composition (%, as-fed basis)

Ingredient (%)	Starter Diet	Grower Diet
Corn	52.53	51.10
Soybean meal (48%)	34.91	29.10
Starch/pregelalinized starch	-	9.63
Soy oil	5.54	3.85
Meat and bone meal (47.9%)	4.00	4.00
Limestone	0.96	0.99
Defluorintated phosphate	0.97	0.49
Salt	0.40	0.29
$_{DL}$-Methionine	0.22	0.11
$_{L}$-Lysine	0.09	0.01
Feed additives[123]	0.36	0.36
Calculated composition		
Metabolizable energy, kcal/kg	3,200	3,200
CP, %	23	20
Lys, %	1.35	1.10
Met, %	0.56	0.42
TSAA[4], %	0.93	0.73
Trp, %	0.30	0.26
Thr, %	0.87	0.76
Ca, %	1.00	0.90
Nonphytate P, %	0.45	0.35
Na, %	0.20	0.16

[1]Supplied at per kilogram of diet manganese, 0.02%; zinc, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg, choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B_6, 1.38 mg; niacin, 27.56 mg; panthothenic acid, 6.61 mg; thiamine, 2.31 mg; menadione, 0.83 mg; vitamin B_{12}, 0.01 mg; vitamin E, 16.53 IU, vitamin D_3, 2,331 ICU, vitamin A, 7,716 IU.
[2]Monensin 0.099 g per kg, Elanco Animal Health, Indianapolis, IN
[3]Bacitracin methylene disalicylate, 0.055 g per kg, Alpharma, Bridgewater, NJ.
[4]Total sulfur amino acids

Table 2.2 The effects of starch gelatinization level and corn particle size on 22 to 42 d old broiler body weight gain, feed consumption and feed conversion

Treatment Gelatinization Level[1]	Particle Size[2]	BWG (kg)	Feed Consumption (kg)	F:G (kg:kg)
0		1.795	77.252	1.745
10		1.775	77.766	1.772
20		1.778	78.940	1.774
SEM (61)		0.016	1.195	0.021
	FP	1.696[b]	76.025	1.780
	MP	1.816[a]	78.044	1.749
	CP	1.836[a]	79.888	1.763
SEM (61)[3]		0.016	1.195	0.021
Treatments Effects			*P*-value	
GL[4]		0.599	0.570	0.544
PS[5]		<0.001	0.067	0.585
Contrasts				
Linear	GL	0.434	0.303	0.330
	PS	<0.001	0.021	0.567
Quadratic	GL	0.522	0.815	0.608
	PS	0.009	0.951	0.385

[a,b] Means within a column with no common superscripts differ significantly ($P \leq 0.05$) as a result of a Tukey-Kramer test

[1]Gelatinization level: 0 = 0 %; 10 = 10 %; 20 = 20 %

[2]Particle sizes: FP = 465 µm; MP = 877 µm; CP = 1239 µm

[3]SEM (61): SEM with 61 df

[4]GL= gelatinization level

[5]PS=particle size

Table 2.3 The effects of starch gelatinization level and corn particle size on carcass dressing percent and gizzard relative weight

Treatment			
Gelatinization Level[1]	Particle Size[2]	Dressing Percent[3] (%)	Gizzard Relative Weight[4] (g/100 g BW)
0		69.93	1.28[ab]
10		70.09	1.21[b]
20		69.96	1.36[a]
SEM		0.23	0.04
	FP	69.65[b]	1.19[b]
	MP	69.82[ab]	1.34[a]
	CP	70.52[a]	1.32[a]
SEM		0.23	0.04
Treatment Effects		*P*-value	
GL[5]		0.872	0.021
PS[6]		0.023	0.014
Contrasts			
Linear	GL	0.934	0.113
	PS	0.01	0.015
Quadratic	GL	0.607	0.019
	PS	0.389	0.083

[a,b] Means within a column with no common superscripts differ significantly (P≤0.05) as a result of a Tukey-Kramer test

[1]Gelatinization level: 0 = 0 %; 10 = 10 %; 20 = 20 %

[2]Particle sizes: FP = 465 µm; MP = 877 µm; CP = 1239 µm

[3]SEM calculated using 45 df

[4]SEM calculated using 43 df

[5]GL= gelatinization level

[6]PS=particle size

Chapter 3 - The Effect of Gelatinized Starch on Broiler Chick Glucose Absorption, Glucose Regulation, and Digesta pH

Abstract

This research explored the effects of gelatinized starch on gastrointestinal pH, glucose absorption, and glucose regulation on male broiler chicks. The broiler chicks were fed experimental diets containing a gelatinized corn starch at 0 or 20% of the total dietary starch content, from 0 to 21 d of age. Starch gelatinization levels were achieved by replacing 20 % of dietary starch with commercial corn starch. The 0 % starch gelatinization level treatment used unmodified corn starch, and the 20 % starch gelatinization level treatment used pregelatinized corn starch. At 20 d of age, feed was removed from pens for 6 hrs and 3 broiler chicks per treatment were selected, and feed was returned to pens. At 30 and 60 min post-feeding 3 more broiler chicks were collected per treatment. The broiler chicks were euthanized and gizzard, duodenum, jejunum, and ileum contents were collected for pH determination. At 21 d of age feed was again removed from pens for 6 hrs, 6 broiler chicks were selected per treatment, and feed was returned to pens. At 30 and 60 min post-feeding 6 more broiler chicks were collected per treatment. Blood was collected via cardiac puncture and analyzed for blood glucose and glucagon. The broiler chicks fed the 20 % starch gelatinization level diet had a higher jejunum pH than the broiler chicks fed the 0 % gelatinization level (P=0.001). Time had a quadratic effect on gizzard (P=0.002) and ileum pH (P=0.03). Blood glucose levels were higher (P=0.037), and glucagon levels were lower (P=0.003) with the 0% starch gelatinization level diet. Similar to pH measurements, a quadratic effect of time post-feeding was observed on glucagon levels (P=0.032). Starch gelatinization level was observed to have little effect on gastrointestinal pH. Increases in starch gelatinization level could cause a greater utilization of stored glucose in broiler chicks..

Introduction

In commercial broiler diets, energy is provided by cereal grains and fats. Energy is stored in cereal grains primarily as starch. Dietary starch is hydrolyzed by α-amylase and brush border carbohydrases, which hydrolyze starch into glucose to be absorbed by the small intestine.

Broilers secrete large amounts of amylase which reaches adult levels within the first few days post-hatch (Nitsan et al., 1991, 1994; Uni et al., 1995). Because of the high amylase secretion, starch digestibility has been reported to be over 90 % in broilers (Uni et al., 1995; Svihus et al., 2004). This high starch digestibility has led to blood glucose levels of 12 to 17 mmol in chickens (Rideau et al., 2008; Proszkoweic-Weglarz et al., 2009). Broilers secrete two hormones to regulate blood glucose level, insulin and glucagon. Insulin lowers blood glucose levels by stimulating liver absorption of glucose, and glucagon raises blood glucose levels by stimulating liver release of stored glucose (Dickson and Langslow, 1978; Hazelwood and Langslow, 1978; McMurtry et al., 1983; Tinker et al., 1984; Ruff and Allen, 1982; Krestel-Rickert et al., 1986; Proszkoweic-Weglarz et al., 2009; Rideau et al., 2010). Glucagon and insulin are inter-related, and increases in glucagon will increase insulin and decrease glucagon (King and Hazelwood, 1976; Hazelwood and Langslow, 1978; Sitbon and Miahle, 1978; Honey and Weir, 1979; Sitbon and Miahle, 1979; Honey et al., 1980). Insulin is more sensitive to glucagon levels than glucose levels, and glucagon is the primary blood glucose regulatory hormone in poultry (Hazelwood, 2000).

Broiler feed is commonly conditioned and formed into pellets to improve body weight gains and feed conversion. Dietary starch is gelatinized during pelleting, which is caused by steam conditioning and forces within a pellet mill die. A range of starch gelatinization can occur during pelleting, and pelleted broiler feed can have a range of starch gelatinization levels from as little as 1 % (Svihus et al., 2004) to as high as 30 % (Moritz et al., 2002). Pellet quality is usually measured using a pellet durability tester, and improvements in pellet quality have been shown to improve broiler growth performance (Mortiz et al., 2001, 2002; Greenwood et al., 2004; McKinney and Teeter, 2004; Quentin et al., 2004; Lemme et al., 2006; Cutlip et al., 2008; Dozier et al., 2010). Measurements of pellet quality are related to how many pellets will survive normal handling practices, and Mckinney and Teeter (2004) reported improvements in metabolizable energy with higher numbers of pellets in feed pans when fed to broilers. A survey of pellet quality revealed that pellet quality is associated with increased starch gelatinization (Moritz et al., 2001, 2002, 2003; Cramer et al., 2003; Svihus et al., 2004; Buchanan et al., 2010).

Changes in starch gelatinization level may affect broiler growth performance. Moritz et al. (2005) reported positive effects of gelatinizing starch on broiler chick starter body weight gain, but no effects on feed conversion. Earlier research conducted in our lab showed decreasing broiler starter body weight gain with increasing levels of starch gelatinization.

While feeding broilers pelleted feed improves growth performance, the feeding of pelleted diets can have negative effects. A negative effect of feeding broilers pelleted diets is reduced gastrointestinal tract size, specifically gizzard size (Choi et al., 1986; Preston et al., 2000; Enberg et al., 2002; Svihus et al., 2004; Amerah et al., 2007). Reduction in gizzard size is believed to have negative effects on nutrient digestion, and research involving different dietary particle sizes reported that increases in gizzard size are beneficial to nutrient retention (Kasim and Edwards, 2000; Kilburn and Edwards, 2001, 2004; Charbeneau and Roberson, 2004). González-Alvarado et al., (2008) found that feeding broilers diets containing cooked corn reduced gizzard relative size. Broiler gizzard size is related to physical stimulation of the gizzard, usually caused by coarse feed particles. Reduction of feed particle size during formation of pellets and the gelatinization of dietary starch may be altering physical stimulation of the gizzard, causing reduced broiler gizzard size.

Altering gastrointestinal size may alter pH, and feeding broilers pelleted rations is reported to increase gizzard pH (Enberg et al., 2002; Huang et al., 2006). Abd El-Khalek et al. (2001) reported that increasing starch gelatinization level from 53 to 74 %, increased pigeon gizzard pH. Changes in pH can alter gastrointestinal bacterial profile and growth. Feeding broilers pelleted diets has been shown to increase enterococci and coliform bacteria (Enberg et al., 2002). *Salmonella typherium* survival rates have been shown to increase with feeding of pelleted diets (Huang et al., 2006). Increased gastrointestinal pH will increase bacteria survivability, increasing the bacterial load at the time of slaughter. Altering pH can affect digestive enzymatic efficiency. Osman (1982) observed that the optimal pH for pancreatic amylase was 7.5. Deviations from the optimal pH will decrease enzymatic digestion rate.

Work in our lab has shown that gelatinized starch levels at 20% will decrease broiler starter performance, but not broiler grower phase growth performance. An experiment was

designed to find if changes in gelatinization level were 1) responsible for changes in gastrointestinal pH associated with pelleting and 2) determine if changes in broiler growth associated with gelatinization level are due to altering glucose absorption levels and glucose regulatory hormones.

Materials and Methods

All animals were reared following protocols established by the Kansas State University Institutional Animal Care and Use Committee. Broilers were housed at the Thomas B. Avery Poultry Research Unit (Manhattan, KS). Male Cobb 500 broiler chicks were obtained from a local hatchery, and 50 chicks were placed into 6 floor pens. Pens were randomly assigned to treatments. The chicks were kept under 24 h of light for the first 3 d, and a schedule of 23L:1D for the remainder of the experiment. The broiler chicks were raised at 33 ^{o}C for the first 3 d, and temperature was reduced 2.5 ^{o}C every 7 d for remainder of the experiment. The experimental diets were formulated to meet or exceed NRC (1994) nutrient requirements (Table 3.1). The broiler chicks were fed experimental diets from 0 to 21 d of age. The experimental diets were designed to replace 20% of dietary starch with commercial corn starch to simulate starch gelatinization levels (GL) found in pelleted diets. A 0% GL was created by including unmodified corn starch, and a 20% GL was created by including pregelatinized corn starch (product number 12030, Cargill Foods, Minneapolis, MN). The broiler chicks were provided feed and water *ad libitum*, and were checked daily for mortalities.

pH Measurements

At 20 d of age all feed was removed from pens for 6 h to ensure emptying of the gastrointestinal tract. After 6 h one bird per pen was selected representing time 0, and then feed was returned to pens. One bird per pen was selected at 30 min post-feeding and at 60 min post-feeding. Selected birds were euthanized using CO_2 administration, and then gizzard, duodenum, jejunum, and ileum contents were harvested by squeezing out intestinal contents and washing using deionized water. The jejunum was defined as the section of the small intestine from the end of the duodenal loop to the Meckel's diverticulum, and the ileum was defined as the section from the Meckel's diverticulum to the ileal-cecal junction. Measurement of pH was modified from the method outlined by Santos et al. (2008). Harvested contents were diluted using

deionized water (1:10), homogenized, and pH was recorded using a pH meter (Orion Model 230A pH meter, Thermo Fisher Scientific, Waltham, MA).

Blood Glucose and Glucagon Levels

At 21 d of age feed was removed from pens for 6 h to ensure emptying of the gastrointestinal tract. After 6 h two birds per pen were selected, representing time 0, and then feed was returned to pens. Two birds per pen were selected at 30 min and at 60 min post-feeding. Blood was collected from broilers via cardiac puncture, injected into a Vacutainer containing EDTA (Becton Dickinson Vacutainer systems 1119801, Fanklin Lakes, NJ), and placed on ice. Blood samples were centrifuged at 500 g for 10 min, and plasma samples were collected and frozen for further analysis. The plasma samples were analyzed for blood glucose level using glucose oxidase peroxidase colorimetry (Brian Luebbe Autoanalyzer 3, Seal Analytical, Mequon, WI). Glucagon levels were analyzed using a method outlined by Lu et al. (2007) and a glucagon radioimmunoassy kit (Gl-32K, Millipore, St. Charles, MO).

Statistical Analysis

Experimental data were analyzed as a completely randomized design, with pen serving as the experimental unit and an alpha of 0.05. All data were analyzed using the MIXED procedure of SAS (Release 9.1 for Windows, SAS Institute, Cary, NC). When *F*-tests indicated significance treatment means were separated using the Tukey-Kramer method. Least square means were calculated, and contrasts were conducted to determine linear and quadratic effects of time post-feeding on measured variables.

Results

Gastrointestinal pH

All pH data is presented in Table 3.2. There was no effect of GL on gizzard, duodenum, and ileum pH, but jejunum pH was higher with the 20 % GL (6.01 pH) than with the 0 % GL (5.75 pH). Time post-feeding significantly affected gizzard and ileum pH, but no effect was detected on duodenum or jejunum pH. Contrasts revealed a significant quadratic effect of time on gizzard and ileum pH. Interactions between GL and time post-feeding were detected on gizzard and jejunum pH. The gizzard pH of broiler chicks were lowest in broiler chicks fed 0 %

GL at time 0 min (2.28 pH) post-feeding and broiler chicks fed 20 % GL diets at time 0 min (3.06 pH) and 60 min(3.04 pH) post-feeding. Broiler chicks fed 20 % GL diet at 0 min post-feeding had higher jejunum pH (6.19 pH) than broiler chicks fed 0 % GL diet at 0 min (5.62 pH) and 30 min (5.75 pH) post-feeding.

Blood parameters

Time post-feeding had no effect on blood glucose level, although it did approach significance (P=0.063). Increasing GL from 0 to 20 % decreased blood glucose level. Interactions of time post-feeding and GL had no effect on blood glucose level. Glucagon levels were decreased by time post-feeding. Contrasts revealed decreases in blood glucagon level due to time post-feeding were quadratic. Starch gelatinization level affected glucagon, with increases in GL increasing glucagon (254 pg/ml to 421 pg/ml). Glucagon was affected by interactions between GL and time post-feeding, with 0 min post-feeding and 20 % GL having the highest glucagon level (686 pg/ml).

Discussion

Increases in time post-feeding had a quadratic effect on gizzard and ileum pH, with gizzard pH peaking at 30 min post-feeding. Research into fasting on pH of laying hens revealed a higher pH in the intestine and proventriculus after 6 hr fast than was observed in the gizzard, and is contrary to this study (Winget et al., 1962). Differences in diet and gastrointestinal tract size between broiler chicks and laying hens may have caused of differences in reported pH levels. Wiseman et al. (1956) observed that fasted broilers had a higher pH in all segments of the gastrointestinal tract when compared to the gastrointestinal pH of fed broilers, in contrast to this research. Quadratic effect of time post-feeding is expected because of feed consumption will stimulate acid and bicarbonate secretion. Feed has a higher pH than observed in gastrointestinal tract, and pH should increase after feeding, especially in the gizzard. As the gizzard empties the relative amount of acid in the gastrointestinal contents should increase.

The results of the current study contrasts the results from other research on pelleting (Enberg et al., 2002; Huang et al., 2006; Preston et al., 2000), with little effect shown on gastrointestinal pH. Huang et al. (2006) reported an increase in gizzard pH with pelleted feed,

which led to increase survival rates of Salmonella. Salmonella has been reported to have an optimal growth at pH of 6.5 to 7.5, which is higher than average pH reported for either treatment for all gastrointestinal sections (Chung and Goepfort, 1970). Enberg et al. (2002) found lower pH in the jejunum, but higher gizzard pH with feeding of pelleted feed, not supporting the results of this research. Higher bacteria levels were observed in the gizzard with pelleting, but no effect on jejunum bacteria levels. Higher bacteria levels in the gizzard demonstrate that gizzard pH is more important for controlling bacteria levels than jejunum pH. Pigeon gizzard pH has been shown to increase with starch gelatinization level increasing from 53 to 74 %, differing from the results of this study (Abd El-Khalek et al., 2001). A lower starch gelatinization level in our studies may have caused differences in results. Heat processing of corn has been shown to not alter gizzard pH in 21 d old broilers, supporting this research (González-Alvarado et al., 2007). At a GL of 20 % gizzard pH does not seem to be affected, but may be altered with a higher GL.

Blood glucose levels were unaffected by time in this study, which is contradictory to previous research, but it did approach significance (P=0.063). Broilers should absorb glucose from diets quickly, as shown by high starch digestion rates (Uni *et al.*, 1995; *Svihus et al.*, 2004). Krestel-Rickert et al. (1996) observed increased glucose levels at 30 min post-feeding, not supporting our experimental results. Similar effects of time post-feeding on blood glucose level were reported by Simon and Rosselin (1978) who observed a peak blood glucose level at 30 min post-feeding with broilers starved for 5-6 h. Decreases in glucagon with increased glucose level has been observed in geese (Sitbon and Miahle, 1978), in chickens injected with synthalin A (Langslow *et al.*, 1973), and chickens injected with glucose (Hazelwood and Langslow, 1978). Glucose and glucagon have been shown to be interrelated and experimental results of the 20% GL diet resulting in higher glucagon and lower blood glucose are within expectations. As blood glucose levels will decrease, glucagon secretions increase blood glucose by mobilizing glucose from glycogen stores in the liver (Ruff and Allen, 1982). Lipocytes (Oscar, 1992) and hepatocytes (Savage *et al*, 1995) have been shown to be desensitized to glucagon if exposed to large amounts of glucagon. Langslow et al. (1973) observed that glucagon concentrations of broilers starved for 48 h was over 900 pg/ml, whereas, the highest glucagon concentration observed in this study was 685 pg/ml. Built up tolerance of high glucagon levels or low glucose storage may have been the cause of high levels of glucagon, and low levels of blood glucose in

the 20% GL treatment. Increased tolerance to glucagon could be a result of reduced glucose absorption during a broiler chick's growth. Liver glycogen stores could have been lower in the 20 % GL fed broiler chicks, which reduces blood glucose and increases glucagon secretion.

Using *in vitro* methods to measure starch digestibility has shown an increase in digestibility with starch gelatinization (Chung et al., 2006; Parada and Aguilera, 2008; Miao et al., 2010; Mahasukhonthachat et al., 2010). Tilapia dietary starch digestibility increased with increased dietary gelatinized starch (Abdolsamad et al., 2006), and is contradictory to this research. Research conducted on juvenile spiny lobsters reported decreased starch digestibilities with higher inclusions of gelatinized corn starch (Simon, 2009), and is supported by blood glucose level results in this study. Differences in gastrointestinal tract sizes between species may have resulted in varying responses to gelatinized starch levels. Lower blood glucose and higher blood glucagon levels may be due to differences in starch digestion rates. *In vitro* methods indicate that native starch has a slower digestive rate, and the broiler chicks could have absorbed more glucose after the feed was removed. More glucose absorption after feed removal would lower the need for the release of stored glucose decreasing glucagon secretion.

In conclusion, broilers fed a higher dietary GL had lower blood glucose level, which resulted in higher glucagon secretion. Lower glucose storage or response to glucagon, caused an increase in glucagon secretion. Higher jejuna pH may be more suitable to bacterial growth, and could lead to decreases in broiler growth performance. Increased GL results in gastrointestinal conditions conducive to bacterial growth, and lowers broiler responses to glucagon, indicative of greater utilization of stored glucose. Starch gelatinization levels found in pelleted diets may be creating gastrointestinal environments more conducive to bacterial growth, and could cause an increase in the utilization of stored glucose.

Table 3.1 Experimental diet formulation and nutrient composition (%, as-fed basis)

Ingredients	Percent in Diet
Corn	43.24
Soybean meal (48%)	36.54
Starch/pregelalinized starch	7.68
Soy oil	5.49
Meat and bone meal (47.9%)	4.00
Limestone	1.13
Dicalcium phosphate	0.85
Salt	0.40
DL-Methionine	0.29
Feed additives[123]	0.36
Calculated composition	
Metabolizable energy, kcal/kg	3,200
CP, %	23
Lys, %	1.29
Met, %	0.63
TSAA[4], %	0.98
Trp, %	0.31
Thr, %	0.88
Ca, %	1.00
Available P, %	0.45
Na, %	0.20

[1]Supplied at per kilogram of diet manganese, 0.02%; zinc, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg, choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B_6, 1.38 mg; niacin, 27.56 mg; panthothenic acid, 6.61 mg; thiamine, 2.31 mg; menadione, 0.83 mg; vitamin B_{12}, 0.01 mg; vitamin E, 16.53 IU, vitamin D_3, 2,331 ICU, vitamin A, 7,716 IU.
[2]Monensin 0.099 g per kg, Elanco Animal Health, Indianapolis, IN.
[3]Bacitracin methylene disalicylate, 0.055 g per kg, Alpharma, Bridgewater, NJ.
[4]Total sulfur amino acids.

Table 3.2 The effect of time post-feeding and gelatinization level on gastrointestinal pH after 6 hr starvation

Treatment		GIT region (pH)			
Gelatinization Level[1]	Time[2]	Gizzard	Duodenum	Jejunum	Ileum
0	0	2.28[b]	5.90	5.62[b]	5.80
	30	3.80[a]	6.16	5.75[b]	6.52
	60	3.25[a]	5.99	5.89[ab]	6.26
20	0	3.06[b]	6.06	6.19[a]	5.91
	30	3.41[a]	6.06	5.88[ab]	6.46
	60	3.04[b]	6.08	5.95[ab]	6.45
SEM (12)		0.21	0.13	0.08	0.18
Treatment Effects			*P*-value		
G[4]		0.711	0.657	0.001	0.596
T[5]		0.002	0.611	0.310	0.010
G*T		0.022	0.570	0.008	0.794
Contrasts					
Linear	T	0.029	0.688	0.777	0.016
Quadratic	T	0.002	0.323	0.141	0.030

[a-c] Means within a column not sharing common superscript differ ($P<0.05$)

[1]Gelatinization level: 0 = 0 %; 20 = 20%

[2]Time: 0 = Collection after 6 hr starvation; 30 = 30 min after consuming feed; 60 = 60 min after consuming feed

[3]SEM(12) = SEM with 12 df

[4]G= gelatinization level

[5]T=time

Table 3.3 The effect of time post-feeding and gelatinization level on glucagon and blood glucose levels after 6 hr starvation

Treatment Gelatinization Level[1]	Time[2]	Blood Glucose (mmol)	Glucagon (pg/ml)
0	0	13.73	303[b]
	30	14.90	249[b]
	60	14.83	209[b]
20	0	12.29	686[a]
	30	14.21	274[b]
	60	13.55	304[b]
SEM[3]		0.56	55
Treatment Effects		*P*-value	
G[4]		0.037	0.003
T[5]		0.063	0.001
G*T		0.796	0.017
Contrasts			
Linear	T	0.073	0.001
Quadratic	T	0.080	0.035

[a,b] Means within a column not sharing common superscript differ ($P<0.05$)

[1]Gelatinization level: 0 = 0 %; 20 = 20%

[2]Time: 0 = Collection after 6 hr starvation; 30 = 30 min after consuming feed; 60 = 60 min after consuming feed

[3]SEM: 11 df for blood glucose; 12 df for glucagon

[4]G= gelatinization level

[5]T=time

The Effect of Gelatinized Starch on Rooster and Broiler Chick Metabolizable Energy, and Broiler Chick Ileal Amino Acid Digestibility

Abstract

Research investigated the effects of gelatinized starch on true metabolizable energy, apparent metabolizable energy, and ileal amino acid digestibility. Broiler diets with three starch gelatinization levels were precision fed to intact Single Comb White Leghorn roosters. Starch gelatinization levels were 0, 50, and 100 %, with 0% containing only unmodified corn starch, 50 % containing a 1:1 blend of unmodified corn starch and pregelatinized corn, and 100% containing only pregelatinized corn starch. Apparent metabolizable energy and amino acid digestibility were determined using broiler chicks. Broiler chicks were fed a standard starter diet from 0 to 17-d of age, and treatment diets from 17 to 21-d of age. Treatments were created by replacing 20 % of dietary starch with unmodified corn starch and pregelatinized corn starch to create 3 different starch gelatinization levels, 0 %, only unmodified corn starch, 10 %, a 1:1 blend of unmodified corn starch and pregelatinized corn starch, and 20 %, only pregelatinized corn starch. True metabolizable energy was affected (P=0.016) by starch gelatinization level, and increasing gelatinization level linearly increased true metabolizable energy (P=0.007). Interestingly, the amount of rooster fecal dry matter was decreased with increasing starch gelatinization level (P=0.023), and the effect was linear (P=0.011). No effect of starch gelatinization level was detected on apparent metabolizable energy (P=0.341) or amino acid digestibility (P>0.05). The effect of gelatinization level on metabolizable energy was only detected in precision fed roosters but was not detected in complete diets. The data indicate that starch gelatinization levels under 20 % do not affect metabolizable energy and amino acid digestibility in the 21-d-old broiler chick.

Introduction

Feeding broilers pelleted diets improves efficiency of gain and body weight. Broilers consuming pelleted diets are reported to spend less time consuming feed and more time resting (Savory, 1974; Skinner-Noble et al., 2005). Because of this, improvements in pellet durability and number of pellets in feed pans improves broiler growth performance (Mortiz et al., 2001, 2002; Greenwood et al., 2004; McKinney and Teeter, 2004; Quentin et al., 2004; Lemme et al.,

2006; Cutlip et al., 2008; Dozier et al., 2010). When broiler diets are pelleted, starch gelatinization occurs due to pellet die friction and steam conditioning. Increased starch gelatinization has been associated with increased pellet durability (Moritz et al., 2001, 2002, 2003; Cramer et al., 2003; Svihus et al., 2004; Buchanan et al., 2010). Pelleting broiler feed has been shown to alter broiler nutrient requirements. Broilers fed pelleted diets have a higher lysine requirement (Greenwood et al., 2005), and lower growth performance responses to additional dietary fat (Plavnik et al., 1997).

Dietary metabolizable energy can be altered by pelleting, and has been observed to increase apparent metabolizable energy (AME) (Gonzalez-Esquerra and Leeson, 2000; Preston et al., 2000; Svihus et al., 2004; Zhang et al., 2009). Pelleting a broiler diet containing flaxseed has been shown to improve AME of flaxseed (Gonzalez-Esquerra and Leeson, 2000). These results are not found throughout the literature, with some reporting no effects of pelleting on AME (Amerah et al., 2007), and others reporting negative effects on AME (Yang et al., 2010). A purified diet based on gelatinized starch has been shown to decrease AME measured with 14 d old broiler chicks, when compared to a purified diet based on regular corn starch (Batal and Parsons, 2004). The results were not repeated in different ages, and there were no ranges of starch gelatinization levels tested.

Research studies have investigated the energy availability of pelleted diets, but little work has been conducted on amino acid availability. Higher lysine requirements have been reported for pellet fed broilers (Greenwood et al., 2005), but amino acid (AA) digestibilities were not investigated. Heat processing cereal grains has been shown to have no effect on total N retention, but no individual AA analysis was conducted (Jiménez-Moreno et al, 2009a). True metabolizable energy (TME) is a measurement of feed ingredients energy availability to broilers for metabolism and growth. Metabolizable energy assays have been conducted for ingredients ranging from rare ingredients such as dried tomato seeds (Persia et al., 2003) to standard soybean meal (Edwards et al., 2000). Little to no work has evaluated gelatinized starch as an ingredient and its effects on dietary metabolizable energy and amino acid availability.

The objective of the research was to evaluate gelatinized starch as an ingredient by using a precision fed rooster assay, and evaluate effects of gelatinized starch independent of feed form on AME and AA digestibility in 21 d old broiler chicks.

Materials and Methods

All animals were reared and experiments conducted following protocols established by the Kansas State University Institution of Animal Care and Use Committee, and chickens were housed at the Kansas State University Thomas B. Avery Poultry Research Unit (Manhattan, KS).

Rooster Assay

For the precision fed rooster assay, 3 different gelatinization levels (GL) were created by blending unmodified corn starch (CS) and pregetlatinized corn starch (PG) (product number 12030, Cargill Foods, Minneapolis, MN). The 0 % GL treatment was only CS, the 50 % GL treatment was a blend of CS to PG in a 1:1 ratio, and the 100 % GL treatment was only PG. Intact Single Comb White Leghorn (SCWL) roosters were starved for 24 h. After food deprivation, 30 g of each treatment diet was precision fed to 3 roosters, and 3 roosters sham fed for measurement of basal secretions. After 48 h all excreta was collected, weighed, dried, and ground. Gross energies were measured using an adiabatic bomb calorimeter, and true metabolizable energy (TME) was calculated using a method outlined by Parsons et al. (1982).

Broiler Chick Assay

The evaluation of AME and AA digestibility was conducted using Cobb 500 male broiler chicks, which were housed in battery brooders (Petersime Incubator Co., Gettysburg, OH). One-day old broiler chicks were obtained from a local hatchery, and 10 chicks placed into each of 18 pens, for a total of 1,800. The chicks were fed a standard corn-soybean meal starter diet formulated to meet or exceed NRC (1994) nutrient requirements from 0 to 17 d of age. Treatment diets were designed to replace 20% of dietary starch with commercial corn starch, and formulated to meet or exceed NRC (1994) nutrient requirements (Table 4.1). To simulate GL typically found in pelleted diets, CS and PG were blended in 3 different ratios, 1 CS: 0 PG for the 0 % GL, 1 CS: 1 PG for the 10% GL, and 0 CS: 1 PG for the 20% GL. Celite (Celite Corp., Lompoc, CA) was included at 1.5 % as a source of acid insoluble ash (AIA). At 17 d of age broiler chicks were weighed, reduced to 6 chicks per pen, and were randomly assigned to

treatments. Each treatment diet was fed from 17 to 21 d of age. On d 21, the broiler chicks were euthanized using CO_2 administration, and ileum contents were collected, frozen, lyophilized, and ground. The ileum was defined as the intestinal section from the Meckel's Diverticulum to 1 cm from the ileal-cecal junction. To obtain sufficient sample for all analyses 2 pens per treatment were combined, reducing samples to 3 per treatment. Analysis of AA's was conducted using acid hydrolysis, and sulfur containing AA's were analyzed using performic acid oxidation. Amino acid concentrations were measured using a HPLC. Gross energy was measured using the same procedure as TME analysis. Analysis of AIA followed the method outlined by Vogtmann et al. (1975). Calculations of AME used method outlined by Scott and Boldaji (1997) and AA digestibility coefficients calculated using method outlined by Garcia et al. (2007). Broiler chicks were provided feed and water *ad libitum*, and were checked daily for mortalities.

Statistical Analysis

The experimental data were analyzed as a completely randomized design using the MIXED procedure of SAS (Release 9.1 for Windows, SAS Institute, Cary, NC), with pen or rooster serving as the experimental unit and an alpha of 0.05. Least square means were calculated, and contrasts were conducted to determine linear and quadratic effects of starch gelatinization level on measured variables.

Results

Gelatinization level significantly increased TME, with CS having a TME of 2560 kcal/kg, and PG having a TME of 3695 kcal/kg (Table 4.2). True metabolizable energy increased linearly with increasing GL. Due to observed differences in excreta collected, the amount of excreta collected was analyzed. Excreta decreased from 14.84 g to 7.13 g when GL increased from 0 to 100 %, and this decrease was linear. No quadratic effects of GL were detected. Contrary to TME data, there was no effect of GL detected on AME (Table 4.3). Amino acid digestibility coefficients are displayed in Table 4.4 and no effect of GL on amino acid digestibility was detected.

Discussion

Linear increases in metabolizable energy of starch with increasing GL is not supported in the literature (Svihus and Hetland, 2001; Svihus et al., 2004). A possible reason for increased

TME was transit time. High amounts of excreta collected from the CS fed SCWL roosters could be indicative of low gastrointestinal retention time, thus decreasing energy availability. Gelatinized starch has been shown to have a higher viscosity than raw starch (Yang and Rao, 1998; Mahasukhonthachat et al., 2010; Horn et al., 2011), and higher viscosity increases retention time (Owusu-Asiedu et al., 2006; Cannon et al., 2010) allowing for greater amylase hydrolyzation of starch. Research involving Tilapia has shown that stomach content viscosities increase with increased starch gelatinization (Abdolsamad et al., 2006). A purified starch source does not have the same gastrointestinal tract physical and chemical signals as a complete diet. Lacy et al. (1986b) observed no responses in Cornish cockerels feed intake to intraduodenal infusions of glucose, demonstrating that chickens do not have the same response to glucose absorption as other macronutrients. A lack of physical and chemical signals may have resulted in SCWL rooster intestinal tract excreting the CS before amylase could hydrolyze the starch, and release the glucose.

The lack of physical and chemical signals is supported by no effects of GL on AME when gelatinized starch is included in a complete diet, and this is supported by the literature (Gonzalez-Esquerra and Leeson, 2000; Preston et al., 2000; Svihus et al., 2004; Zhang et al., 2009). The starch gelatinization level was lower in the broiler chick assay, and could be the cause of differences in results. The experimental results are contradictory to research conducted with spiny lobsters, with increased starch gelatinization level reducing starch digestibility (Simon, 2009). Earlier work in our lab showed a decrease in glucose absorption with increased gelatinization level, differing from AME results. Blood glucose measurements were recorded over a shorter period of time, and an increase in the experimental time period may have caused differences. Amino acid digestibility coefficients were unaffected by gelatinization level, which is supported by the work of Jiménez-Moreno et al. (2009), who observed no differences in N retention. Greenwood et al. (2005) observed higher lysine requirements due to feed pelleting. Broiler behavior has been shown to be altered by pelleting (Savory, 1974; Skinner-Noble et al., 2005), and this may have caused the differences in lysine requirement, not reduced lysine availability. Amino acid availability not affected by starch gelatinization levels of 20 % or lower.

The higher TME values obtained using the precision fed rooster assay may be unreliable due to high amounts of excreta collected in the CS treatment. A more reliable assay for determining GL effects on metabolizable energy is the broiler chick AME, due to feedback from lipids and proteins in the diet. No effects on AME or AA digestibility were detected using 21 d old broiler chicks, indicating GL has no effect on dietary energy or protein availability.

Table 3.4 Experimental diet formulation and composition (%, as is basis)

Ingredients	Percent in Diet
Corn	44.82
Soybean meal (48%)	35.14
Starch/pregelatinized starch	7.96
Meat and bone meal (50%)	4.00
Soy oil	3.91
Celite	1.50
Deflourinated phosphate	1.00
Limestone	0.70
Salt	0.37
DL-Methionine	0.23
Feed additives[123]	0.36
Calculated composition	
Metabolizable energy, kcal/kg	3,200
CP, %	23
Lys, %	1.26
Met, %	0.57
TSAA[4], %	0.92
Trp, %	0.30
Thr, %	0.86
Ca, %	1.00
Available P, %	0.45
Na, %	0.23

[1]Supplied at per kilogram of diet manganese, 0.02%; zinc, 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg, choline, 386 mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B_6, 1.38 mg; niacin, 27.56 mg; panthothenic acid, 6.61 mg; thiamine, 2.31 mg; menadione, 0.83 mg; vitamin B_{12}, 0.01 mg; vitamin E, 16.53 IU, vitamin D_3, 2,331 ICU, vitamin A, 7,716 IU.
[2]Monensin 0.099 g per kg, Elanco Animal Health, Indianapolis, IN
[3]Bacitracin methylene disalicylate, 0.055 g per kg, Alpharma, Bridgewater, NJ.
[4]Total sulfur containing amino acids

Table 3.5 The effect of starch gelatinization level on true metabolizable energy values measured using intact Single Comb White Leghorn roosters

Gelatinization Level (%)	Excreta (g of DM[2])	TME[1] (kcal/kg)
0	14.84	2561
50	8.55	3409
100	7.13	3695
SEM (6)[3]	1.50	198
Treatment Effects	*P*-value	
GL	0.023	0.016
Linear	0.011	0.007
Quadratic	0.233	0.291

[1]True metabolizable energy
[2]Dry matter
[3]SEM(6):SEM with 6 df

Table 3.6 The effect of starch gelatinization level on apparent metabolizable energy values measured using 21 d old broiler chicks

Gelatinization Level (%)	AME[1] (kcal/kg)
0	3130
10	3036
20	3187
SEM (5)	58
Treatment Effects	*P*-value
GL	0.341

[1]Apparent metabolizable energy
[2]Calculated with 5 df

Table 3.7 The effect of starch gelatinization level on amino acid digestibility coefficients measured using 21 d old broiler chicks

	Gelatinization Level (%)				Treatment
Amino Acid	0[1]	10[2]	20[1]	SEM	Effects
Indispensable					
Arginine	83.03±2.52	83.41±3.09	83.47±2.52	0.98	0.945
Glycine	81.07±4.81	79.90±5.89	79.61±4.81	1.87	0.853
Histidine	84.69±4.26	91.65±5.21	84.57±4.26	1.66	0.076
Isoleucine	83.37±4.02	83.32±4.92	82.34±4.02	1.56	0.881
Leucine	85.32±3.59	84.65±4.39	83.55±3.59	1.40	0.684
Lysine	86.20±3.65	85.73±4.47	84.75±3.65	1.42	0.774
Methionine	92.42±1.99	93.79±2.44	90.99±1.99	0.77	0.164
Phenylalanine	86.23±3.15	84.81±3.86	86.66±3.15	1.23	0.645
Threonine	78.01±4.46	76.71±5.47	72.83±4.46	1.74	0.190
Valine	83.39±5.42	83.21±6.63	82.13±5.42	2.11	0.906
Dispensable					
Alanine	84.97±5.00	85.19±6.12	81.73±5.00	1.95	0.458
Aspartic acid	81.97±3.87	81.66±4.74	80.71±3.87	1.50	0.835
Cysteine	71.16±5.14	73.20±6.29	70.62±5.14	2.00	0.720
Glutamic acid	88.18±2.60	88.27±3.19	87.76±2.60	1.01	0.936
Serine	81.60±7.94	88.65±9.72	80.69±7.94	3.09	0.309
Tyrosine	86.06±3.68	86.69±4.50	86.56±3.68	1.43	0.953

[1]Means represent 3 observations per diet, ± 95% confidence limit
[2]Means represent 2 observations per diet, ± 95% confidence limit

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