Evaluation of Feed Ingredients and Feed Additives on Poultry Performance and Health

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ACADEMIC ABSTRACT

The grinding of feed ingredients is a major contributor to the costs of poultry production and there is no optimal ingredient particle size for corn or calcium (Ca). Optimization of ingredient particle size can alleviate costs, improve growth and production, and promote sustainability. Intestinal health is also a key factor of poultry production, but with the limitations of conventional strategies, alternative methods to promote health and performance are necessary. This dissertation investigates corn particle size, alternative feed ingredients (aragonite), and feed additives (direct fed microbials) on their potential to improve turkey and laying hen health and performance. The second chapter is a literature review on ingredient particle size, Ca source, and varying feed additives. The third chapter determined that a smaller corn particle size (581 µm) was necessary for turkeys during the starter 1 phase, but a larger corn particle size (964 µm) is able to maintain performance later on. Turkey poults that consumed the 581 µm corn had an increase in BW ($P \leq 0.01$) over the starter 1 phase (0-21d) compared to those consuming the 964 µm corn. By 42 d of age, there were no differences in BW, FI, or FCR indicating that larger corn particle sizes can be used later on to maintain performance and potentially reduce feed costs associated with grinding of ingredients. The fourth chapter evaluated varying Ca sources (blend of 50:50 fine and coarse limestone and fine aragonite) and concentrations (2.46, 3.28, or 4.10%) on laying hen performance, eggshell quality, and bone mineralization. There were minimal differences in performance

regardless of Ca source and concentration, however the inclusion of 4.10% limestone resulted in the lowest performance. There was a Ca source main effect for all eggshell quality parameters including increased breaking force, shell thickness, relative shell weight, and specific gravity for birds fed the blend of fine and coarse limestone compared to fine aragonite ($P < 0.05$). There was a linear increase in bone mineralization for birds fed limestone ($P = 0.02$) whereas there was no difference for those fed fine aragonite ($P = 0.70$). These data indicate that fine aragonite was able to maintain production and bone mineralization, but birds fed lower levels of limestone started to pull Ca from the bone to support egg production. Fine aragonite may be used as a higher bioavailable Ca source and less Ca can be added into the diet to reduce costs. A combination of fine and coarse calcium is needed to support eggshell quality. Direct fed microbial (DFM) supplementation (Novela ECL® (ECL), Novela® (NOV), and Amnil® (AMN)) was investigated in the fifth chapter and its effects on laying hen performance, egg quality, and energy metabolism. Supplementation of all DFM altered the energy metabolism within the hen and allowed the birds to partition energy either towards egg production energy and storage. Egg weight was highest in the ECL fed birds followed by AMN then NOV and the control ($P \leq 0.01$). An increase in egg weight resulted in a higher egg mass for ECL and AMN ($P \leq 0.01$) which improved the feed conversion ratio by 7 and 9 points, respectively ($P \leq 0.01$). Inclusion of NOV increased the body weight and stored energy within the hen ($P = 0.05$) whereas ECL and AMN diverted energy down a productive pathway and improved productive performance. Inclusion of both NOV and AMN increased egg breaking force compared to other treatments ($P \leq 0.01$). The sixth chapter investigated the effects of a larger corn particle size on feed milling efficiency

and the amelioration of a coccidial challenge in turkey poults using performance, intestinal permeability, nutrient digestibility, and litter moisture. The coarse corn (1,049 µm) had a higher mill load than the fine corn (597 µm; $P < 0.01$) but pellet quality was not different ($P > 0.05$). No interactions occurred for any of the measured parameters between corn particle size and coccidiosis challenge from 21 to 42 d. Inclusion of the coarse corn reduced FI and BW ($P \leq 0.01$). Coccidiosis reduced all performance parameters and apparent ileal crude fat digestibility compared to the non-challenged birds ($P \leq 0.01$). Litter moisture increased on D35 (d 7 post coccidiosis vaccination) for challenged birds ($P = 0.02$). A coccidiosis challenge reduced performance in turkey poults from 21 to 42 d and large particle corn was not able to overcome the coccidia challenge.

This dissertation conveys possible ways to maximize turkey and laying hen production parameters through feed additives and optimization of ingredient particle size. These experiments provide insight on potential strategies to lower feed costs and varying feed additives to improve or maintain turkey and laying hen production and performance.

Evaluation of Feed Ingredients and Feed Additives on Poultry Performance and Health

Alyssa M. Lyons

GENERAL AUDIENCE ABSTRACT

Feed manufacture and grinding of feed ingredients is a significant cost to the poultry industry. There is no optimal particle size of different feed ingredients such as corn or calcium to promote growth, production, and sustainability. An important aspect of poultry production is intestinal health which has traditionally been promoted via antibiotics or conventional strategies. Alternative strategies to promote gut health and performance have gained traction in recent years because of consumer concerns over antibiotic resistance. This dissertation investigates how varying ingredient particle sizes (corn and calcium) and feed additives (aragonite and direct fed microbials) promote bird performance and intestinal health.

Corn particle size has been shown to affect growth performance of turkeys and a larger particle size may improve intestinal health and litter dry matter. The effects of a larger corn particle size (964 vs 581 μm) were investigated in chapter three and the larger particle size decreased performance over 0 to 21 days but was able to maintain performance over the 21 to 42 day period. However, intestinal health and litter dry matter were not affected by the larger corn particle size, showing that the larger particle corn can be used later on to reduce feed costs and maintain bird performance. Varying particle sizes and concentrations of limestone (50:50 fine and coarse) and aragonite (100% fine), an alternative calcium source with a potentially higher bioavailability, on the performance, eggshell quality, and bone mineralization was investigated in chapter four.

There were minimal differences in laying hen performance regardless of the calcium source or concentration, however eggshell quality was improved with the inclusion of fine and coarse limestone. Bone mineralization decreased as the concentration of limestone increased but aragonite maintained bone mineralization, indicating that less fine aragonite can be used in laying hen diets to reduce feed costs. The effects of various direct fed microbials on laying hen performance, eggshell quality, and energy metabolism were investigated in chapter five. All direct fed microbials altered energy metabolism within the hen, reducing the energy needed for maintenance and partitioning energy either towards productive or stored energy. Inclusion of two of the direct fed microbials increased the eggshell quality. In the sixth chapter, effects of a larger corn particle size (1,049 vs 597 μm) on feed milling efficiency and amelioration of a coccidial vaccine challenge in turkeys showed that a larger corn particle size increased mill load but was unable to ameliorate the coccidial challenge. The coccidial vaccine challenge reduced turkey performance and fat digestibility.

This research provides insight on ingredient particle size and feed additives for turkeys and laying hens. These ingredients and additives help reduce feed costs and improve bird performance.

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CHAPTER 1:

General Introduction

A primary goal of poultry production is to improve the efficiency of products such as meat and eggs using sustainable practices. This includes nutritional advancements such as optimizing the particle size of feed as well as exploring alternatives to antibiotics and ingredients to improve poultry production. Feed manufacture makes up approximately 70% of the costs in an integrated poultry operation (Donohue and Cuningham, 2009). There is a need to explore alternative ingredients that help to reduce feed and overall production costs since companies utilize least cost formulation while also maximizing bird performance and health. Therefore, the focus of this dissertation is to investigate optimal ingredient particle size, alternative calcium sources and additives to improve laying hen health and energy metabolism, and different methods to improve turkey performance and health.

Grinding of feed ingredients represents one of the largest costs of feed manufacture with the cost of grinding decreasing as ingredient particle size (PS) increases (Flores et al., 2021). Larger particles of feed stimulate the gizzard and are retained for a longer period of time within the gizzard which increases hydrochloric acid secretion and decreases gizzard pH (Gabriel et al., 2003). The decrease in pH helps to lower counts of pathogenic bacteria such as *E. Coli* or *Salmonella* (Engberg et al., 2004). In turn, this may have a beneficial downstream effect on gut health and overall performance in birds. Published work on corn particle size effects on performance has been extensively studied in broilers (Reece et al., 1986; Amerah et al., 2007a; Amerah et al., 2008; Rubio et al., 2020), but less of an emphasis has been put on how corn PS might affect turkey performance. Therefore, the first objective was to determine the effects of corn particle size on turkey hen performance, intestinal permeability, and litter dry matter (Chapter 3).

Calcium is essential in laying hens for skeletal health and to maintain egg production. Recently, there has been a push to find sustainable calcium sources that have a higher bioavailability compared to traditional limestone. A higher bioavailable calcium could save costs by not diluting the diet with excess calcium and therefore including less protein (soybean meal) or energy (oil) sources which are the two most expensive ingredients in poultry diets (Liu et al., 2023). There is also the potential to use one ingredient rather than the typical commercial blend of fine and coarse calcium if it is more bioavailable to the laying hen. One alternative is aragonite, a renewable calcium source mined from the ocean floor. However, laying hens are typically fed a blend of fine and coarse calcium because the coarse calcium is retained in the gizzard for longer and is necessary for eggshell calcification when the bird is not consuming feed at night (Scott et al., 1971; Roland and Harms, 1973). The second objective was to determine the bioavailability of fine ground aragonite on broiler tibia ash. The validation of fine ground aragonite as a sole calcium source in laying hen diets was evaluated using performance, bone mineralization, and egg quality (Chapter 4).

Direct fed microbials (DFM) are live microorganisms that have a health benefit to the host through various mechanisms that enhance intestinal health or digestion of dietary nutrients. The most common DFM are *Bacillus* spp. (Ray et al., 2022) because they are spore forming facultative anaerobes that are resistant to the low pH in the intestinal tract allowing it to travel further down the intestinal tract and act on lower parts of the intestine (Elshaghabee et al., 2017; Grant et al., 2018). Modes of action vary for each DFM and include competitive exclusion (Patterson and Burkholder, 2003), volatile fatty acid production (Chichlowski et al., 2007), and digestive enzyme secretion (Elshaghabee et al., 2017). Direct fed microbials have been studied for their health benefits but there is the potential to improve energy utilization within the bird. In previous work, laying hens were fed low energy diets (2,550 kcal/kg) and supplemented with DFM, resulting in performance and abdominal fat pad weights that were similar to birds fed a high energy diet (2,650 kcal/kg) with no DFM supplementation (Mikulski et al., 2020). This indicates that DFM may alter energy metabolism in laying hens although the different pathways and mechanisms are unclear. Therefore, the third objective was to determine the effects of various direct fed microbials on laying hen performance, energy partitioning, and egg quality (Chapter 5).

Coccidiosis is a detrimental disease in the poultry industry stemming from a protozoa infection that damages the intestinal lining. This causes economic losses due to decreased nutrient digestibility and mortality (Blake et al., 2020). Typically, anticoccidial drugs or vaccinations have been used to control coccidiosis in turkeys, but alternative strategies are needed with the push to no antibiotics ever in the poultry industry. A natural strategy to help control coccidiosis in turkeys may be the inclusion of larger particle corn in the diet to improve overall intestinal health and subsequent performance. Recent published work demonstrated that fine corn may be included in the starter 1 phase but coarse corn can be used in the starter 2 diet to maintain bird performance at breeder standards. Inclusion of the larger particle corn would decrease costs associated with feed manufacture due to less energy needed to grind (Flores et al., 2021). Additionally, larger particles are kept in the gizzard longer, releasing more hydrochloric acid and decreasing the pH (Gabriel et al., 2003) which acts as a barrier to prevent pathogenic bacteria from entering distal portions of the intestine (Bjerrum et al., 2005) and improving gastrointestinal health and thus performance and potentially nutrient digestibility. The fourth objective was to determine the effects of large particle corn on the amelioration of a coccidial challenge in turkey hen poults using performance, intestinal permeability, nutrient digestibility, and litter moisture (Chapter 6).

This dissertation investigates alternative methods and strategies to improve poultry health and performance while decreasing costs associated with feed manufacture and poultry production.

REFERENCES

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

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. Poult. Sci. 87:2320-2328. doi:10.3382/ps.2008-00149.

Bjerrum, L., K. Pedersen, and R. M. Engberg. 2005. The influence of whole wheat feeding on salmonella infection and gut flora composition in broilers. Avian Dis. 49:9-15. https://doi.org/10.1637/7223-061504R.

Blake, D. P., J. Knox, B. Dehaeck, B. Huntington, T. Rathinam, V. Ravipati, S. Ayoade, W. Gilbert, A. O. Adebambo, I. D. Jatau, and M. Raman. 2020. Re-calculating the cost of coccidiosis in chickens. Vet. Res. 51:115.

Chichlowski, M. J. Croom, B. W. McBride, G. B. Havenstein, and M. D. Koci. 2007. Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: A brief review of current knowledge. Int. J. Poult. Sci. 10:694-704.

Donohue, M. and D. L. Cunningham. 2009. Effects of grain and oilseed prices on the costs of US poultry production. J. Appl. Poult. Res. 18:325-337.

Elshaghabee, F. M. F., N. Rokana, R. D. Gulhane, C. Sharma, and H. Panwar. 2017. Bacillus as potential probiotics: Status, concerns, and future perspectives. Front. Microbiol. 8:1490. doi: 10.3389/fmicb.2017.01490.

Engberg, R. M., M. S. Hedemann, S. Steenfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925-938.

Flores, K. R., A. Fahrenholz, P. R. Ferket, T. J. Biggs, and J. L. Grimes. 2021. Effect of methionine chelated Zn and Mn and corn particle size on Large White male turkey live performance and carcass yields. Poult. Sci. 100:101444. https://doi.org/10.1016/j.psj.2021.101444.

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

Grant, A., C. G. Gay, and H. S. Lillehoj. 2018. *Bacillus* spp. As direct-fed microbial antibiotic alternatives to enhance growth, immunity, and gut health in poultry. Avian Pathol. 47:339-351. DOI: 10.1080/03079457.2018.1464117.

Liu, G, F. L. S. Castro, and W. K. Kim. 2023. Applied research note: Exogenous protease supplementation to reduced-energy, reduced-protein, and reduced-amino acid diets for broiler chickens from days 1 to 42. J. Appl. Poult. Res. 32:100362. https://doi.org/10.1016/j.japr.2023.100362.

Mikulski, D., J. Jankowski, M. Mikulska, and V. Demey. 2020. Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on productive performance, egg quality, and body composition in laying hens fed diets varying in energy density. Poult. Sci. 99:2275-2285. https://doi.org/10.1016/j.psj.2019.11.046.

Patterson, J. A. and K. M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82:627-631.

Ray, B. C., S. D. Chowdhury, S. C. Das, B. Dey, A. Khatun, B. C. Roy, and M. A. Siddik. 2022. Comparative effects of feeding single- and multi- strain probiotics to commercial layers on the productive performance and egg quality indices. J. Appl. Poult. Res. 31:100257. https://doi.org/10.1016/j.japr.2022.100257.

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

Roland, D. A. and R. H. Harms. 1973. Calcium metabolism in the laying hen 5. Effect of various sources and sizes of calcium carbonate on shell quality. Poult. Sci. 52:369-372.

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

Scott, M. L., S. J. Hull, and P. A. Mullenhoff. 1971. The calcium requirements of laying hens and effects of dietary oyster shell upon egg shell quality. Poult. Sci. 50:1055-1063. https://doi.org/10.3382/ps.0501055.

CHAPTER 2:

Review of Literature

Corn is the primary cereal grain that is used in the United States livestock industry and accounts for 95% of the total grain fed (USDA, 2025). Approximately 40% of the corn produced is used as the primary source of energy in livestock production in the United States. Additionally, approximately 45% of corn produced in the US is used for ethanol production, a renewable fuel source (USDA, 2025). With an increase in ethanol production in recent years, the price of corn has also increased resulting in higher feed prices in the poultry industry. In a typical commercial broiler or turkey formulation, approximately 50-70% of the diet is composed of a cereal grain. Corn is made up primarily of carbohydrates containing starches that are highly digestible to poultry along with about 3% fat. With the high carbohydrate content and moderate oil content, corn can contribute around 65% of the metabolizable energy and about 20% of the protein to a commercial poultry diet (Naderinejad et al., 2016). Corn usage for livestock has remained relatively constant, but the use of corn for ethanol production has increased the need for corn production and supported an increased cost (USDA, 2025). An increased demand for corn means that poultry producers are seeking methods to increase the efficiency of corn in poultry diets. One way to make corn and cereal grains more available to poultry is through the grinding and processing of the whole grains to reduce particle size, to improve feed uniformity, as well as digestibility and utilization of nutrients within the feed.

Feed Milling Factors

Particle Size of Feed Ingredients

The particle size (**PS**) of feed ingredients is an important aspect of poultry production and nutrition. Particle size reduction, or grinding of feed ingredients, is one of the first steps in feed manufacture. The grinding process involves disruption of the outer seed coat to expose the endosperm. Hammermills are the most commonly used machines to grind ingredients and the screen size has a large part in energy consumption for a hammermill since smaller screen sizes will require more energy to push particles through the screen. The grinding of cereal grains accounts for the second largest expense during the feed manufacturing process only following the actual pelleting process (Reece et al., 1985). There is a direct relationship between reduction of feed ingredient PS and cost (Reece, et al., 1986a; Flores et al., 2021). A smaller screen size requires a longer grinding time in the hammermill, translating into increased electricity usage at the mill and higher overall feed costs. By using larger hammermill screen sizes and grinding ingredients to a larger PS, feed mills and producers could potentially save on feed costs by reducing electricity use and time to grind. A 7.5 kWh/ton energy reduction was reported when hammermill screen size was adjusted from 1.6 to 9.5 mm during corn grinding (Wondra et al., 1993). As hammermill screen size was increased from 4.8 to 6.4 mm, the energy costs to grind corn was reduced by approximately 27% (Reece et al., 1986b). Grinding of corn to a larger PS (2,595 µm) resulted in a 36% decrease in energy consumption and a 27% reduction in motor load compared to reducing the PS to 276 µm (Flores *et al.*, 2021). A common method to calculate ingredient PS is the Ro-Tap sieve shaker using ASAE method S319.4 (ASABE, 2022). The PS is reported as the geometric mean diameter (GMD) in µm with the variation explained by the geometric standard deviation (GSD; Nir et al., 1994a). Ingredients that have a low GSD have been reported to have positive impacts on broiler performance because the diet provides a more uniform distribution of nutrients throughout (Nir et al., 1994b). The grinding of feed ingredients is required, but adds to feed manufacture costs due to the use of electricity, labor, and the costs of equipment such as hammermills and roller mills. Reduction of the size of whole feed ingredients increases the surface area for enzymes to facilitate digestion while also reducing ingredient segregation during feed manufacture (Behnke, 1996). Although ingredient PS impacts various parameters of poultry production, there is no optimal PS for bird performance, nutrient digestibility, or pellet quality (Kiarie and Mills, 2019; Table 2.1).

Table 2.1. Previous research with varying corn particle size (CPS) on broiler performance, nutrient digestibility, and pellet quality.

CPS (µm)	Pellet Quality	Age	Response	Experiment
297 528	297 µm ↑ PDI	0-21 d	297 µm ↑ FI and FCR	Amerah et al., 2008
267 1,132 2,028	N/A[2]	7-21 d	1,132 and 2,028 µm ↑ BW, FI, gain:feed	Nir et al., 1994b
490 651 796	796 µm ↑ PDI 5%	0-21 d	796 µm ↑ starch and AME	Naderinejad et al., 2016
781 950 1,042 1,109 2,242	N/A[2]	0-21 d	Increasing PS ↑ FI and ↓ FCR Increasing PS ↑ N retention	Parsons et al., 2006
832 1,432 2,036	N/A[2]	0-21 d	2,036 µm ↑ FCR	Downs et al., 2023
750 1,150 1,550	Increasing PS ↑ PDI	0-39 d	750 µm ↑ BW and FI Increasing PS ↑ crude protein	Rueda et al., 2024

[1] No difference.
[2] Parameter is not applicable, or not measured.

The age of birds needs to be considered for PS reduction because particles that are too small may not be utilized efficiently for digestibility or growth. Broilers fed a mash diet at 8 d of age demonstrated a preference for feed particles ranging in PS from less than 60 to 85 µm with a decrease in consumption for particles above 1,180 µm (Portella et al., 1988). By 24 and 32 d of age, broilers consumed all PS with a preference for particles above 2,360 µm in size, indicating that as birds age, their preference for larger particles increases. These data indicate that the PS distribution in a mash diet affects feed intake and subsequent growth performance, demonstrating that pelleting diets has positive effects on performance.

Particle size may be more influential in mash diets compared to crumbled or pelleted diets as the pelleting process may influence ingredient PS through additional grinding or agglomeration of particles. Pelleting provides a more uniform diet whereas feed fed in a mash form allows birds to pick out specific feed particles. Typically, cereal grains and protein sources compose the largest PS in the feed, and poultry have a preference to pick out those larger particles (Portella et al., 1988). When birds pick out the larger particles, they may be lacking in vitamin and mineral uptake which are typically found in more the fines of the feed (Tang et al., 2006).

Feed Milling Efficiency

Costs that are associated with the pelleting process include electricity, specialized equipment, and labor (Reece et al., 1986b; Flores et al., 2021). Steam conditioning is a practice used in commercial feed mills that adds moisture and heat to which aids in the binding of feed particles and lubrication of the pellet die. Energy consumption at the feed mill demonstrated a decrease when increasing steam conditioning temperatures from 65 to 80°C were implemented ($P < 0.05$; Skoch et al., 1981). However, excess moisture could lead to an increase in frictional forces at the pellet die and possibly plug the die (Hott et al., 2008). This could potentially reduce feed production rate and increase electricity and energy needed for the pelleting process. An important consideration is to ensure that the maximum production rate is being utilized to reduce energy required to push feed through the pellet die (Farenholz, 2012). If the maximum production is not being utilized, it will take longer to pellet feed which translates to increased energy and electrical consumption to continue pushing feed through the die.

Pellet Quality

Pellet quality (**PQ**) is a priority in both the broiler and turkey industries as pelleting helps improve flow through the feed mill, reduce nutrient segregation, and provide a more uniform diet to the bird. There are a variety of factors that influence PQ including diet formulation, particle size, die size, conditioning temperature, and pellet cooling time (Reimer, 1992; Behnke, 2001; Figure 2.1). The physical quality of pellets is important to understand the ability of the pellet to withstand physical force and abrasion that occurs during manufacture, handling, and transport. A variety of methods are used to determine overall PQ of feed. These methods were developed to provide standard testing procedures for feed mills to compare across diets.

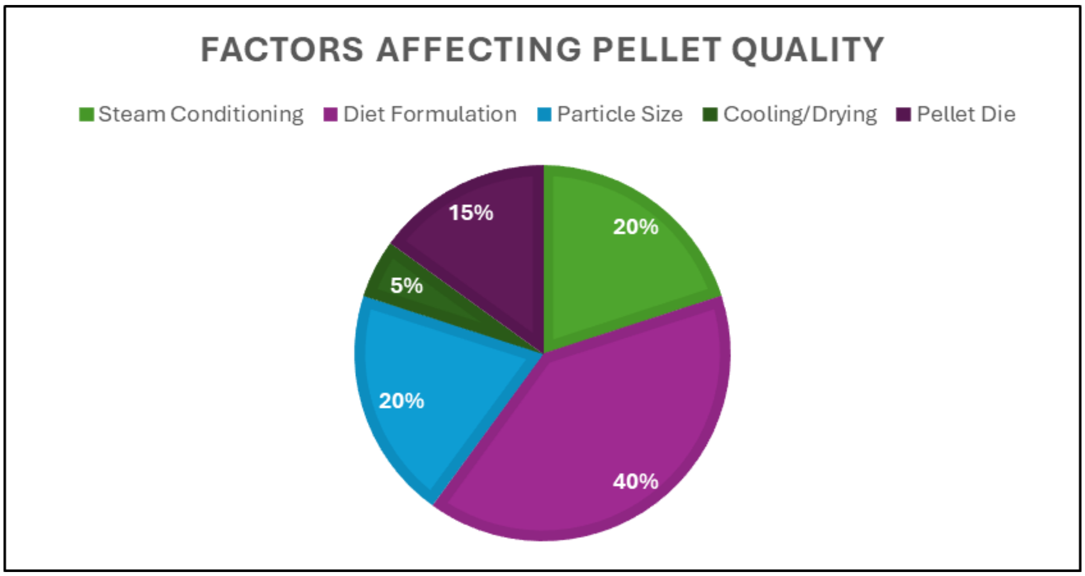

Figure 2.1. Factors that affect pellet quality (Reimer, 1992).

Fine particles have a larger surface area with more contact points which helps the agglomeration and increased binding of the pellet, thus improving durability and PQ (Behnke, 2001). Finer particles also have an increased water adsorption capacity with the increased surface area which in turn assists with formation of a stronger pellet (Hemmingsen et al., 2008). An increase in the pellet durability may be associated with a reduction in the amount of fines which also decreases the amount of time and energy a bird spends picking out larger particles in the feed (Amerah et al., 2007b). A 2% increase in pellet durability was reported when finely ground corn (297 µm) or wheat (284 µm) was fed in a broiler diet compared to coarsely ground corn (528 µm) and wheat (890 µm; Amerah et al., 2008). A 6.5% improvement in pellet durability was reported when wheat was finely ground using a 3 mm hammermill screen compared to a 6.1 mm hammermill screen (Svihus et al., 2004). Conversely, corn that was ground using a 4.73 mm or 6.35 mm hammermill screen did not alter PQ when included in a broiler diet (Reece et al., 1986). Previous research has demonstrated that pelleting may minimize differences in particle distribution because coarser particles are more likely to be ground by the pellet mill from frictional forces within the pellet die (Svihus et al., 2004; Amerah et al., 2007a). The steam that is used during the pelleting process helps to soften the feed particles before they enter the pellet die, allowing for additional grinding to occur. More recent research supported the idea that coarse particles are further reduced in PS compared to fine particles during the pelleting process when mash diets with an average PS of 936 µm were reduced in PS by 36% whereas diets with a mean PS of 1,567 µm were reduced by 44% (Bonilla, 2022).

Ingredient Particle Size Effects on the Gizzard and Intestinal Health

Researchers have hypothesized that the feeding of larger PS ingredients may influence gizzard function as well as help improve intestinal health. Consideration of the PS inclusion is important because the energy that the bird is putting towards mechanical digestion of feed in the gizzard is energy that the bird could be partitioning towards growth. Coarse particles in a mash diet have demonstrated an increased retention time in the gizzard which results in an increased gizzard weight (Nir et al., 1994a). The increased retention time with the larger particles is likely attributed to a slower feed passage rate resulting in increased gizzard size when 50% coarse corn

(1,362 vs 294 µm) was included in the diet compared to 0% coarse corn inclusion (Xu *et al.*, 2015). A similar response was reported when 21 d old broilers fed corn ground through a 7mm hammermill screen had heavier gizzards compared to those fed corn ground with a 1mm hammermill screen (Amerah et al., 2008). It is expected that both the increased retention time and increased gizzard weight will be supported by larger PS because the larger PS will be retained in the gizzard for a longer time to allow the gizzard to mechanically reduce the PS. Therefore, when PS reduction is completed outside of the bird during corn grinding, the smaller PS has no need to be held in the gizzard nor to activate the gizzard to muscularly grind the corn decreasing retention time and resulting in less muscle (weight) development within the gizzard. The increased grinding function of the gizzard increases the surface area of the feed that enters the small intestine, potentially exposing the feed to more digestive enzymes and possibly improving nutrient digestibility (Hetland et al., 2002). Diets containing corn PS of 297 and 528 µm were analyzed by wet sieving and differences in PS persisted after the pelleting process but the PS of duodenal contents after exiting the gizzard were not different between the two PS (Amerah et al., 2008). Improved nutrient digestibility with feeding larger particle corn is supported by previous work where feeding of 1,109 µm corn increased nitrogen retention and tended to increase lysine retention in broilers (Parsons et al., 2006). Finely ground particles have been shown to negatively impact the gizzard because they have a faster passage rate and therefore, do not stimulate the gizzard to the extent that the larger particles do (Péron et al., 2005; Abdollahi et al., 2011). With fine particles in the diet, the gizzard may not develop to the extent that it would with large particle addition and therefore these diets result in a lower feed capacity and decreased gut motility. Gut motility in birds enhances pancreatic enzyme secretion such as trypsin, chymotrypsin, and lipase, so with a faster passage rate, the exposure of nutrients to digestive enzymes may be lower and not as sufficient if larger particles were present in the diet to stimulate gizzard function and gut motility (Amerah et al., 2008). Contractions of the gizzard result in a reflux of material into the proventriculus where pepsinogen and hydrochloric acid are secreted, resulting in additional exposure of feed material to enzymatic digestion (Svihus, 2011).

Stimulation of the gizzard through mechanical grinding of larger feed particles is important because it typically results in a decrease in the pH of the gizzard through the increased release of hydrochloric acid from the proventriculus (Gabriel et al., 2003; Engberg et al., 2004). A similar response has been shown in pelleted diets where inclusion of 796 µm corn decreased the gizzard pH from 3.43 to 2.90 in 21 d broilers compared to inclusion of 490 µm corn (Naderinejad et al., 2016). Hydrochloric acid is crucial during the digestive process since it aids in protein digestion by the activation of digestive enzymes (Gabriel et al., 2003) as well as creating an acidic environment. Increased feed retention time in a low pH (acid environment) in the gizzard is beneficial for gut health because it acts as a barrier for and possibly inactivating harmful bacteria reducing bacterial numbers entering the distal portions of the intestine (Engberg et al., 2004). It has been speculated that harmful bacteria like *Salmonella* or *E. coli* are more susceptible to these acidic conditions than commensal bacteria (Zaefarian et al., 2016). Feeding 100% whole wheat v. 100% ground wheat resulted in reductions in lactose-negative enterobacteria, such as *Salmonella*, in the gizzard (Engberg et al., 2004). The increased exposure to an acid environment in the gizzard has the potential to reduce bacteria from traveling down the intestinal tract resulting in reduced dysbiosis and potential negative effects on growth and production.

Pathogenic bacteria in the intestines could lead to increased fecal water content and diarrhea (Francesch and Brufau, 2003; Dunlop et al., 2016). An increase in water excretion could result in increased litter moisture (Collett, 2012) and potentially volatize higher concentrations of ammonia increasing respiratory issues in both birds and human caretakers (Liu et al., 2007). Large particle corn reduced litter nitrogen concentration (Xu, 2014; Wang-Li et al., 2020) which was attributed to an increase in nitrogen retention that occurred with feeding larger particle corn (1,109 µm) in comparison to small particle corn (950 µm; Parsons et al., 2006). Previous work also reported a decrease in litter moisture when 50% of the dietary corn was replaced with coarse corn (1,100-1,300 µm) in broilers which was a result of improved digestibility and overall intestinal health (Xu, 2014).

In addition to reduced bacteria, increased gizzard retention time may also improve nutrient digestibility. As mentioned previously, larger particles are retained in the gizzard for a longer period and exposed to more enzymes through enhanced gut motility as mentioned above (Amerah et al., 2008) and possibly improving nutrient absorption and digestibility (Amerah et al., 2007b). When 50% of the fine corn in a diet was replaced with coarse corn, dietary retention time increased 18% and this translated into a 8% increase in apparent ileal energy digestibility and a 12% increase in apparent ileal nitrogen digestibility (Xu et al., 2015). Broilers that consumed 1,550 or 1,150 µm corn in a pelleted finisher diet exhibited a higher apparent ileal crude protein digestibility compared to 750 µm corn (Rueda et al., 2024). In addition to the numerous broiler reports above, 21 d old turkeys were fed 380 or 806 µm corn in a crumble or micro pellet with the turkeys fed the larger corn particle resulting in increased apparent ileal nitrogen digestibility (Favero et al., 2012). These data indicate that inclusion of a larger PS cereal grain in the diet has the potential to improve nutrient digestibility due to the increased time the feed spends in the gizzard and exposure to digestive enzymes with an increase in gut motility and gizzard contractions.

Effects of Ingredient Particle Size on Broiler and Turkey Performance

Reports that have looked at how ingredient PS affects poultry performance have primarily been in broilers. A 33 g reduction in BWG was observed when broilers were fed 1,387 µm corn compared to 858 µm corn in a mash diet from 0-21d (Jacobs et al., 2010). There was a similar response when broilers were fed diets containing 600 µm corn compared to 300 µm corn from 0-21d (Chewning et al., 2012). Many publications have reported reductions in performance when broilers are fed diets with increasing ingredient PS, especially in younger birds (Parsons et al., 2006; Chewning et al., 2012). The decrease in performance with younger birds may be from the increased energy expenditure in the gizzard so the birds are then partitioning energy towards maintenance and grinding of the feed rather than growth and performance.

Limited work has been conducted pertaining to ingredient PS and effects on turkey performance. Hybrid Converter turkeys consumed feed with 1,094 µm corn and had decreased BWG from 0-7 or 0-15 d compared to turkeys that consumed either 606, 701, or 832 µm corn (Charbeneau and Roberson, 2004). A diet containing 606 µm corn improved BWG and FI with no difference in FCR from 0-21 d in BUT 9 male turkeys compared to diets with 380 or 806 µm corn (Favero et. al., 2009). Coarse corn may reduce turkey performance during the starter period as indicated by previous work, so inclusion of finer grain particles should be considered when feeding younger turkeys early on. Although larger particles may be detrimental to turkey growth and performance over the starter 1 phase, there is the potential to begin feeding coarser corn particles after the starter 1 phase. The idea of feeding larger particles after starter 1 would help to alleviate feed production costs by reducing energy needed to grind ingredients (Flores et al., 2021) as well as the potential to maintain growth and performance and improve overall gut health, although these parameters have not been evaluated as a whole in turkeys.

Calcium Needs of Poultry

Calcium **(Ca)** is an essential mineral for life that supports both growth and performance. Approximately 99% of the Ca within the body is stored in the skeletal system as hydroxyapatite which is necessary for structural integrity of bones (Berndt et al., 2005). Another important function pertaining to skeletal health is the formation of the medullary bone at the onset of sexual maturity in laying hens which has been regarded as a skeletal Ca reserve that supplies Ca during periods of low Ca consumption or the dark period (Leeson and Summers, 2001). While Ca is necessary for skeletal development and mineralization, the remaining 1% of the Ca is essential for a majority of metabolic processes such as muscle and nerve function, enzyme activation, and blood clotting (Berndt et al., 2005).

Calcium is not only important for skeletal health and metabolic processes, but egg production as well. Laying hens have been genetically selected to produce one egg every 24 hours with a production cycle that lasts 100 weeks or more (Sinclair-Black et al., 2023). Ionic blood Ca supplies the Ca necessary for eggshell formation, which sums to approximately 2 grams a day (Gautron et al., 2021). While some of the Ca is provided directly from the diet and intestinal absorption, a majority of the Ca for eggshell formation, approximately 40%, is provided from the medullary bone (Gautron et al., 2021). As mentioned previously, the medullary bone is a Ca reservoir and is important for when hens are not consuming feed or during periods of darkness, which is primarily when eggshell formation and calcification are occurring (Leeson and Summers, 2001). With the intensive production cycles of laying hens, adequate Ca is necessary to maintain both egg production and skeletal health to not compromise hen welfare.

Calcium Digestion and Absorption in Poultry

Ingredients from plants like grain or protein sources often contain low levels of Ca that are available to the bird because dietary Ca is absorbed in the ionic form. Therefore, supplemental Ca sources must be added to poultry diets since most major ingredients do not contain sufficient amounts of Ca. Absorption of Ca primarily occurs in the duodenum and proximal portion of the small intestine and is regulated by the parathyroid hormone and calcitonin (David et al., 2023). When there are low levels of serum Ca, the parathyroid gland has a Ca sensing receptor that signals the release of parathyroid hormone which stimulates bone Ca mobilization (Sinclair-Black et al., 2023). Parathyroid hormone also stimulates 1α-hydroxylase which indirectly increases production of the biologically active form of vitamin D ($1,25(OH)_2D_3$) in the kidney (Figure 2.2; Proszkowiec-Weglarz and Angel, 2013). These events increase the absorption of Ca in the small intestines and reabsorption in the kidney. Calcitonin is also regulated by $1,25(OH)_2D_3$ however it has the opposite effect of parathyroid hormone so it inhibits bone resorption and decreases serum Ca concentration.

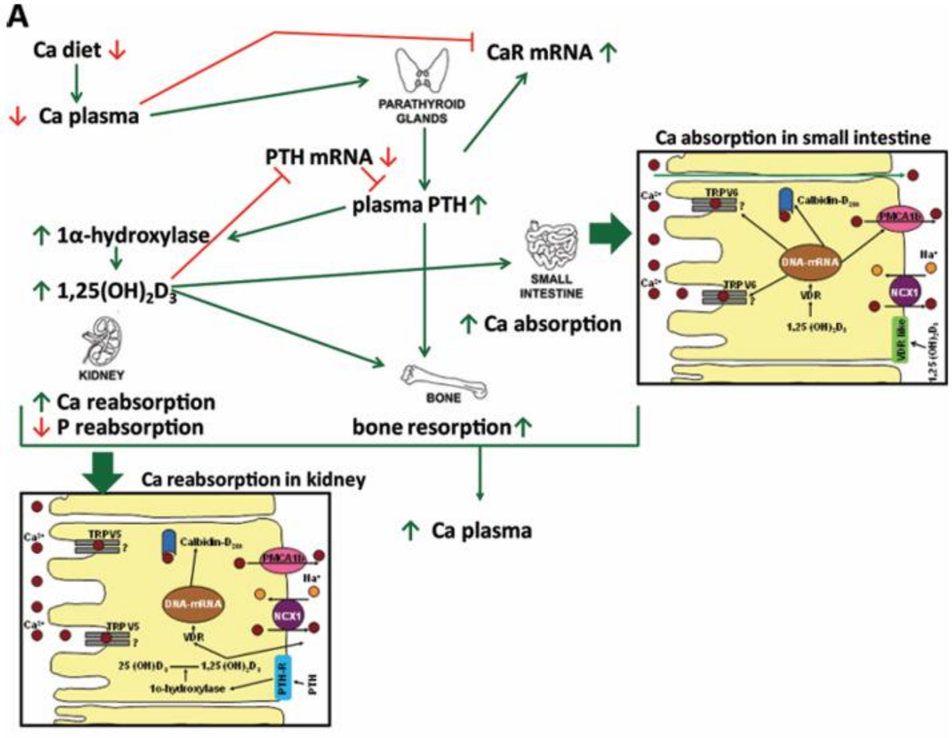

Figure 2.2. Calcium metabolism in poultry (Adapted from Proszkowiec-Weglarz and Angel, 2013)

Calcium absorption in the small intestine is mediated through both active and passive processes. The active transport, or transcellular, requires ATP and is dependent on vitamin D. Passive transport, or paracellular, does not require ATP or vitamin D and ions move from the intestinal lumen to circulation (David et al., 2023). Adequate or high concentrations of Ca will inhibit active transport since passive transport predominates when there is high luminal Ca concentration (Walk et al., 2021).

Since Ca is inexpensive and limestone (LS) is highly available, there is little consideration given to oversupplying dietary Ca. The high levels of Ca in the diet may have bound other minerals such as zinc or phosphorous leading to some nutrients being unavailable to the bird and reducing growth and performance (Walk et al., 2021). There is an assumption that the Ca sources used are 100% available to the bird so therefore, Ca is considered on a total basis (David et al., 2023). However, Ca sources such as LS are not 100% available and there should be a move towards digestible Ca when formulating poultry diets. Limited studies using digestible Ca have yielded inconsistent results where the standard ileal digestibility of Ca needed to maximize tibia ash ranged from 3.32 to 5.30 g/kg, 3.05 to 5.15 g/kg, and 3.5 to 3.7 g/kg in the starter, grower, and finisher diets, respectively (David et al., 2023). With the discrepancies reported in previous work, Ca sources that are highly available with a potential higher bioavailability compared to LS should be considered.

Dietary Ca supplementation increases as laying hens age because Ca absorption and utilization tends to decline as hens age, potentially from a reduction in intestinal uptake of Ca. A linear reduction in duodenal Ca uptake from 37 to 58 wk of age was reported in three strains of commercial laying hens (Al-Batshan et al., 1994). A decreased intestinal uptake of Ca could potentially reduce eggshell quality and increase shell breakage. Previous research reported a decrease in the percentage shell and shell thickness in laying hens from 22 to 57 weeks of age, suggesting a reduction in eggshell calcification (Al-Batshan et al., 1994). Research demonstrated that egg weight increased approximately 14% from 35 to 74 wk of age and the shell weight only increased about 3% (Roland, 1979) so the eggshell is being calcified over a larger surface area. The difference between egg weight and shell weight as laying hens age likely contributes to the eggshell quality decline that is often associated with aged laying hens and demonstrates the need for adequate dietary Ca.

A more neutral pH in the intestines results in phytate forming mineral chelates that are rendered insoluble (Selle et al., 2009). The calcium-phytate interaction is complex as Ca has a low affinity for phytate, however, it is added at high concentrations in poultry diets, especially laying hens, and the concentration overpowers the low affinity to form Ca-phytate complexes that can make both Ca and phosphorous unavailable (Selle et al., 2009). The formation of insoluble complexes is why it becomes important not to add excess Ca into the diet because high concentrations will impede availability of not only phosphorus and Ca itself, but other minerals such as zinc or magnesium (David et al., 2023). High concentrations of Ca in poultry diets is an antinutrient because the binding of Ca to the other minerals makes them insoluble (Pizzolante et al., 2009). Published work also reported a reduction in apparent ileal digestibility of N and energy in broilers at 21d of age when fed increased concentrations of Ca (Walk et al., 2012). Laying hens have a higher requirement for Ca compared to broilers in order to maintain both egg production and eggshell quality. The NRC (1994) recommends approximately 3.25% of dietary Ca regardless of hen age. However, Ca requirements vary depending on a number of factors including egg production rate, bird age, and environmental factors such as heat or cold stress since feed intake will fluctuate (Pesti et al., 2005).

Alternative Sources of Calcium

Various Ca sources were determined by feeding laying hens a diet supplemented with 3% dietary Ca in the form of LS, aragonite, oyster shell, clam shell, and eggshells. Laying hens supplemented with aragonite, oyster shell, clam shell, and eggshells had similar performance and eggshell quality to those consuming LS (Muir et al., 1976), indicating that they may be used as alternative Ca sources. Aragonite is a renewable source of Ca that is harvested from the ocean floor and composed of marine sediments (Sulpis et al., 2022). Aragonite is primarily produced from shelled organisms in the ocean that when they die, the aragonite settles to the ocean floor. This new source of Ca has a potentially higher bioavailability due to its increased density and solubility (Sulpis et al., 2022) compared to mined LS that is typically used in commercial laying hen diets. Utilization of a higher bioavailable Ca source would potentially allow producers to formulate less Ca into the diet and alleviate issues associated with high Ca in the diet as well as possibly decrease overall feed costs. With less Ca in the diet, there is the potential to decrease concentrations of other major ingredients such as protein and energy sources which are the two most expensive ingredients in a poultry diet (Liu et al., 2023).

Calcium Particle Size and Solubility

A number of factors affect Ca utilization such as solubility and the ability to form complexes with phytate and oxalate which reduce the overall bioavailability (Soares, 1995). Calcium can only be absorbed in the intestine if it is able to be solubilized. Solubilization of Ca is related to the pH of the small intestine (approximately 5 to 7) as well as dietary phytate concentration (Walk et al., 2012). Typically, a combination of small and large Ca are fed to laying hens to ensure adequate Ca is provided to support egg production, eggshell quality, and skeletal health. The benefits of feeding coarse Ca include a prolonged retention time in the gizzard which slows the rate of solubilization and subsequently increases the degree of bioavailability to the bird (de Witt et al., 2006; Zhang et al., 2017). A slower solubilization of Ca allows a more constant release of Ca into the bloodstream, making Ca more available during the dark period when birds are not typically consuming feed and when eggshell formation and calcification is more intensive (Roland and Harms, 1973). Commercial laying hens are reared on 16 hours of light and 8 hours of darkness to allow the bird to maintain maximum egg production and performance, so the large particle calcium is particularly during the 8 hours. An interaction was reported for Ca PS and dietary Ca level when laying hens were fed large particle LS (2 to 5 mm) or small particle LS (0.5 to 0.8 mm) at 1.5, 3.0, 4.5, and 6.0% dietary Ca. The hens fed 4.5% dietary Ca in the form of large particle LS utilized 25% more Ca compared to those fed the small particle LS ($P < 0.01$; Rao and Roland, 1990). This was similar to previous work where insolubilized Ca in the excreta was measured and it was determined that laying hens consuming large particle LS solubilized an average of 25% more Ca compared to hens fed small particle LS ($P < 0.05$; Rao and Roland, 1989), however the rate of solubilization was reduced by one-third with the larger particle LS.

Intestinal Health and Coccidiosis in Poultry

Background on Intestinal Structure and Protection

The gastrointestinal tract is composed of enterocytes that each contain villi and crypts to assist in digestion and absorption of nutrients (Snoeck et al., 2005; Lee et al., 2018). Tight junctions between enterocytes in the gastrointestinal tract act as a barrier to prevent bacteria and microorganisms from entering the intestinal tissue and bloodstream (Lee et al., 2018; Zhang et al, 2019). During a period of stress such as heat stress or coccidiosis, the tight junctions are weakened which allows pathogens and bacteria into surrounding tissue, resulting in increased inflammation and damage to the epithelial lining of the intestine (Lee et al., 2018). Intestinal enterocytes have a rapid cellular turnover rate to maintain structural integrity of the intestines while also helping to remove any pathogenic bacteria or harmful organisms (Arike et al., 2020).

Coccidiosis is a disease that is caused by infection from protozoa of the genus *Eimeria* that is ubiquitous across all species (Shivaramaiah et al., 2014). The disease has distinct *Eimeria* species that affect the intestinal tract of birds from the duodenum down to the ceca. Coccidiosis is prevalent in the poultry industry, especially in broilers and turkeys and poses a significant challenge to poultry producers since it is difficult to eradicate (Price, 2012). Typically, coccidial vaccines or ionophores have been used as methods to control coccidiosis (Allen and Fetterer, 2002; Price, 2012). The loss of antibiotic control has resulted in major economic losses due to reductions in bird performance, increases in mortality and intestinal damage (Adhikari et al., 2020). While coccidiosis is a major disease in the poultry industry, intestinal damage is also a predisposing factor for secondary bacterial infections such as *Salmonella*, *E. coli*, or *Clostridium perfringens* (Collier et al., 2008; Dunlop et al., 2016).

***Eimeria* Transmission**

The life cycle of *Eimeria* involves three phases: sporogony, schizonogy, and gametonogy (Chapman et al., 2013). Transmission of coccidiosis occurs when a bird ingests fecal material that contains coccidia oocysts excreted from an infected bird (Chapman et al., 2013). Oocysts that are shed into the litter are not yet infective since they have not sporulated. Infected birds will continue to shed unsporulated oocysts into the litter to be picked up by susceptible birds. In order for these oocysts to sporulate and become infective (sporulation), proper conditions of heat, moisture, and oxygen must occur (Shivaramaiah et al., 2014). Broiler and turkey houses typically have optimal conditions for sporulation to occur. A sporulated oocyst contains 4 sporocysts with each containing two infective sporozoites (Price, 2012). Birds become infected with coccidiosis after they ingest the sporulated oocyst and the gizzard and proventriculus ruptures the oocyst cell wall through mechanical and chemical digestion. The sporozoites are then released and penetrate the intestinal epithelial cells in the gastrointestinal tract which begins schizonogy, the second stage of the life cycle. Sporozoites undergo asexual reproduction to produce trophozoites which continue to enlarge and absorb nutrients from the host (Price, 2012). Trophozoite replication results in schizont containing numerous merozoites (McDonald and Shirley, 2009). When the schizont burst, the merozoites are released and infect surrounding epithelial cells (Fayer, 1980). The last phase of the life cycle is gametogony where merozoites ultimately differentiate into either microgametes (male) or macrogametes (female; Innes and Vermeulen, 2006). The microgametes and macrogametes undergo sexual reproduction to produce a zygote which form an oocyst wall and develop into an unsporulated oocyst. Oocysts are released from the epithelial cells and released into the lumen of the intestines so that it can be excreted out (Fayer, 1980).

Effects of Coccidial Infection

Disruption of the oocyst wall in the gizzard and proventriculus may cause damage to the epithelial lining, impair the digestion of nutrients, and lead to dysbiosis within the intestines (Lu et al., 2021). On day of hatch, turkeys were given no vaccination against coccidiosis, an anticoccidial drug, or a commercial coccidiosis vaccine and on day 21, all turkeys were infected with sporulated oocysts via crop intubation. On day 28, body weight was reduced by approximately 17 and 14% in the birds given no anticoccidial drug or vaccine compared to those given the anticoccidial or commercial vaccine, respectively (Milbradt et al., 2014). When turkeys were infected with 0, 30, or 90 oocysts per bird on day of hatch, there was a significant increase in oocysts per gram of excreta and a reduction in BW on days 14, 21, and 28 for birds given the 30 and 90 oocyst per bird compared to those not infected with oocysts (Yeboah et al., 2025). Broilers were infected with increasing doses of mixed *Eimeria* species and a linear reduction in body weight and feed intake was found as the dosage of *Eimeria* species increased ($P < 0.05$; Teng et al., 2020). In addition to reduced performance with a coccidia infection, intestinal permeability was compromised and apparent ileal energy digestibility decreased on day 6 post infection compared to the control group that was not infected (Teng et al., 2020).

Control of Coccidiosis

Primary methods to mitigate or control coccidiosis have been through the use of anticoccidials or vaccines. Anticoccidial drugs include both ionophores and chemical or synthetic drugs (Price, 2012). Ionophores, produced through fermentation, disturb the transport of ions across the protozoan membrane, thereby disrupting the coccidia life cycle (Chapman and Jeffers, 2014; Noack et al., 2019). Previously, ionophores have been used since they do not completely suppress coccidia development and allow for the bird to build immunity against the pathogen (Noack et al., 2019). Chemical and synthetic drugs may inhibit mitochondrial respiration as well as the folic acid pathway which is necessary for cellular replication (Chapman and Jeffers, 2014). However, with the loss of antimicrobial treatments and concerns over antibiotic resistance, there is a push towards feed additives and vaccination programs. Coccidiosis vaccines in the broiler and turkey industries are designed so that broilers will ingest the sporulated oocysts or the infectious stage of the coccidiosis life cycle to generate an immune response. Sometimes, the vaccine may produce a subclinical infection where birds have damage to the intestinal lining, resulting in decreased absorption of nutrients and reduced growth performance (de Freitas et al., 2023). The vaccination helps the bird build immunity against future infections with the *Eimeria* species, but only with that particular species (Adhikari et al., 2020). The adaptive immune response is stimulated with a coccidial infection, providing long term immunity against the disease. A part of adaptive immunity is the cell mediated immune response which is the most important in a coccidia challenge and involves both helper T cells and cytotoxic T cells. The cytotoxic T cells select and identify host cells infected with the *Eimeria* pathogen and kill those cells (Shivaramaiah et al., 2014). In the United States turkey industry, coccidiosis ranked 16th among 38 health issues that were surveyed in 2024 (Clark and Chiaia, 2024). There are seven major species of *Eimeria* in turkeys but only four are highly pathogenic and pose a significant threat to the turkey industry. The four pathogenic species include *E. adenoids, E. meleagrimitis, E. gallopavonis,* and *E. dispersa* (Milbradt et al., 2014). Sporulation times for *E. adenoeides, E. meleagrimitis, E. gallopavonis* are approximately 24, 18, and 15 hours, respectively (Durairaj et al., 2024). The *Eimeria meleagrimitis* and *E. dispersa* primarily affect the duodenum where fat digestion occurs whereas *Eimeria adenoeides* and *E. gallopavonis* infect the lower portion of the small intestine where protein absorption primarily occurs. A coccidial infection will result in malabsorption of nutrients, dehydration, a reduction in feed intake, and a poor feed conversion ratio (Chapman, 2008). Coccidiosis infections are more severe in younger poults around two to three weeks of age and mortality begins to decrease as poults age and build immunity against the disease (Chapman, 2008).

Until recently, only one live coccidial vaccine existed for turkeys and it contained live oocysts from *E. adenoeides* and *E. meleagrimitis*. Vaccination against one species of *Eimeria* does not mean that the bird then has immunity against other *Eimeria* species (Adhikari et al., 2020). Since there are four highly pathogenic strains of *Eimeria* spp. in turkeys, the vaccine containing only *E. adenoeides* and *E. meleagrimitis* likely did not provide sufficient protection against turkey coccidiosis. A second live vaccine containing *E. adenoeides*, *E. meleagrimitis*, and *E. gallopavonis* was recently approved by the USDA in 2024 to use against coccidiosis in turkeys. A vaccine containing an additional *Eimeria* species provides better protection against coccidiosis in the turkey industry because it encompasses three out of the four highly pathogenic species of *Eimeria* in turkeys. While broiler coccidiosis models have been developed using concentrated doses of the broiler vaccine *Eimeria* strains, a turkey model has not been generated using concentrated doses of a commercially available turkey coccidiosis vaccine.

Direct Fed Microbials

In the commercial laying hen industry, direct fed microbials (DFM) help to improve overall gut health and performance. Direct fed microbials are live microorganisms that are beneficial to the host by improving gastrointestinal health through a variety of mechanisms including enhanced nutrient utilization and intestinal microbial balance (Fuller, 1989; Elshaghabee et al., 2017). There are multiple bacterial species that are used as DFM such as *Lactobacillus* and *Bacillus*. The most common bacterial species used as DFM are *Bacillus* spp. *Bacillus* spp. are spore forming and facultative anaerobes, so they are thermostable and able to survive in low pH conditions of the gastrointestinal tract (Grant et al., 2018). Thermostability and survival in low PH is important since temperatures during feed processing can be high and the gastrointestinal tract has a low pH during digestion. This allows the spores to travel further down the intestinal tract and act on the lower intestines.

Modes of Action

There is a limited amount of resources and space within the gastrointestinal tract to provide nutrients and substrate for growth. Addition of DFM in the diet has been reported to alter microbiota in the intestines and reduce competition for resources between microbes and the host (Grant et al., 2018). *Bacillus* spores attach to the epithelial lining of the intestine thereby preventing harmful pathogens from adhering to the intestinal walls (Patterson and Burkholder, 2003). Prevention of pathogenic bacteria from adhering to the epithelial lining reduces host interactions with pathogenic bacteria allowing them to be moved out of the intestinal tract faster and limiting the need for immune responses (Grant et al., 2018). A reduction in competition for nutrients between bacteria and the host potentially decreases the need to generate an immune response. By not spending energy on generating an immune response, this would potentially decrease maintenance energy costs and allow the bird to partition this energy towards performance and production.

Direct fed microbials containing *Bacillus* spp. have been reported to aid in the digestion of nutrients through an increase in exogenous enzyme production and secretion (Callaway et al., 2017). Many DFM have been reported to secrete lipases, proteases, and amylases (Chowdhury et al., 2020). These enzymes have the potential to enhance lipid, protein, and starch digestion within the host. Improvements in performance were reported when DeKalb XL Single Comb White Leghorn laying hens were fed a DFM as well as increased retention of nitrogen, phosphorous, and Ca measured through excreta analysis (Nahashon et al., 1994). Similar improvements in crude protein, phosphorous, and Ca retention were also reported when increasing doses of a single strain *Bacillus* based DFM were supplemented in the diet of 19 to 28 week old laying hens (Neijat et al., 2019).

Lactic acid producing bacteria such as *Lactobacillus* have been used to lower the pH in the intestines of poultry (Wu et al., 2011). Pathogenic bacteria like *E. coli* or *Salmonella* do not thrive in this lowered pH providing the host with some additional protection. However, beneficial bacteria like *Lactobacillus* and *Bifidobacteria* thrive in acidic conditions further limiting pathogenic exposure and improving gut health and subsequent bird performance (Mazotto et al., 2011).

The fermentation that occurs in the ceca results in the synthesis of short chain fatty acids, or VFA. Volatile fatty acids are produced in the ceca and then absorbed and metabolized to potentially act as an energy source and increase the energy status of the bird (Chichlowski et al., 2007). Accumulation of short chain fatty acids can then reduce the pH of their surrounding environment in the intestinal tract to inhibit the colonization and growth of pathogenic bacteria

(Fooks and Gibson, 2002). The three major VFA that are produced are propionate, acetate, and butyrate. A major substrate for energy production is butyrate. Short chain fatty acids are needed for the maintenance of epithelial cells in the gastrointestinal tract as well as pathogenic bacteria inhibition (Grant et al., 2018). Butyrate has been shown to provide energy for enterocytes to promote proliferation and maturation of the intestinal epithelial cells (Hu and Guo, 2007).

DFM and Energy Metabolism

Energy within a laying hen is balanced between three major pathways which are maintenance, egg production, and storage (Murugesan and Persia, 2013; Lyons et al, 2023). Laying hens will continue to produce eggs at the expense of body energy reserves (storage). The mechanisms of how DFM affect the energy status of laying hens can be determined via direct and indirect measurements. Various doses of *B. subtilis* fed to laying hens from 19 to 48 week directly increased dietary apparent metabolizable energy (AME) compared to control diet not containing the DFM (Neijat et al., 2019). An increase in retention of crude protein, phosphorous, and Ca was also noted with DFM supplementation (Neijat et al., 2019). A direct increase in dietary energy with DFM supplementation may allow for a decrease in energy sources such as oil, which is an expensive component of poultry diets (Liu et al., 2023). An increase in excreta GE retention for birds that were fed a DFM compared to birds that were not was reported when Hy-Line Brown laying hens consuming low energy diets (2,550 kcal/kg) demonstrated egg production, egg mass, and FCR similar to the higher energy diet (2,650 kcal/kg; Mikulski et al., 2020). Direct fed microbials likely improve intestinal health and structure as well as increased fermentation and production of VFA, resulting in increased energy retention and utilization.

DFM and Food Safety

Poultry products are often associated with foodborne illnesses (Patterson and Burkholder, 2003) and one way to mitigate this food borne illness risk is through the use of feed additives such as DFM. Broilers were infected with *Salmonella enterica* and then fed a standard diet with no DFM supplementation or a standard diet + *B. subtilis*. Broilers infected with *Salmonella* and fed *B. subtilis* had a 58% reduction in *Salmonella* positive drag swabs and a 3-log reduction cecal *Salmonella* load (Knap et al., 2011). Similarly, broilers were infected with *E. coli* and birds that were fed a DFM in the diet had significant reductions in cecal *Salmonella* and *E. coli* bacterial counts (Manafi et al., 2017). Hy-Line laying hens were fed a control diet with no DFM, control + 0.1% *L. acidophilus*, or control + 0.05% *B. subtilis* from 16 to 48 wk of age and inclusion of both DFM resulted in reduced counts of *Enterococcus* spp. and increased counts of beneficial bacteria like *Lactobacillus* and *Bifidobacterium* (Forte et al., 2016). Reduced intestinal pathogenic bacterial counts are an indicator of improved gut health that likely translates into food safety improvements since *Salmonella* is a major bacteria contributing to foodborne illnesses (Chowdhury et al., 2020).

DFM and Laying Hen Performance/Egg Shell Quality

Direct fed microbials are often supplemented in laying hen diets to improve overall performance and egg production. Hy-Line Brown laying hens supplemented with *Pediococcus acidilactici* demonstrated improvements in egg mass and FCR compared to hens not supplemented with the probiotic from 23 to 46 weeks of age (Mikulski et al., 2012). Feed conversion ratio was improved by 7 points when Hy-Line Brown laying hens were fed B. subtilis from 72 to 79 weeks of age (Wang et al., 2021). Similarly, FCR was reduced 14 points when a DFM was included in the diet of Lohmann Brown laying hens along with a 4 gram reduction in feed intake (Xu et al., 2006).

As laying hens age, they begin to reduce egg production and the overall shell quality and strength declines potentially due to the impact of age on nutrient efficiency such as a reduction in intestinal Ca uptake (Al-Batshan et al., 1994). Since laying hens have been genetically selected to produce an egg a day and have a long laying cycle, Ca utilization should be maximized, especially as hens age. Direct fed microbials have been reported to improve nutrient digestion through exogenous enzyme production which would increase Ca digestibility, resulting in more Ca available for egg calcification and bone formation (Leeson and Summers, 2001). Inclusion of *B. subtilis* increased both eggshell thickness and breaking strength when fed to Lohmann Brown laying hens from 18 to 42 wk of age (Sobczak and Kozlowski, 2015). Other reports of improved eggshell quality have been demonstrated when DFM are included in the diet (Zhang et al., 2012; Mikulski et al., 2012; Mikulski et al., 2020). Previous reports indicate that improvements in egg quality are possibly attributed to digestive enzyme secretion from the DFM, potentially increasing Ca digestibility and thus increasing shell thickness or strength.

REFERENCES

Abdollahi, M.R., V. Ravindran, T. J. Wester, G. Ravindran, D. V. Thomas. 2011. Influence of feed form and conditioning temperature on performance, apparent metabolizable energy and ileal digestibility of starch and nitrogen in broiler starters fed wheat-based diet. Anim. Feed Sci. Technol. 168:88-99. doi:10.1016/j.anifeedsci.2011.03.014.

Adhikari, P., A. Kiess, R. Adhikari, and R. Jha. 2020. An approach to alternative strategies to control avian coccidiosis and necrotic enteritis. J. Appl. Poult. Res. 29:515-534. https://doi.org/10.1016/j.japr.2019.11.005.

Al-Batshan, H. A., S. E. Scheideler, B. L. Black, J. D. Garlich, and K. E. Anderson. 1994. Duodenal calcium uptake, femur ash, and eggshell quality decline with age and increase following molt. Poult. Sci. 73:1590-1596.

Allen, P. C., and R. H. Fetterer. 2002. Recent advances in biology and immunobiology of Eimeria species and in diagnosis and control of infection with these coccidian parasites of poultry. Clin. Microbiol. Rev. 15:58–65.

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

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007b. Feed particle size: Implication on the digestion and performance of poultry. World’s Poult. Sci. J. 63:439-451.

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

Arike, L., A. Seiman, S. van der Post, A. M. R. Pineiro, A. Ermund, A. Schütte, F. Bäckhed, M. E. V. Johansson, and G. C. Hansson. 2020. Protein turnover in epithelial cells and mucus along the gastrointestinal tract is coordinated by the spatial location and microbiota. Cell Rep. 30:1077-1087. https://doi.org/10.1016/j.celrep.2019.12.068.

ASABE. 2022. Method of determining and expressing fineness of feed materials by sieving. American Society of Agricultural and Biological Engineer Standards. 319.4. Am. Soc. Agric. Biol. Eng., St., Joseph, MI.

Behnke, K. C. 1996. Feed manufacturing technology: current issues and challenges. Anim. Feed Sci. Technol. 62:49-57.

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

Berndt, J. T., S. Schiavi, and R. Kumar. 2005. “Phosphatonins” and the regulation of phosphorus homeostasis. 289:F1159-F1387. https://doi.org/10.1152/ajprenal.00072.2005.

Bonilla, S. M. 2022. A new methodology to measure internal particle size in pellets and to quantify the degree of grinding during pelleting. Master’s Thesis. Auburn University, Auburn, AL.

Callaway, T. R., T. S. Edrington, J. A. Byrd, D. J. Nisbet, S. C. Ricke. 2017. Use of direct-fed microbials in layer hen production – Performance response and *Salmonella* control. 15: 301- 322. http://dx.doi.org/10.1016/B978-0-12-802582-6.00015-X.

Chapman, H. D. 2008. Coccidiosis in the turkey. Avian Pathol. 37(3):205-223. DOI: 10.1080/03079450802050689.

Chapman, H.D., J. R. Barta, D. Blake, A. Gruber, M. Jenkins, N. C. Smith, X. Suo, and F. M. Tomley. 2013. A selective review fo advances in coccidiosis research. Adv. Parasitiol. 83:93-171. http://dx.doi.org/10.1016/B978-0-12-407705-8.00002-1.

Chapman, H. D., and T. K. Jeffers. 2014. Vaccination of chickens against coccidiosis

ameliorates drug resistance in commercial poultry production. Int. J Parasitol. Drugs Drug Resist. 4:214-217. http://dx.doi.org/10.1016/j.ijpddr.2014.10.002.

Charbeneau, R. A. and K. D. Roberson. 2004. Effects of corn and soybean meal particle size on phosphorous use in turkey poults. J. Appl. Poult. Res. 13:302-310.

Chichlowski, M. J. Croom, B. W. McBride, G. B. Havenstein, and M. D. Koci. 2007. Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: A brief review of current knowledge. Int. J. Poult. Sci. 10:694-704.

Chowdhury, S. D., B. C. Ray, A. Khatun, M. R. A. Redoy, and A. S. Afsana. 2020. Application of probiotics in commercial layer diets: A review. Bang. J. Anim. Sci. 49:1-12.

Clark, S. R. and L. Chiaia. 2024. Current Health Issues Facing the US Turkey Industry (HVP.PO.100324.1). Proceedings 128th Annual Mtg of the USAHA, Virtual; Committee on Poultry and Other Avian Species. Pending Publication. Presented Sep 30, 2024.

Collett, S. R. 2012. Nutrition and wet litter problems in poultry. Anim. Feed Sci. Technol. 173:65-75. https://doi.org/10.1016/j.anifeedsci.2011.12.013.

David, L. S., M. N. Anwar, M. R. Abdollahi, M. R. Bedford, and V. Ravindran. 2023. Calcium nutrition of broilers: Current perspectives and challenges. Animals. 13:1590. https://doi.org/10.3390/ani13101590.

de Freitas, L. F. V. B., N. K. Sakomura, M. de Paula Reis, A. B. Mariani, W. Lambert, I. Andretta, and M. P. Létourneau-Montminy. 2023. Coccidiosis infection and growth performance of broilers in experimental trials: insights from a meta-analysis including modulating factors. Poult. Sci. 102:103021.

de Witt, F. H., H. J. van der Merwe, J. P. Hayes, and M. D. Fair. 2006. Influence of particle size distribution on *in vivo* and *in vitro* limestone solubility. South Afr. J. Anim. Sci. 36:95-98.

Driver, J. P., G. M. Pesti, R. I. Bakalli, and H. M. Edwards, Jr. 2005. Calcium requirements of the modern broiler chicken as influenced by dietary protein and age. Poult. Sci. 84:1629-1639.

Dunlop, M. W., A. F. Moss, P. J. Groves, S. J. Wilkinson, R. M. Stuetz, and P. H. Selle. 2016. The multidimensional causal factors of 'wet litter' in chicken-meat production. Sci. Total Environ. 562:766-776.

Durairaj, V., R. V. Veen, and S. Clark. 2024. Turkey coccidiosis: Understanding highly pathogenic Eimeria species in turkeys. Modern Poultry. Published March 12, 2024.

Elshaghabee, F. M. F., N. Rokana, R. D. Gulhane, C. Sharma, and H. Panwar. 2017. Bacillus as potential probiotics: Status, concerns, and future perspectives. Front. Microbiol. 8:1490. doi: 10.3389/fmicb.2017.01490.

Engberg, R. M., M. S. Hedemann, S. Steenfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925-938.

Fallah, H., A. Karimi, G. H. Sadeghi, and N. Behroozi-Khazaei. 2018. The effects of calcium source and concentration on performance, bone mineralization and serum traits in male broiler chickens from 1 to 21 days of age. Anim. Prod. Sci. 59:1090-1097. https://doi.org/10.1071/AN18166.

Favero, A., A. Maiorka, F. Dahlke, R. F. P. Meurer, R. S. Oliveira, and R. F. Sens. 2009. Influence of feed form and corn particle size on the live performance and digestive tract development of turkeys. J. Appl. Poult. Res. 18: 772-779. doi: 10.3382/japr.2009-00069.

Fayer, R. 1980. epidemiology of protozoan infections: The coccidia. Vet. Parasitol. 6:75–103.

Flores, K. R., A. Fahrenholz, P. R. Ferket, T. J. Biggs, and J. L. Grimes. 2021. Effect of methionine chelated Zn and Mn and corn particle size on Large White male turkey live performance and carcass yields. Poult. Sci. 100:101444. https://doi.org/10.1016/j.psj.2021.101444.

Forte, C., G. Acuti, E. Manuali, P. Casagrande Proietti, S. Pavone, M. Trabalza-Marinucci, L. Moscati, A. Onofri. C. Lorenzetti, and M. P. Franciosini. 2016. Effects of two different probiotics on microflora, morphology, and morphometry of gut in organic laying hens. Poult. Sci. 95:2528-2535. http://dx.doi.org/10.3382/ps/pew164.

Fooks, L. J. and G. R. Gibson. 2002. Probiotics as modulators of the gut flora. Brit. J. Nutr. 88:39-49. DOI: 10.1079/BJN2002628.

Francesch, M. and J. Brufau. 2003. Nutritional factors affecting excreta/litter moisture and quality. Worlds Poult. Sci. 60:64-75. https://doi.org/10.1079/WPS20035.

Fuller, R. 1989. Probiotics in man and animals. J Appl. Bacteriol. 66:365-378.

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

Gautron, J., L. Stapene, N. L. Roy, Y. Nys, A. B, Rodriguez-Navarro, and M. T. Hincke. 2021. Avian eggshell biomineralization: an update on its structure, mineralogy and protein tool kit. BMC Molec. Cell Biol. 22:11. https://doi.org/10.1186/s12860-021-00350-0.

Grant, A., C. G. Gay, and H. S. Lillehoj. 2018. *Bacillus* spp. As direct-fed microbial antibiotic alternatives to enhance growth, immunity, and gut health in poultry. Avian Pathol. 47:339-351. DOI: 10.1080/03079457.2018.1464117.

Jensen, L. S., L. H. Merrill, C. V. Reddy, and J. McGinnis. 1962. Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. Poult. Sci. 41:1414-1419.

Hemmingsen, A. K. T., A. M. Stevik, I. C. Claussen, K. K. Lundblad, E. Prestløkken, M. Sørensen, and T. M. Eikevik. 2008. Water adsorption in feed ingredients for animal pellets at different temperatures, particle size, and ingredient combinations. Dry. Technol. 26:738-748.

Hott, J. M., N. P. Buchanan, S. E. Cutlip, and J. S. Moritz. 2008. The effect of moisture addition with a mold inhibitor on pellet quality, feed manufacture, and broiler performance. J. Appl. Poult. Res. 17:262-271.

Hu, Z. and Y. Guo. 2007. Effects of dietary sodium butyrate supplementation on the intestinal morphological structure, absorptive function and gut flora in chickens. Anim. Feed Sci. Tech. 132:240-249.

Innes, E. A., and A. N. Vermeulen. 2006. Vaccination as a control strategy against the coccidial parasites Eimeria, Toxoplasma, and Neospora. Parasitology. 133:S145–S168.

Kiarie, E. G. and A. Mills, 2019. Role of feed processing on gut health and function in pigs and poultry: Conundrum of optimal particle size and hydrothermal regimens. Front. Vet. Sci. 6:19. https://doi.org/10.3389/fvets.2019.00019.

Knap, I., A. B. Kehlet, M. Bennedsen, G. F. Mathis, C. L. Hofacre, B. S. Lumpkins, M. M. Jensen, M. Raun, and A. Lay. 2011. *Bacillus subtilis* (DSM17299) significantly reduces *Salmonella* in broilers. Poult. Sci. 90:1690-1694. doi: 10.3382/ps.2010-01056.

Lee, B., K. M. Moon, and C. Y. Kim. 2018. Tight junction in the intestinal epithelium: Its association with diseases and regulation by phytochemicals. J. Immunol. Res. 2018:1-11. https://doi.org/10.1155/2018/2645465.

Liu, Z., L. Wang, D. Beasley, and E. Oviedo. 2007. Effect of moisture content on ammonia emissions from broiler litter: A laboratory study. J. Atmos. Chem. 58:41-53. DOI 10.1007/s10874-007-9076-8.

Liu, G, F. L. S. Castro, and W. K. Kim. 2023. Applied research note: Exogenous protease supplementation to reduced-energy, reduced-protein, and reduced-amino acid diets for broiler chickens from days 1 to 42. J. Appl. Poult. Res. 32:100362. https://doi.org/10.1016/j.japr.2023.100362.

Lu, C., Y. Yan, F. Jian, and C. Ning. 2021. Coccidia-microbiota interactions and their effects on the host. Front. Cell. Infect. Microbiol. 11:751481.

Lyons, A. M., C. A. Granghelli, and M. E. Persia. 2023. Estimating energy utilization in laying hens: what are the best response criteria?. J. Appl. Poult. Res. 32:100357. https://doi.org/10.1016/j.japr.2023.100357.

MacIsaac, J. L., D. M. Anderson, and A. Safamehr. 2016. Evaluation of whelks as an alternative source of calcium in laying hens. J. Appl. Poult. Res. 25:492-505. http://dx.doi.org/10.3382/japr/pfw031.

Manafi, M., S. Khalaji, M. Hedayati, and N. Pirany. 2017. Efficacy of *Bacillus subtilis* and bacitracin methylene disalicylate on growth performance, digestibility, blood metabolites, immunity, and intestinal microbiota after intramuscular inoculation with *Escherichia coli* in broilers. Poult. Sci. 96:1174-1183. http://dx.doi.org/10.3382/ps/pew347.

Mazotto, A. M., R. R. Rodrigues-Coelho, S. M. Lage-Cedrola, M. F. de Lima, S. Couri, E. Paraguai de Souza, and A. B. Vermelho. 2011. Keratinase production by three Bacillus spp. using feather meal and whole feather as substrate in a submerged fermentation. Enz. Res. 4:523780. http://dx.doi.org/10.4061/2011/523780.

McDonald, V., and M. W. Shirley. 2009. Past and future: Vaccination against Eimeria. Parasitology. 136:14771489.

Mikulski, D., J. Jankowski, J. Naczmanski, M. Mikulska, and V. Demey. 2012. Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. Poult. Sci. 91:2691-2700. http://dx.doi.org/ 10.3382/ps.2012-02370.

Mikulski, D., J. Jankowski, M. Mikulska, and V. Demey. 2020. Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on productive performance, egg quality, and body composition in laying hens fed diets varying in energy density. Poult. Sci. 99:2275-2285. https://doi.org/10.1016/j.psj.2019.11.046.

Milbradt, E. L., A. A. Mendes, J. G. Ferreira, I. Almeida Paz, M. B. Martins, C. Sanfelice, B. C. Fernandes, and A. S. Okamoto. 2014. Use of live oocyst vaccine in the control of turkey coccidiosis: Effect on performance and intestinal morphology. J. Appl. Poult. Res. 23:204-211. http://dx.doi.org/ 10.3382/japr.2013-00877.

Muir, F. V., P. C. Harris, and R. W. Gerry. 1976. The comparative value of five calcium sources for laying hens. Poult. Sci. 55:1046-1051.

Murugesan, G. R. and M. E. Persia. 2013. Validation of the effects of small differences in dietary metabolizable energy and feed restriction in first-cycle laying hens. Poult. Sci. 92:1238-1243. http://dx.doi.org/10.3382/ps.2012-02719.

Naderinejad, S., F. Zaefarian, M. R. Abdollahi, A. Hassanabadi, H. Kermanshahi, and V. Ravindran. 2016. Influence of feed form and particle size on performance, nutrient utilisation, and gastrointestinal tract development and morphometry in broiler starters fed maize-based diets. Anim. Feed Sci. Tech. 215:92-104. https://doi.org/10.1016/j.anifeedsci.2016.02.012.

Neijat, M., R. B. Shirley, J. Barton, P. Thiery, A. Welsher, and E. Kiarie. 2019. Effect of dietary supplementation of Bacillus subtilis DSM29784 on hen performance, egg quality indices, and apparent retention of dietary components in laying hens from 19 to 48 weeks of age. Poult. Sci. 98:5622-5635. http://dx.doi.org/10.3382/ps/pez324.

Nir, I., R. Hillel, G. Shefet, and Z. Nitsan. 1994a. Effect of grain particle size on performance. 2. Grain texture interactions. Poult. Sci. 73:781-791.

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

Noack, S., H. D. Chapman, and P. M. Selzer. 2019. Anticoccidial drugs of the livestock industry. Parasitol. Res. 118:2009-2026. https://doi.org/10.1007/s00436-019-06343-5.

Nusairat, B. and J.J. Wang. 2020. Xylanase and direct-fed microbials (DFM) potential for improvement of live performance, energy digestibility, and reduction of environmental microbial load of broilers. Front. Vet. Sci. 7:1014.

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

Patterson, J. A. and K. M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82:627-631.

Pelícia, K., E. A. Garcia, M. R. S. Scherer, C. Móri, J. A. Dalanezi, A. B. G. Faitarone, A. B. Molino, and D. A. Berto. 2007. Alternative calcium source effects on commerical egg produciton and quality. Braz. J. Poult. Sci. 9:105-109.

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

Pesti, G. M., R. I. Bakalli, J. P. Driver, A. Atencio, and E. H. Foster. 2005. Poultry nutrition and feeding. Pages 13-7 to 13-8 in Bone & Egg Shell Structure. Trafford Publishing, Victoria, BC.

Pizzolante, C. C., E. S. P. B. Saldanha, C. Laganá, S. K. Kakimoto, and C. K. Togashi. 2009. Effects of calcium levels and limestone particle size on the egg quality of semi-heavy layers in their second production cycle. Braz. J. Poult. Sci. 11:79-86.

Portella, F. J., L. J. Caston, and S. Leeson. 1988. Apparent feed particle size preference by broilers. Can. J. Anim. Sci. 68:923-930.

Price, K. R. 2012. Use of live vaccines for coccidiosis control in replacement layer pullets. J. Appl. Poult. Res. 21:679-692. http://dx.doi.org/10.3382/japr.2011-00486.

Ray, B. C., S. D. Chowdhury, S. C. Das, B. Dey, A. Khatun, B. C. Roy, and M. A. Siddik. 2022. Comparative effects of feeding single- and multi- strain probiotics to commercial layers on the productive performance and egg quality indices. J. Appl. Poult. Res. 31:100257. https://doi.org/10.1016/j.japr.2022.100257.

Rao, K. S., and D. A. Roland. 1989. Influence of dietary calcium level and particle size of calcium source on in vivo calcium solubilization by commercial leghorns. Poult. Sci. 68:1499-1505.

Rao, K. S., and D. A. Roland. 1990. *In vivo* solubilization in commercial leghorns: Role of dietary calcium level, limestone particle size, *in vitro* limestone solubility rate, and the calcium status of the hen. Poult. Sci. 69:2170-2176.

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

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

Reece, F. N., B. D. Lott, and J. Deaton. 1986b. Effects of environmental temperature and corn particle size on response of broilers to pelleted feed. Poult. Sci. 65:636–641.

Reimer, L. 1992. Conditioning. Northern Crops Institute Feed Mill Management and Feed Manufacturing Technology Short Course. Northern Crops Institute, Fargo, North Dakota, USA, 7.

Roland, D. A. and R. H. Harms. 1973. Calcium metabolism in the laying hen 5. Effect of various sources and sizes of calcium carbonate on shell quality. Poult. Sci. 52:369-372.

Roland, D. A. Sr. 1979. Factors influencing shell quality of aging hens. Poult. Sci. 58:774-777.

Rueda, M. S., S. Bonilla, C. de Souza, J. D. Starkey, C. W. Starkey, L. Mejia, and W. J. Pacheco. 2024. Evaluation of particle size and feed form on performance, carcass characteristics, nutrient digestibility, and gastrointestinal tract development of broilers at 39 d of age. Poult. Sci. 103:103437. https://doi.org/10.1016/j.psj.2024.103437.

Scott, M. L., S. J. Hull, and P. A. Mullenhoff. 1971. The calcium requirements of laying hens and effects of dietary oyster shell upon egg shell quality. Poult. Sci. 50:1055-1063. https://doi.org/10.3382/ps.0501055.

Selle, P. H., A. J. Cowieson, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livestock Sci. 124:126-141. http://dx.doi.org/10.1016/j.livsci.2009.01.006.

Shivaramaiah, C., J. R. Barta, X. H-Velasco, G. Téllez, and B. M. Hargis. 2014. Coccidiosis: recent advancements in the immunobiology of *Eimeria* species, preventative measures, and the importance of vaccination as a control tool against these Apicomplexan parasites. Vet. Med. Res. Reports. 5:23-34. http://dx.doi.org/10.2147/VMRR.S57839.

Sinclair-Black, M., R. A. Garcia, and L. E. Ellestad. 2023. Physiological regulation of calcium and phosphorous utilization in laying hens. Front. Physiol. 14:1112499. https://doi.org/10.3389/fphys.2023.1112499.

Skoch, E. R., K. C. Behnke, C. W. Deyoe, and S. F. Bidner. 1981. The effect of steam conditioning rate on the pelleting process. Anim. Feed Sci. Technol. 6:83-90.

Snoeck, V., B. Goddeeris, and E. Cox. 2005. The role of enterocytes in the intestinal barrier function and antigen uptake. Micr. Infect. 7:997-1004. doi:10.1016/j.micinf.2005.04.003.

Soares, J. H. 1995. Calcium bioavailability. Pages 95-118 in Bioavailability of nutrients for animals: Amino acids, minerals, and vitamins. Ammerman CB, Baker DH, Lewis AJ (eds) Academic, Oxford.

Sobczak, A. and K. Kozlowski. 2015. The effect of a probiotic preparation containing Bacillus subtilis ATCC PTA-6737 on egg production and physiological parameters of laying hens. Ann. Anim. Sci. 15:711-723. DOI: 10.1515/aoas-2015-0040.

Sulpis, O., P. Agrawal, M. Wolthers, G. Munhoven, M. Walker, and J. J. Middelburg. 2022. Aragonite dissolution protects calcite at the seafloor. Nature. 13:1104. https://doi.org/10.1038/s41467-022-28711-z.

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

Svihus, B. 2011. The gizzard: function, influence of diet structure and effects on nutrient availability. 67:207-224. doi:10.1017/S0043933911000249.

Tang, P., P. H. Patterson, and V. M. Puri. 2006. Effect of feed segregation on the commercial hen and egg quality. J. Appl. Poult. Res. 15:564–573.

Teng, P-Y., S. Yadav, F. L. de Souza Castro, Y. H. Tompkins, A. L. Fuller, and W. K. Kim. 2020. Graded *Eimeria* challenge linearly regulated growth performance, dynamic change of gastrointestinal permeability, apparent ileal digestibility, intestinal morphology, and tight junctions of broiler chickens. Poult. Sci. 99:4203-4216. https://doi.org/10.1016/j.psj.2020.04.031.

USDA Economic Research Service. Corn and Other Feed Grains - Feed Grains Sector at a Glance | Economic Research Service. Accessed 7/21/2025.

Walk, C. L., E. K. Addo-Chidie, M. R. Bedford, and O. Adeola. 2012. Evaluation of a highly soluble calcium source and phytase in the diets of broiler chickens. Poult. Sci. 91:2255-2263. http://dx.doi.org/10.3382/ps.2012-02224.

Wang-Li, L., Y. Xu, A. P. Shivkumar, M. Williams, and J. Brake. 2020. Effect of dietary coarse corn inclusion in broiler live performance, litter characteristics, and ammonia emission. Poult. Sci. 99:869-878. http://dx.doi.org/10.1016/j.psj.2019.10.010.

Wang, J., W. Wang, G. Qi, C. Cui, S. Wu. H. Zhang, L. Xu. And J. Wang. 2021. Effects of dietary Bacillus subtilis supplementation and calcium levels on performance and eggshell quality of laying hens in the late phase of production. Poult. Sci. 100:100970. https://doi.org/10.1016/j.psj.2020.12.067.

Wondra, K. J., C. R. Stark, R. H. Hines, J. D. Hancock, and K. C. Behnke. 1993. Effects of hammermills and roller mills on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. Kansas State University Swine Day Report of Progress. 0:140-143.

Wu, B. Q., T. Zhang, L. Q. Guo, and J. F. Lin. 2011. Effects of *Bacillus subtilis* KD1 on broiler intestinal flora. Poult. Sci. 90:2493-2499. doi: 10.3382/ps.2011-01529.

Xu, C. L., C. Ji, Q. Ma, K. Hao, Z. Y. Jin, and K. Li. 2006. Effects of a dried *Bacillus subtilis* culture on egg quality. Poult. Sci. 85:364-368.

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

Yeboah, P, D. Ibiwoye, J. C. Bielke, W. N. Briggs, K. M. Chasser, A. F. Duff, and L. R. Bielke. 2025. Assessment of Eimeria gallopavonis dose for vaccination strategies in turkeys. J. Appl. Poult. Res. 34:100533. https://doi.org/10.1016/j.japr.2025.100533.

Zaefarian, F., M. R. Abdollahi, and V. Ravindran. 2016. Particle size and feed form in broiler diets: impact on gastrointestinal tract development and gut health. World's Poult. Sci. 72:277-290. https://doi.org/10.1017/S0043933916000222.

Zhang, J. L., Q. M. Xie, J. Ji, W. H. Yang, Y. B. Wu, C. Li, J. Y. Ma, and Y. Z. Bi. 2012. Different combinations of probiotics improve the production performance, egg quality, and immune response of layer hens. Poult. Sci. 91:2755-2760. http://dx.doi.org/10.3382/ps.2012-02339.

Zhang, B., J. V. Caldas, and C. N. Coon. 2017. Effect of dietary calcium intake and limestone solubility on eggshell quality and bone parameters for aged laying hens. Int. J. Poult. Sci. 16:132-138. DOI: 10.3923/ijps.2017.132.138.

Zhang, H., D. Li, L. Liu, L. Xu, M. Zhu, X. He, and Y. Liu. 2019. Cellular composition and differentiation signaling in chicken small intestinal epithelium. Animals. 9:870. http://dx.doi.org/10.3390/ani9110870.

CHAPTER 3:

Evaluation of corn particle size on the growth performance, intestinal permeability, pellet quality, and litter moisture of turkey hens raised without antibiotics

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SUMMARY

An experiment was conducted to determine the effects of corn particle size (CPS) on turkey hen poult performance, intestinal permeability (IP), and litter dry matter. A total of 1,584 Hybrid turkey hen poults were raised in floor pens from 0 to 42d. Poults were fed a crumbled starter 1 and pelleted starter 2 diet. The experiment was a 2 x 2 factorial with diet phase (starter 1 and starter 2) and CPS (fine or coarse), resulting in 4 treatments of 18 replicates of 22 turkeys. Corn was ground using a hammermill with a 4.76 mm screen (581 μm; fine) or a 6.35 mm screen (964 μm; coarse). Pellet quality was determined using pellet durability index (PDI), modified pellet durability index (MPDI), and New Holmen Pellet Tester (NHPT). Body weight and feed intake were measured on D0 and D42. Feed conversion ratio was calculated from 0 to 42d. Litter dry matter content and IP were measured on D42. Data were analyzed using JMP Pro 16.0 ($P \leq 0.05$) and means were separated using Student's t-test. Corn particle size did not impact starter 2 pellet quality (PDI, MPDI, and NHPT; $P > 0.05$). There were no interactions between dietary phase and CPS over 0 to 42d. Overall, there was a CPS main effect over the starter 1 phase and hens fed coarse corn had decreased FI ($P \leq 0.01$) and BW ($P \leq 0.01$), however FCR was not affected ($P > 0.05$). Day 42 IP and litter dry matter content were not influenced by CPS in either diet phase ($P > 0.05$). These data indicate that coarse corn decreases both FI and BW of turkey hens when fed during starter 1, but had no effects on FCR, IP, and litter dry matter content when fed in starter 2. Although fine ground corn was needed to maximize performance in the starter 1 phase, coarse ground corn may be able to maintain turkey hen performance in the starter 2 phase while reducing milling costs.

DESCRIPTION OF PROBLEM

The grinding of feed ingredients is required but adds to the costs of feed manufacture using specialized equipment, electricity, and labor. The cost of grinding decreases as ingredient particle size (**PS**) increases (Reece et al., 1986; Flores et al., 2021). Although coarse grinding of ingredients reduces milling costs, the effects of ingredient PS on bird performance and nutrient digestibility can vary.

Coarse particles in a mash diet have been shown to result in increased retention time in the gizzard, thus increasing gizzard weight while decreasing gizzard pH (Gabriel et al., 2003; Engberg et al., 2004). A lower pH was observed in the gizzards of broilers fed diets containing whole wheat as well as lower counts of lactose-negative enterobacteria, including *Salmonella*, in the gizzard ($P = 0.02$; Engberg et al., 2004). The low pH of the gizzard may kill the bacteria and prevent the bacteria from entering the distal portion of the intestine (Engberg et al., 2004). However, previous work has shown that pellets may not stimulate the gizzard to the extent of mash diets due to the breakdown of coarse particles that occurs during the pelleting process, resulting in decreased gizzard stimulation, higher pH, and the potential for harmful bacteria in the intestine (Gabriel et al., 2003; Engberg et al., 2004; Svihus et al., 2004).

There have been many published studies that have looked at ingredient PS and its effects on broiler performance and nutrient digestibility (Reece et al., 1986; Nir et al., 1994; Amerah et al., 2007b; Amerah et al., 2008; Jacobs et al., 2010; Xu et al., 2015a, b; Rubio et al., 2020), but little work has been done with regards to ingredient PS in turkey diets. Previous work demonstrated that 1,094 µm corn reduced BWG compared to 606, 701, and 832 µm corn when fed to Hybrid Converter turkey hens from D0-7 (Charbeneau and Roberson, 2004). Various corn particle sizes (380, 606, and 806 µm) were fed to BUT 9 male turkeys from 0 to 21d and the turkeys consuming the 606 µm corn had increased BWG and FI with no effect on FCR (Favero et al., 2009). These data indicate that coarse corn may decrease turkey performance over the starter period, but there is limited work that has investigated the effects over a longer feeding period. Therefore, an experiment was conducted to determine the effects of corn particle size on pellet quality, 0-42 d turkey hen performance, intestinal permeability, and litter dry matter.

MATERIALS AND METHODS

Corn and experimental diet manufacture

The same batch of whole corn was used to manufacture the fine and coarse corn particle sizes (**CPS**) to ensure similar nutritional quality. Corn was ground at West Virginia University using a hammermill fitted with a 6.35 mm or 4.76 mm screen to produce coarse and fine corn PS, respectively. The corn was ground with the 4.76 mm screen twice. All corn was then transported to Virginia Tech for diet generation and bird feeding.

Diets were nutritionally similar and only differed in the CPS. Hybrid Turkey breeder recommendations were used as the basis for diet formulation with energy and digestible amino acids adjusted to align with industry practices at the time the experiment was conducted. A basal diet was manufactured with no corn and experimental diets were manufactured by adding corn to the basal diet. Mash diets were manufactured at Virginia Tech and then transported to a local toll milling facility to be pelleted and crumbled. Diets were sent to a commercial laboratory for nutrient analysis (University of Missouri; Table 3.1). Particle size of the corn and mash diets was determined by ASAE method S319.4 (ASABE, 2022). Dry sieving of the diets and corn was completed using a Ro-Tap tester plus agitators (Ro-Tap tester, Model RX-29, WS Tyler company, Mentor, OH). The Ro-Tap tester was fitted with a set of 14 sieves ranging from 4,760 μm to 38 μm and was used to shake 100g samples for 10 min. A dispersing agent was not added to the samples. After 10 min, individual sieves were weighed to determine average PS for each dietary treatment and corn sample.

Pellet quality analyses

Pellets from the starter 2 diet were collected after feed manufacturing and sent to Pennsylvania State University for pellet quality analyses. All pellet quality analyses were conducted in duplicate. Pure pellets were obtained by using a No. 5 W. S. Tyler testing sieve (Mentor, OH). Pellet durability index and modified pellet durability index were conducted using a Pfost tumble box (Gamet Manufacturing Inc., Saint Paul, MN). The New Holmen Pellet Tester was used to determine pellet survivability (New Holmen's Pellet Tester, TekPro Ltd., Norfolk, UK).

Experimental design

All animal procedures used in the experiment were approved by the Virginia Tech Animal Care and Use Committee. A total of 1,584 one-day-old Hybrid turkey hens were randomly allocated to floor pens (4.5' x 8') with 18 replicate pens per treatment and 22 birds per pen. The experiment was a 2 x 2 factorial with CPS in starter 1 vs CPS in starter 2 as main effects. Each experimental unit received one of two dietary treatments varying only in CPS (fine or coarse) from 0-21 (starter 1) and 22-42 (starter 2) d of age. The experiment was a randomized complete block design with the experimental unit being the pen. Experimental diets were randomly assigned to pens within blocks that were arranged within the building by location. Birds were fed ad libitum and provided a crumbled starter 1 diet from D0-21 and a pelleted starter 2 diet from D22-42. Water was provided ad libitum throughout the experiment with nipple drinkers in each pen. Temperature and lighting were adjusted according to breeder recommendations and the age of the bird (Hybrid Turkey Management Guide, 2021).

Turkey performance

Feed intake (**FI**) was determined at the end of each diet phase and reported from 0-42 d. Feed intake was calculated by subtracting the weight of the remaining feed at the end of the period from the amount of feed added to the feeder over the duration of the feeding phase. Initial body weight (**BW**) was measured on D0 and final weights were determined on D42. Feed conversion ratio (**FCR**) was calculated and reported over the 0-42 d period. Due to the factorial analysis across the feeding phases, data were not calculated for the individual 0-21 and 22-42 d periods.

Intestinal permeability

A FITC-dextran (**FITC-d**) assay was used to estimate intestinal permeability in turkeys on D42 (Baxter et al., 2017). The large insoluble dextran molecule has limited ability to pass through the tight junctions in the intestine and pass into the blood in a healthy bird. However, intestinal structure is compromised when a bird is presented with a challenge and the dextran molecule can pass through the tight junctions, resulting in an increased concentration in the blood. On D42, one turkey per pen was orally gavaged with 8.32 mg/kg of FITC-d that was dissolved in ultra-pure water. Approximately 1 h after FITC-d inoculation, blood was collected from the brachial vein and was allowed to clot at room temperature in serum tubes. Blood samples were centrifuged at 2000 × g for 10 min at 4˚C to isolate serum from the blood. Serum was diluted 1:5 in 0.9% saline to a total volume of 100 µl in a 96 well flat bottom black plate. A multi-mode plate reader (Infinite M200 Pro, Tecan, Morrisville, NC) was used to analyze serum for FITC-d at an excitation wavelength of 485 nm and emission wavelength of 528 nm. A standard curve was generated by adding known amounts of FITC-d to sera collected from turkeys that had not received a dose of FITC-d.

Litter dry matter

Litter samples were collected from each pen on D42 to determine dry matter content. A composite litter sample was collected by taking litter from the center of the pen and the center of each quadrant when the pen is divided into 4 equal squares (Sizmaz et al., 2022). Areas near drinkers were avoided. Samples were mixed for each pen to create one composite sample. Approximately 100 g of each sample was weighed into an aluminum weigh dish and oven dried at 100˚C for 24 h. All samples were conducted in duplicate. Dried samples were then weighed and litter dry matter was calculated using the following equation:

(Dry wt of litter (g)/Wet wt of litter (g)) x 100

Statistical analysis

Performance data, litter dry matter, and intestinal permeability were analyzed as a 2 x 2 factorial with CPS in the starter 1 phase (581 and 964 μm) and CPS in the starter 2 phase as main effects in JMP Pro 16.0 (SAS Institute Inc., Cary, NC). If significance was detected for the interaction, ($P \leq 0.05$), means were further separated using Student's t-test (Sarsour and Persia, 2022). Data for the starter 1 phase could not be analyzed as a factorial and was therefore analyzed using only the particle size over the starter 1 phase as the effect. Pellet quality data for the starter 2 diet was analyzed in JMP Pro 16.0 ($P \leq 0.05$). Trends were determined at $P \leq 0.10$. All data were analyzed using the pen as the experimental unit.

RESULTS AND DISCUSSION

Corn particle distribution and starter 2 pellet quality

The PS distribution of the corn used in the starter 1 and starter 2 diets is shown in Figure 3.1A. Particle size of the corn averaged 581 µm for the fine corn and 964 µm for the coarse corn. The average PS of the starter 1 mash diets was 690 µm when 581 µm corn was included and 795 µm when 964 µm corn was used. As expected, 964 µm corn inclusion in the starter 2 diet increased the PS of the mash diet ($P \leq 0.01$; Table 3.2; Figure 3.1B). Particle size of the starter 2 mash diet containing the 581 µm corn was 736 µm and the diet containing the 964 µm corn was 879 µm. Although starter 2 mash diets differed in PS, there were no differences in starter 2 pellet quality for NHPT, PDI, or MPDI ($P > 0.05$). Data from the current experiment agree with previous literature in broiler diets showing no differences in pellet quality in diets containing 629, 763, 814, or 1,779 µm corn (Rubio et al., 2020). Previous work has demonstrated that the pelleting process may minimize differences in particle distribution because coarse particles are more susceptible to grinding by the pellet mill due to frictional forces within the pellet die (Svihus et al., 2004; Amerah et al., 2007b). Diets containing 490 µm and 796 µm corn were pelleted and wet sieving demonstrated that final diet particle sizes were reduced to 253 µm and 299 µm, respectively (Naderinejad et al., 2016). Recent research from Bonilla (2022) demonstrated that the degree of grinding that occurs during pelleting increases as the PS of the mash diet increases. These data indicate that diet particle size after pelleting is an important consideration when discussing ingredient PS.

Turkey performance

There were no interactions throughout the 0-42d experiment so only the main effects are discussed. Birds fed 964 µm corn during the starter 1 phase had a lower BW at D42 ($P < 0.01$; Table 3.4) than those fed 581 µm corn. There were no differences in any of the other measured performance parameters including FI, FCR, and mortality from 0-42d ($P > 0.05$). Previous work demonstrated a linear decrease in BWG when Hybrid Converter turkeys were fed increasing CPS of 606, 701, 832, and 1,094 µm corn from 0-7d ($P < 0.01$) and 0-15d ($P = 0.02$; Charbeneau and Roberson, 2004). When BUT 9 male turkeys were fed 606 µm corn from 1-21d, they had increased BWG and FI compared to birds fed 380 and 806 um corn ($P < 0.01$), however, there were no differences in FCR ($P > 0.05$; Favero et al., 2009). Similar responses in broilers have been shown with a reduced BWG when fed diets containing 1,387 or 600 µm corn compared to 858 or 300 µm corn from 0-21 d (Jacobs et al., 2010; Chewning et al., 2012). The difference in PS preference between broilers and turkeys may be due to beak size and the mechanoreceptors of the beak, which has been reported to affect FI (Moran, 1982). Data from 0-21 d was analyzed using only starter 1 CPS as the effect (Table 3.3). Turkey poults that consumed 964 µm corn during the starter 1 phase had lower BWG and FI as well as an increased FCR compared to those fed 581 µm corn ($P \leq 0.05$). This is in agreement with previous work demonstrating that coarse corn decreased BW in poults when fed from 0-15 or 0-21 d ($P \leq 0.05$; Charbeneau and Roberson, 2004; Favero et al., 2009). Data from the current experiment and previous research indicate that feeding coarse corn early decreases BW in both broilers and turkeys. However, there were no differences in the current experiment in FI, BWG, or FCR when poults were fed 581 or 964 µm corn in the starter 2 phase ($P > 0.05$; data not shown). This indicates that turkeys may be fed fine corn (581 µm) during the starter 1 phase to improve performance and coarse corn (964 µm) after starter 1 to save costs at the feed mill and maximize turkey performance in the starter 2 phase.

Intestinal permeability

No main effects were observed for intestinal permeability measured on D42 ($P > 0.05$; Table 3.5). Previous work demonstrated that inclusion of coarse particle corn in a pelleted diet decreased gizzard pH compared to when fine particle corn was fed to broilers over a 21 d period ($P < 0.05$; (Naderinejad et al., 2016). Although whole wheat inclusion in broiler diets has been shown to reduce gizzard pH, resulting in reduced counts of harmful bacteria such as *Salmonella* in the gizzard (Gabriel et al., 2003; Engberg et al., 2004), this has not been reported in turkeys. However, pelleted diets containing coarse particles may not stimulate the gizzard to the extent of mash diets due to a greater degree of grinding that occurs in the pellet die (Svihus et al., 2004).

Litter dry matter

There was a trend for birds that were fed 964 µm corn during both the starter 1 and starter 2 phases to have higher litter dry matter at 42 d ($P = 0.07$). Whole wheat inclusion in broiler diets increased retention time in the gizzard and lowered gizzard pH, thus decreasing harmful bacteria counts, such as *Salmonella,* in the gizzard (Engberg et al., 2004). This reduction in bacteria could positively impact intestinal microbial populations and improve gut health, although this has not been demonstrated in turkeys. The presence of harmful bacteria in the intestines could lead to diarrhea and increased fecal water content which may decrease litter dry matter (Collett, 2012). Inclusion of 50% coarse corn (1,100-1,300 µm) in broiler diets has been shown to increase litter dry matter and decrease litter nitrogen (Xu, 2014; Wang-Li et al., 2020), possibly due to an increase in nitrogen retention by the bird (Parsons et al., 2006). In contrast, litter dry matter was not different at 35d when broilers were fed diets containing 941 or 2,982 µm corn ($P = 0.73$; Kheravii et al., 2017), however, it is important to note the variation in PS across previous work and the current experiment. Although increased corn PS in broiler diets may reduce harmful bacteria and improve litter dry matter, there are limited data focusing on how ingredient PS affects microbial populations and subsequent litter dry matter in turkeys.

CONCLUSIONS AND APPLICATIONS

1. Pellet quality was not affected when turkey starter 2 diets containing 581 or 964 µm corn were pelleted.
2. Although 581 µm corn is beneficial when fed during the starter 1 phase, 964 µm corn may be able to maintain performance at breeder standards in the starter 2 phase while decreasing milling costs.
3. Corn particle size (581 um, 964 um) did not alter intestinal permeability in 42d old turkey hen poults.

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DISCLOSURES

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

REFERENCES

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2007a. Feed particle size: Implications on the digestion and performance of poultry. World Poult. Sci. 63:439-455. DOI: 10.1017/S0043933907001560.

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

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. Poult. Sci. 87:2320-2328. doi:10.3382/ps.2008-00149.

ASABE. 2022. Method of determining and expressing fineness of feed materials by sieving. American Society of Agricultural and Biological Engineer Standards. 319.4. Am. Soc. Agric. Biol. Eng., St., Joseph, MI.

Baxter MF, R. Merino-Guzman, J.D. Latorre, B.D. Mahaffey, Y. Yang, K.D. Teague, L.E. Graham, A.D. Wolfenden, X. Hernandez-Velasco, L.R. Bielke, and B.M. Hargis. 2017. Optimizing fluorescein isothiocyanate dextran measurement as a biomarker in a 24-h feed restriction model to induce gut permeability in broiler chickens. Fron. Vet. Sci. 4:56.

Bjerrum, L., K. Pedersen, and R. M. Engberg. 2005. The influence of whole wheat feeding on salmonella infection and gut flora composition in broilers. Avian Dis. 49:9-15. https://doi.org/10.1637/7223-061504R.

Charbeneau, R. A. and K. D. Roberson. 2004. Effects of corn and soybean meal particle size on phosphorous use in turkey poults. J. Appl. Poult. Res. 13:302-310.

Chewning, C. G., C. R. Stark, and J. Brake. 2012. Effects of particle size and feed form on broiler performance. J. Appl. Poult. Res. 21:830-837. http://dx.doi.org/10.3382/japr.2012-00553.

Collett, S. R. 2012. Nutrition and wet litter problems in poultry. Anim. Feed Sci. Technol. 173:65-75. https://doi.org/10.1016/j.anifeedsci.2011.12.013.

Engberg, R. M., M. S. Hedemann, S. Steenfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925-938.

Favero, A., A. Maiorka, F. Dahlke, R. F. P. Meurer, R. S. Oliveira, and R. F. Sens. 2009. Influence of feed form and corn particle size on the live performance and digestive tract development of turkeys. J. Appl. Poult. Res. 18: 772-779. doi: 10.3382/japr.2009-00069.

Flores, K. R., A. Fahrenholz, P. R. Ferket, T. J. Biggs, and J. L. Grimes. 2021. Effect of methionine chelated Zn and Mn and corn particle size on Large White male turkey live performance and carcass yields. Poult. Sci. 100:101444. https://doi.org/10.1016/j.psj.2021.101444.

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

Jacobs, C. M., P. L. Utterback, and C. M. Parsons. 2010. Effects of corn particle size on growth performance and nutrient utilization in young chicks. Poult. Sci. 89:539–544. doi: 10.3382/ps.2009-00434.

Kheravii, S. K., R. A. Swick, M. Choct, and S. Wu. 2017. Coarse particle inclusion and lignocellulose-rich fiber addition in feed benefit performance and health of broiler chickens. Poult. Sci. 96:3272-3281. http://dx.doi.org/10.3382/ps/pex123.

Moran, E. T. 1982. Comparative Nutrition of Fowl and Swine: The Gastrointestinal Systems. University of Guelph, Ontario, Canada.

Naderinejad, S., F. Zaefarian, M. R. Abdollahi, A. Hassanabadi, H. Kermanshahi, and V. Ravindran. 2016. Influence of feed form and particle size on performance, nutrient utilisation, and gastrointestinal tract development and morphometry in broiler starters fed maize-based diets. Anim. Feed Sci. Tech. 215:92-104. https://doi.org/10.1016/j.anifeedsci.2016.02.012.

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

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

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

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

Sarsour, A. H. and M. E. Persia. 2022. Effects of sulfur amnio acid supplementation on broiler chickens exposed to acute and chronic cyclic heat stress. Poult. Sci. 101:101952. https://doi.org/10.1016/j.psj.2022.101952.

Sizmaz, O, N. W. Barrett, J. Lewis, H. Yakout, and M. E. Persia. 2022. Effect of various concentration of butyric acid on growth performance, intestinal lesion scores, and body composition of broilers raised on used litter. J. Appl. Poult. Res. 31:100296. https://doi.org/10.1016/j.japr.2022.100296.

Svihus, B., K. H. Kløvstad, V. Perez, O. Zimonja, S. Sahlström, R. B. Schuller, W. K. Jeksrud, and E. Prestløkken. 2004. Physical and nutritional effects of pelleting of broiler chicken diets made from wheat gro9und to different coarsenesses by the use of roller mill and hammer mill. Anim. Feed Sci. Tech. 117:281-293.

Hendrix Genetics. Technical guide for Hybrid Turkeys commercial products. 2021.

Wang-Li, L., Y. Xu, A. P. Shivkumar, M. Williams, and J. Brake. 2020. Effect of dietary coarse corn inclusion in broiler live performance, litter characteristics, and ammonia emission. Poult. Sci. 99:869-878. http://dx.doi.org/10.1016/j.psj.2019.10.010.

Xu, Y. 2014. Interaction of dietary coarse corn with litter conditions on broiler live performance and gastrointestinal tract function. Dissertation. NC State Univ. Raleigh, NC.

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

Xu, Y., C. R. Stark, P. R. Ferket, C. M Williams, S. Auttawong, and J. Brake. 2015b. Effects of dietary coarsely ground corn on broiler live performance, litter characteristics, gastrointestinal tract development, apparent ileal digestibility of energy and nitrogen, and intestinal morphology. Poult. Sci. 94:353-361.

Zaefarian, F., M. R. Abdollahi, and V. Ravindran. 2016. Particle size and feed form in broiler diets: impact on gastrointestinal tract development and gut health. World's Poult. Sci. 72:277-290. https://doi.org/10.1017/S0043933916000222.

Table 3.1. Composition of experimental diets containing 581 μm and 964 μm corn fed to Hybrid turkey hen poults from 0-42 d of age[1].

Ingredient	Starter 1		Starter 2	
	581 μm	964 μm	581 μm	964 μm
	(%)			
Corn	42.37	42.37	43.89	43.89
Soybean meal (48% CP)	39.74	39.74	42.28	42.28
Poultry by-product meal	10.00	10.00	4.09	4.09
Soybean oil	3.37	3.37	4.73	4.73
Dicalcium phosphate	1.52	1.52	1.71	1.71
Limestone	1.30	1.30	1.60	1.60
Vitamin and mineral premix[2]	0.60	0.60	0.50	0.50
DL-Met (99%)	0.43	0.43	0.42	0.42
L-Lys•HCl (78.5%)	0.24	0.24	0.23	0.23
L-Thr (98.5%)	0.12	0.12	0.13	0.13
Sodium chloride	0.27	0.27	0.30	0.30
Sodium bicarbonate	0.04	0.04	0.11	0.11
Phytase[3]	0.01	0.01	0.01	0.01
Calculated nutrient content[4]				
Crude protein	27.50 (28.27)	27.50 (29.21)	26.00 (25.93)	26.00 (26.77)
ME (kcal/kg)	2,998	2,998	3,042	3,042
Calcium	1.40	1.40	1.39	1.39
Available phosphorous	0.73	0.73	0.68	0.68
Crude fat	7.12 (4.83)	7.12 (4.81)	7.80 (5.46)	7.80 (5.71)
Digestible Lys	1.66	1.66	1.59	1.59
Digestible Met	0.81	0.81	0.77	0.77
Digestible SAA	1.15	1.15	1.09	1.09
Digestible Thr	1.05	1.05	1.01	1.01
Digestible Trp	0.29	0.29	0.29	0.29
Digestible Ile	1.05	1.05	1.03	1.03
Sodium	0.17	0.17	0.18	0.18
Chloride	0.25	0.25	0.24	0.24

[1] Diets only varied in corn particle size for starter 1 and starter 2.
[2] Provided per kg of diet: vitamin A, 1,320,000 IU; vitamin D3, 440,000 ICU; vitamin E, 2860 IU; menadione, 176 mg; biotin, 6.6 mg; vitamin B12, 1.9 mg; choline, 71.5 g; niacin, 6.6 mg; pantothenic acid, 1.8 g; selenium, 40 mg; riboflavin, 880 mg; Cu, 4.4 g; Fe, 45 g; I, 135 mg; Mn, 44 g; Zn, 44 g; Co, 4.4 g.
[3] Quantum blue (500 FTU/kg) was formulated to provide 0.10% of calcium and nonphytate phosphorus
[4] Values within parentheses are analyzed values for complete diets.

Table 3.2. Effect of corn particle size on mash particle size and pellet quality parameters of starter 2 feed fed to Hybrid turkey hens from 22-42 d of age.

Particle Size[1]	Mash Particle Size	Pellet Durability Index[2]	Modified Pellet Durability Index[3]	New Holmen Pellet Tester[4]
581 µm	736[b]	77.8	63.1	58.0
964 µm	879[a]	77.0	67.1	65.3
Pooled SEM	8	1.5	2.0	3.6
Starter 2 CPS[5]	≤ 0.01	0.72	0.21	0.20

[1] Represents the corn particle size.
[2] 500 g of sifted pellets were placed into the P:Fost tumbler box (Gamet Manufacturing Inc., Saint Paul, MN) and tumbled for 10 minutes at 53 RPM, after which remaining pellets were weighed.
[3] Similar to pellet durability index but modified by adding five hexagonal nuts to the sample before tumbling.
[4] 100 g of sifted pellets were placed into the chamber, 30 seconds of forced air was applied, and remaining pellets were weighed.
[5] Corn particle size.
[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

Table 3.3. Effects of corn particle size across the starter 1 feeding period on the feed intake (FI), body weight gain (BWG)[1], feed conversion ratio (FCR), and mortality of Hybrid turkey hen poults from 0-21 d of age.

Starter 1[2]	0 to 21d FI	0 to 21d BW	0-21d FCR	0-21d Mortality
	(kg)	(kg/bd)	(kg/kg)	(%)
581 µm	16.4[a]	0.683[a]	1.244[a]	3.9
964 µm	15.9[b]	0.658[b]	1.264[b]	4.2
Pooled SEM	0.18	0.005	0.005	NA
Starter 1 CPS[3]	0.05	≤ 0.01	0.01	0.80

[1] IBW (g/bird): 56.2g (P = 0.48; SEM = 0.14)
[2] Represents the corn particle size; fine: 581 µm; coarse: 964 µm
[3] Corn particle size
[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

Table 3.4. Effect of corn particle size across both the starter phase 1 and 2 feeding periods on the feed intake (FI), body weight (BW), feed conversion ratio (FCR), and mortality of Hybrid turkey hen poults from 0-42 d of age.[1]

Corn Particle Size		0 to 42d FI	42d BW	0 to 42d FCR	0 to 42d Mortality
Starter 1	Starter 2	(kg)	(kg/bd)	(kg/kg)	(%)
581 µm	---	68.8	2.49[a]	1.349	4.67
964 µm	---	67.2	2.44[b]	1.352	4.92
Pooled SEM		0.70	0.014	0.004	N/A
Starter 1	Starter 2				
---	581 µm	68.1	2.47	1.354	5.05
---	964 µm	67.9	2.46	1.347	4.55
Pooled SEM		0.70	0.014	0.004	N/A
Starter 1	Starter 2				
581 µm	581 µm	69.3	2.50	1.350	4.55
581 µm	964 µm	68.4	2.48	1.348	4.80
964 µm	581 µm	67.0	2.44	1.358	5.56
964 µm	964 µm	67.5	2.44	1.347	4.29
Pooled SEM		1.00	0.020	0.006	N/A
Starter 1 CPS[2]		0.12	≤ 0.01	0.58	0.67
Starter 2 CPS		0.87	0.71	0.30	0.93
Starter 1 CPS x Starter 2 CPS		0.46	0.50	0.48	0.83

[1] IBW (g/bird): 56.2g ($P = 0.48$; SEM = 0.14)
[2] Corn particle size.
[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

Table 3.5. Effect of corn particle size across both the starter phase 1 and 2 feeding periods on gut permeability and litter dry matter of Hybrid turkey hen poults at 42 d of age.

Corn Particle Size		Litter Dry Matter	Serum FITC-Dextran
Starter 1	Starter 2	(%)	(ng/ml)
581 µm	---	66.4	179.9
964 µm	---	68.3	177.3
Pooled SEM		N/A	3.33
Starter 1	Starter 2		
---	581 µm	67.0	181.0
---	964 µm	67.6	176.2
Pooled SEM		N/A	3.33
Starter 1	Starter 2		
581 µm	581 µm	67.2	184.6
581 µm	964 µm	65.5	175.1
964 µm	581 µm	66.8	177.3
964 µm	964 µm	69.8	177.3
Pooled SEM		N/A	4.70
Starter 1 CPS[2]		0.13	0.59
Starter 2 CPS		0.62	0.31
Starter 1 CPS x Starter 2 CPS		0.07	0.32

[1] Corn particle size

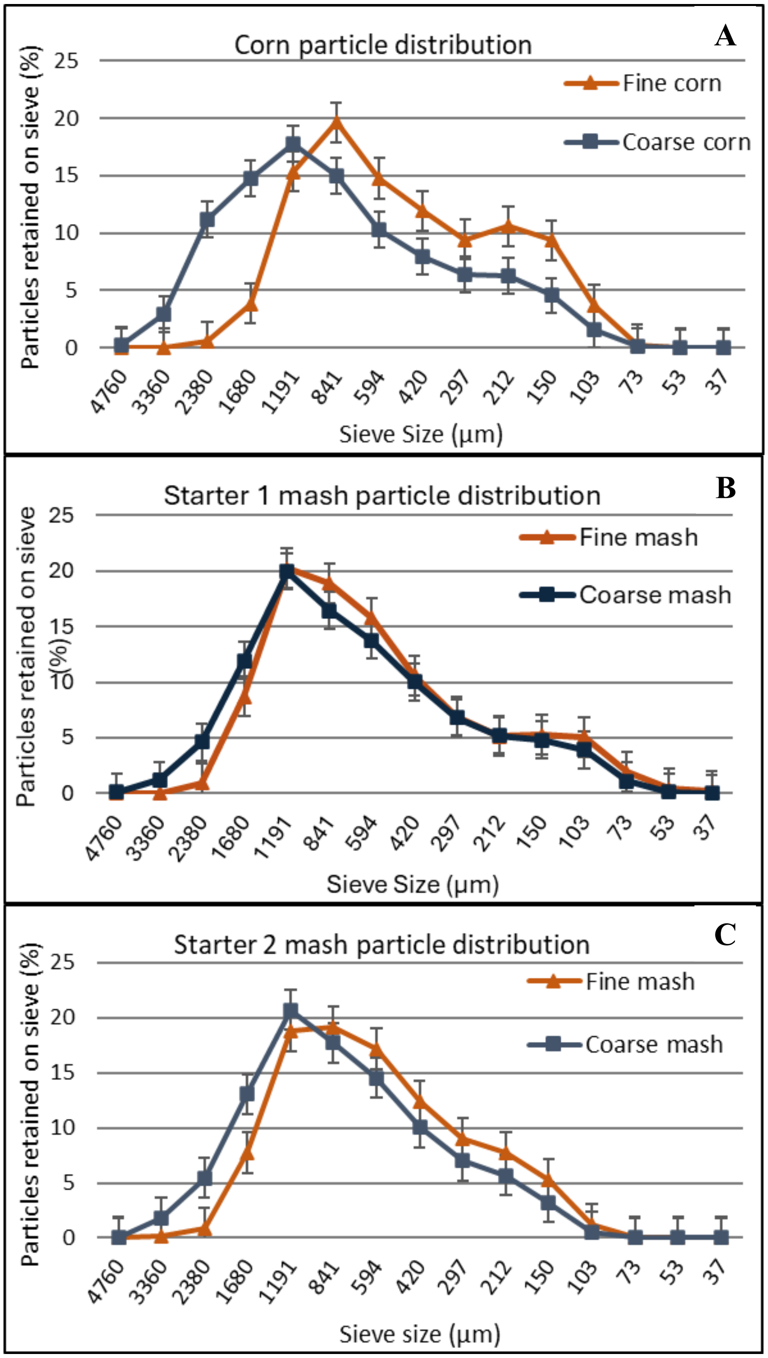

Figure 3.1. Particle distribution of corn (A), starter 1 mash feed (B), and starter 2 mash feed (C). Percentages retained on each sieve for each treatment represent the averages across two replicates.

CHAPTER 4:

Evaluation of aragonite as a calcium source in laying hen diets

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SUMMARY

Experiments were conducted to determine the bioavailability of fine ground aragonite (FGA) using 0-14 d old broilers and to validate the use of FGA as a single particle size Ca ingredient on performance, eggshell quality, and tibia ash (TA) of laying hens from 28-56 wk. In Experiment 1, 450 Ross 708 broilers were fed 0.35, 0.43, 0.51, 0.59, and 0.67% Ca using the addition of 0, 0.08, 0.16, 0.24 and 0.32% Ca from either FGA or limestone (LS). Body weight, feed intake (FI), feed conversion ratio (FCR) and TA were measured at the end of 14 d. The relative bioavailability of FGA to LS was 179% using slope ratio analysis of chick TA. In Experiment 2, 180 Hy-Line W-36 laying hens were fed diets containing 50/50 fine and coarse LS or 100% FGA at 2.46, 3.28, or 4.10% Ca using a 2 x 3 factorial. Interactions occurred for hen day egg production, egg weight, egg mass and feed efficiency. There were limited differences in performance regardless of Ca source or concentration, but the 4.10% LS resulted in the lowest performance. An interaction was noted in that all FGA maximized TA in the laying hens, however, reduced LS feeding resulted in linear reductions in TA indicating Ca insufficiency at lower dietary concentrations. The fine/coarse mixture of LS increased shell quality compared to FGA ($P \leq 0.05$). These data indicate that FGA may be used as a higher available alternative Ca source in laying hens based on performance and tibia ash, but the combined use of coarse and fine Ca should continue to be used to support eggshell quality.

DESCRIPTION OF PROBLEM

Laying hens require high amounts of calcium (**Ca**) in the diet to maintain egg production and eggshell quality. Mined limestone (**LS**) sources have traditionally been used to provide the needed Ca in a laying hen ration, but this non-renewable Ca source has a low bioavailability within the digestive tract and the excess Ca can cause digestibility issues (David et al., 2023). Alternative Ca sources that potentially have a higher bioavailability compared to the traditional mined LS should be evaluated.

Aragonite is a renewable source of Ca that is harvested from the ocean floor and is composed of marine sediments (Sulpis et al., 2022). Current laying hen research on this Ca source is limited, but aragonite has been directly substituted for LS with minimal effects on egg production or egg quality (Muir et al., 1976). One of the limitations of this research is that Ca digestibility was not considered and direct replacement in an adequate diet was tested. This aragonite has increased density and solubility compared to mined LS and might result in increased Ca bioavailability or digestibility (Sulpis et al., 2022). Previous work reported an increase in both shell weight and thickness when diets containing two thirds aragonite and one third oyster shell were fed to Babcock B-300V hens (Brister et al., 1981). This indicates that aragonite may have a higher bioavailability compared to traditional mined LS, but the large particle Ca is necessary to maintain eggshell quality. Increased bioavailability would suggest that a lower concentration of total Ca can be fed while maintaining egg production and skeletal reserves.

The use of both fine and coarse Ca sources allows for the finely ground Ca to be immediately absorbed and slower Ca release and absorption with increased retention time in the gizzard for the larger particle size Ca (Scott et al., 1971). The prolonged retention of the larger Ca particles in the gizzard allows for decreased solubilization of Ca (de Witt et al., 2006; Zhang et al., 2017) making it more available at night when the bird is not consuming feed, but when eggshell formation and calcification are more intensive (Roland and Harms, 1973). This use to both large and small particle LS has been shown to increase eggshell quality, but the question remains if this excess dietary Ca is from the low soluble large particle LS having negative effects within the diet. The first potential cost savings is decreased dilution of diet by the excess Ca. Although Ca sources are typically inexpensive, outside of Ca they contain few nutrients and can increase the need for higher cost ingredients like soybean meal and feed oil (Liu et al., 2023). Moving from two ingredients (small and large particle LS) at a mill to one (finely ground aragonite) ingredient would save both time and space within the mill and simplify ingredient ordering. Finally, high dietary Ca has been associated with decreased nutrient digestibility of other nutrients in broiler diets (Pizzolante et al., 2009; Selle et al., 2009; Walk et al., 2012; David et al., 2023) also resulting in increased dietary costs.

Therefore, the objectives of the current experiments were to determine the bioavailability of fine ground aragonite using 14 d broiler tibia ash. Validation of fine ground aragonite was studied using hen performance, eggshell quality, and tibia ash to determine if a single source of finely ground aragonite can be fed in replacement of a combination of fine and coarse limestone.

MATERIALS AND METHODS

Calcium source analysis and particle size

All diets were analyzed for total dietary Ca and phosphorous. Diet samples were ground, 0.5 g was weighed into a digestion vessel, and 10 mL of nitric acid was added to each tube. Samples were then run through a Mars 6 Microwave Digestor (CEM Corporation, Charlotte, NC) using a OneTouch Method for feed analysis. The digested samples were analyzed using a Spectro ARCOS II MV ICP-AES (Ametek Inc., Berwyn, PA) and Teledyne ASX-560 Autosampler (TRS-RenTelco, Dallas, TX). Calibrations were performed using nitric acid and certified reference standards purchased from Inorganic Ventures (I.V. Labs Inc., Christiansburg, VA).

Particle size (**PS**) of the fine ground aragonite (**FGA**) and fine and coarse LS sources was determined by ASAE method S319.4 (ASABE, 2022). Dry sieving of the Ca sources was completed using a Ro-Tap tester plus agitators (Ro-Tap tester, Model RX-29, WS Tyler company, Mentor, OH). The Ro-Tap tester was fitted with a set of 14 sieves ranging from 4,760 µm to 38 µm and was used to shake 100 g samples for 10 min without any dispersing agents. After 10 min, individual sieves were weighed to determine average PS for each calcium source.

Animal Experiments

All animal procedures used in the experiments were approved by the Virginia Tech Animal Care and Use Committee.

Experiment 1. A total of 450 as hatched Ross 708 broilers were housed in Petersime starter battery cages (Petersime Brood Unit, Gettysburg, OH; 679 cm^2/bird) with 10 replicate cages per treatment and five birds per cage. Birds were provided *ad libitum* access to experimental diets and water throughout the experiment. Temperature and lighting were adjusted according to breeder recommendations (Aviagen, 2018). The experiment was a completely randomized design with the experimental unit being one cage of five birds. Each experimental unit received one of nine dietary treatments varying in Ca source and concentration. A basal diet was formulated and manufactured to contain 0.35% Ca. Generation of the experimental diets was completed with Ca supplementation to the basal diet in increments of 0.08% from LS or FGA to generate treatment diets containing 0.43, 0.51, 0.59, or 0.67% Ca supplied from either LS or FGA (Table 4.1). Body weight, feed intake **(FI)**, and mortality corrected feed conversion ratio

(**FCRm**) were calculated over the 0-14 d period. The right tibia was harvested from all remaining birds on d 14, pooled by cage and used for tibia ash determination. Tibia samples were autoclaved to remove excess flesh from the bone and then ashed in a muffle furnace (Thermo Fisher Scientific, Asheville, NC) at 600˚C for 24 hours.

Experiment 2. A total of 180 Hy-Line W-36 laying hens were housed in cages (465 cm^2/bird) with 10 replicates per treatment and three birds per cage. Hens were weighed at the start of the experiment and egg production was recorded for two weeks prior to ensure there were no differences among treatment groups. The experiment was a randomized complete block design with the experimental unit being one cage of three birds. Hens were control-fed 95 g of feed daily from 28 to 40 wk and 97 g from 40 to 56 wk to align with Hy-Line W-36 breeder recommendations (Hy-Line Management Guide, 2020). The lighting program was maintained at 16L:8D throughout the experiment and hens had *ad libitum* access to water. Experimental diets were manufactured to contain LS or FGA at 2.46, 3.28, or 4.10% Ca, corresponding to 60, 80, or 100% of the Hy-Line W-36 breeder recommended Ca from 28-56 wk of age (Table 4.2). The LS treatments were composed of a 50:50 blend of fine and coarse particles. Each experimental unit received one of six dietary treatments varying in Ca source and concentration: LS60, LS80, LS100, FGA60, FGA80, FGA100.

Laying Hen Performance. Feed intake, hen day egg production **(HDEP)**, egg weight **(EW)**, egg mass **(EM)**, and FE were calculated every two weeks over the 28-week experimental period. Each cage was allocated the respective treatment feed in a bucket at the beginning of the feeding period. Feed intake was determined by weighing the remaining feed in the respective bucket for each cage at the end of two-week periods and subtracting it from the weight of the total feed added. Old feed was discarded and a fresh diet was weighed and added to the respective bucket for each cage. Eggs were collected and weighed daily to measure HDEP, EW, and EM. Feed efficiency was calculated on a cage basis using the following formula: EM/g of FI. Initial BW was measured at 28 weeks on a cage basis and then every four weeks throughout the experiment. Body weight was then reported as an average BW/bird by dividing the total cage weight by the number of birds in the respective cage.

Eggshell Quality. Eggs were collected over a five-day period every two weeks and refrigerated for one week prior to eggshell quality analysis. One egg per cage per day over the first 3 days of collection, or three eggs total, were weighed, cracked and opened and the contents removed, dried for 24 hours and measured for relative shell weight and shell thickness was measured using a digital caliper (Visbrite, Guangdong, China). Shell breaking force was determined using one egg per cage per day over the first 3 days, three eggs total, with a TA.XT plus texture analyzer (Stable Micro Systems, Goldaming, UK). All remaining eggs were subjected to specific gravity to measure the density of the eggshell relative to saltwater concentrations ranging from 1.060 to 1.100 (Wen et al., 2019).

Fat-Free Tibia Ash. All remaining hens were euthanized on wk 56 and the right leg was collected for fat-free tibia ash determination. Legs were pooled by cage, wrapped in aluminum foil, and autoclaved to remove adhering flesh and cartilage from the tibia. The tibias were dried in a 100 ˚C oven (Thermo Fisher Scientific, Model 645, Asheville, NC) for 24 h and then placed into a Soxhlet (Borosil Technologies Ltd., Mumbai, India) where petroleum ether was used to extract the fat for 48 hours. Tibias were removed from the Soxhlet and allowed to air dry under a fume hood to allow any residual petroleum ether to evaporate before being dried in an oven at 100˚C for 24 h. All tibias were weighed to determine the dry fat-free tibia weight before being placed into a muffle furnace (Thermo Fisher Scientific, Asheville, NC) at 600˚C for 24 h. Tibia ash was re-weighed to determine ash weight, and the following formula was used to calculate fat-free tibia ash:

$$Fat - free\ tibia\ ash\ (\%) = \frac{fat - free\ tibia\ ash\ weight\ (g)}{fat - free\ tibia\ dry\ weight\ (g)} \times 100$$

Statistical analysis

All performance data in Experiment 1 were analyzed using ANOVA in JMP Pro 16.0 using Student's t-test to further separate the means ($P \leq 0.10$). Slope ratio analysis was conducted on tibia ash from Experiment 1 to determine the relative bioavailability of FGA to LS using the Fit Curve option in JMP Pro 16.0. Performance and eggshell quality data in Experiment 2 were analyzed as a 2 x 3 factorial with repeated measures using ANOVA in SAS and means were further separated using Fisher's LSD test when $P \leq 0.10$. Linear analysis was conducted on tibia ash using the two Ca sources in JMP Pro 16.0 and significance was accepted at $P \leq 0.10$.

RESULTS AND DISCUSSION

Calcium source analysis and particle size

Dietary Ca concentrations for Experiment 1 were within acceptable ranges although slightly higher than formulated and increased Ca was achieved with supplementation (Table 4.1). The analyzed Ca in Experiment 2 was within acceptable ranges (Table 4.2). The PS of the FGA, fine LS, and coarse LS was 73, 503, and 3,002 µm, respectively. The smaller PS associated with the FGA may affect Ca digestibility and subsequent performance and bone mineralization (David et al., 2023).

Experiment 1

Body weight and FCRm were not different among treatments from 0-14 d ($P > 0.10$; Table 3) although the birds receiving higher concentrations of dietary Ca did result in the highest BW. As expected, increasing the supplemental Ca in the diet increased tibia ash in broilers at 14 d ($P \leq 0.01$; Table 4.3). The concentrations selected for this bioassay were based on previous research that showed a linear increase in tibia ash when broiler chicks were fed up to 0.85 to 0.90% Ca (Driver et al, 2005; Fallah et al., 2018). Slope ratio analysis of tibia ash percent estimated that the relative bioavailability of Ca in FGA was 179% in comparison to LS (Figure 4.1). Calcium sources vary in solubility and subsequent absorption depending on the PS (Zhang et al., 2017), therefore, the increase in bioavailability reported with the FGA may be due to the reduced PS and a higher solubility compared to the fine LS.

Experiment 2

Laying Hen Performance. There was a Ca source by concentration interaction for HDEP, EW, EM, and FE (Table 4.4). Hen day egg production was highest in birds fed LS80 and lowest in birds fed LS100 with no other differences among all other treatment groups ($P = 0.03$), however, there is little evidence in the literature to suggest this is a biologically relevant response. Minimal differences in egg production were noted when varying dietary Ca concentrations (2.5 to 5.5%) were fed to laying hens (Clunies et al., 1992; Keshavarz and Nakajima, 1993; Leeson et al., 1993; Pelicia et al., 2009; Zhang et al., 2017). Both egg mass and FE ($P \leq 0.01$) followed a similar pattern as the hens fed LS100 resulted in the lowest EM and FE compared to other treatments. The reduced performance seen with the LS100 treatment was mostly due to the decreased HDEP as EM and FE are both dependent upon HDEP. Egg weights were increased with LS60, FGA80, and FGA100 feeding ($P \leq 0.01$), but these responses are minor, with an approximate 1 g difference. These lack of differences could indicate that FGA may be used as a single Ca source in a laying hen diet based on performance parameters alone. Reducing the need for multiple Ca sources at a mill is positive as it reduces pressure on bin space and simplifies dietary formulation. As expected with the controlled feeding approach, FI was not different regardless of Ca source or concentration ($P > 0.05$). Egg production and performance seem to be fairly well conserved in modern laying hen genetics, but other response criteria are compromised before egg production suggesting that Ca requirement estimates should be validated with response criteria such as eggshell quality, hen BW or skeletal composition.

Eggshell Quality. There was a main effect of Ca source for all measured eggshell quality parameters (Table 4.5). The 50:50 blend of fine and coarse LS increased relative shell weight, breaking strength, shell thickness, and specific gravity ($P < 0.05$) in comparison to the single FGA treatment. The increased retention time in the gizzard with the larger particles allowed the Ca to be metered out of the gizzard into the bloodstream at a more constant rate (Scott et al., 1971). Laying hens supplemented with coarse Ca particles improved eggshell quality compared to hens that were fed only fine Ca alone (Brister et al., 1981; Guinotte and Nys, 1991; Lichovnikova, 2007) in agreement with the current data. A constant rate of Ca in the bloodstream is particularly important when the hen is not consuming feed during periods of darkness and eggshell calcification is occurring (Roland and Harms, 1973). Eggshell thickness and breaking strength were increased when 69-week-old ISA Brown laying hens were fed diets containing 25 or 50% large LS compared to 0% inclusion of large particles, regardless of the concentration (Swiatkiewicz et al., 2015). This is similar to the current work where sole inclusion of FGA resulted in decreased eggshell quality compared to a blend of small and large particle Ca. Other researchers have demonstrated that Ca level in the diet (3.0 to 4.5%) did not affect eggshell thickness or weight in 58 wk old Hisex Brown laying hens (Pelicia et al., 2009). Previous and current research demonstrates that the PS of Ca sources is an important aspect to consider when formulating laying hen diets. The larger particle Ca results in improved eggshell quality due to slower release from the gizzard in comparison to small particle Ca. This slower release allows for more constant Ca in the blood stream longer into the dark period when FI stops. Limited data exists on aragonite in laying hen diets but one study demonstrated increased shell weight and thickness when Babcock B-300V hens consumed diets supplemented with two thirds aragonite and one third oyster shell (Brister et al., 1981), again suggesting that larger particle Ca can help maximize eggshell quality. This indicates that FGA may be used as a more bioavailable source of Ca but it should be used in combination with a larger particle Ca to support and maintain eggshell quality. A higher bioavailable Ca source could reduce the dietary Ca concentration and potentially limit negative effects of feeding excess Ca.

Body Weight and Fat-free Tibia Ash. There was a Ca source by concentration interaction for BW ($P = 0.08$; Table 4.6) over the duration of the experiment. Birds consuming the FGA100 diet had an increased BW in comparison to the FGA60, FGA80, and LS60 treatments This suggests that the lower concentrations of dietary Ca may have limited body weight gain even though FI was not different among these groups. Linear analysis of 56 wk hen tibia ash resulted in a linear decrease in bone mineralization when hens were fed decreasing concentrations of LS ($P = 0.02$), however, tibia ash was maximized when hens consumed FGA ($P = 0.70$), regardless of dietary concentration (Table 4.6; Figure 4.2). These data support results from Experiment 1 and suggest increased bioavailability of the FGA in comparison to LS. The current data indicate that hens consuming the diet with 60% of the recommended Ca (2.46%) as

LS began to pull Ca from their bone reserves to support egg production while the 2.46% FGA was able to fully support egg production and tibia ash. This suggests that FGA is a higher bioavailable source of Ca compared to LS when considering both the performance and bone reserves of Hy-Line W-36 laying hens. The current data due indicate that there is a higher Ca requirement for tibia ash in comparison to eggshell quality or production in line with previous research (Zhang et al., 2017). The use of a more bioavailable source of Ca allows for an overall reduction in the amount of Ca in the diet which would potentially save costs at the mill. These higher concentrations of lower energy and low amino acid Ca sources act to dilute the diet and require the use of additional oil or soybean meal adding to the overall cost of the diet.

CONCLUSIONS AND APPLICATIONS

1. Relative bioavailability of aragonite was 179% in comparison to limestone when using tibia ash of 14-day old broilers.
2. Fine ground aragonite maintained egg production and maximized tibia ash at 60% (2.46% Ca) of the Hy-Line W-36 breeder recommended calcium when limestone was not able to maintain tibia ash. This validates increased bioavailability of fine ground aragonite in laying hens.
3. Larger particles of calcium are needed to increase or maintain eggshell quality.
4. Finley ground aragonite should not be used as a sole source of Ca but can replace and reduce the amount of fine Ca in the diet of laying hens.

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DISCLOSURES

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

REFERENCES

ASABE. 2022. Method of determining and expressing fineness of feed materials by sieving. American Society of Agricultural and Biological Engineer Standards. 319.4. Am. Soc. Agric. Biol. Eng., St., Joseph, MI.

Aviagen. 2018. Broiler Management Handbook. Accessed July. 2021. https://aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf.

Brister, R. D. Jr., S. S. Linton, and C. R. Creger. 1981. Effects of dietary calcium sources and particle size on laying hen performance. Poult. Sci. 60:2648-2654.

Clunies. M., D. Parks, and S. Leeson. 1992. Calcium and phosphorus metabolism and eggshell formation of hens fed different amounts of calcium. Poult. Sci. 71:482-489.

David, L. S., M. N. Anwar, M. R. Abdollahi, M. R. Bedford, and V. Ravindran. 2023. Calcium nutrition of broilers: Current perspectives and challenges. Animals. 13:1590. https://doi.org/10.3390/ani13101590.

de Witt, F. H., H. J. van der Merwe, J. P. Hayes, and M. D. Fair. 2006. Influence of particle size distribution on *in vivo* and *in vitro* limestone solubility. South Afr. J. Anim. Sci. 36:95-98.

Driver, J. P., G. M. Pesti, R. I. Bakalli, and H. M. Edwards, Jr. 2005. Calcium requirements of the modern broiler chicken as influenced by dietary protein and age. Poult. Sci. 84:1629-1639.

Fallah, H., A. Karimi, G. H. Sadeghi, and N. Behroozi-Khazaei. 2018. The effects of calcium source and concentration on performance, bone mineralization and serum traits in male broiler chickens from 1 to 21 days of age. Anim. Prod. Sci. 59:1090-1097. https://doi.org/10.1071/AN18166.

Guinotte, F. and Y. Nys. 1991. Effects of particle size and origin of calcium sources on eggshell quality and bone mineralization in egg laying hens. Poult. Sci. 70:583-592.

Keshavarz, K. and S. Nakajima. 1993. Re-evaluation of calcium and phosphorus requirements of laying hens for optimum performance and eggshell quality. Poult. Sci. 72:144-153.

Leeson, S., J. D. Summers, and L. Caston. 1993. Response of brown-egg strain layers to dietary calcium or phosphorous. Poult. Sci. 72:1510-1514.

Lichovnikova, M. 2007. The effect of dietary calcium source, concentration and particle size on calcium retention, eggshell quality and overall calcium requirement in laying hens. Brit. Poult. Sci. 48:71-75. DOI: 10.1080/00071660601148203.

Liu, G, F. L. S. Castro, and W. K. Kim. 2023. Applied research note: Exogenous protease supplementation to reduced-energy, reduced-protein, and reduced-amino acid diets for broiler chickens from days 1 to 42. J. Appl. Poult. Res. 32:100362. https://doi.org/10.1016/j.japr.2023.100362.

Lohmann Tierzucht GmbH, 2022. Lohmann LSL-Lite Layer Management Guide, Cage Housing, North American Addition. LohmannTierzucht, Cuxhaven, Germany.

Pelicia, K., E. Garcia, C. Móri, A. B. G. Faitarone, A. P. Silva, A. B. Silva, F. Vercese, and D. A. Berto. 2009. Calcium levels and limestone particle size in the diet of commercial layers at the end of the first production cycle. Braz. J. of Poult. Sci. 11:87-94.

Pizzolante, C. C., E. S. P. B. Saldanha, C. Laganá, S. K. Kakimoto, and C. K. Togashi. 2009. Effects of calcium levels and limestone particle size on the egg quality of semi-heavy layers in their second production cycle. Braz. J. Poult. Sci. 11:79-86.

Roland, D. A. and R. H. Harms. 1973. Calcium metabolism in the laying hen 5. Effect of various sources and sizes of calcium carbonate on shell quality. Poult. Sci. 52:369-372.

Scott, M. L., S. J. Hull, and P. A. Mullenhoff. 1971. The calcium requirements of laying hens and effects of dietary oyster shell upon egg shell quality. Poult. Sci. 50:1055-1063. https://doi.org/10.3382/ps.0501055.

Selle, P. H., A. J. Cowieson, and V. Ravindran. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livestock Sci. 124:126-141. http://dx.doi.org/10.1016/j.livsci.2009.01.006.

Sulpis, O., P. Agrawal, M. Wolthers, G. Munhoven, M. Walker, and J. J. Middelburg. 2022. Aragonite dissolution protects calcite at the seafloor. Nature. 13:1104. https://doi.org/10.1038/s41467-022-28711-z.

Swiatkiewicz, S., A. Arczewska-Wlosek, J. Krawczyk, M. Puchala, and D. Jozefiak. 2015. Effects on performance and eggshell quality of particle size of calcium sources in laying hens' diets with different Ca concentrations. Arch. Anim. Breed. 58:301-307. doi:10.5194/aab-58-301-2015.

Walk, C. L., E. K. Addo-Chidie, M. R. Bedford, and O. Adeola. 2012. Evaluation of a highly soluble calcium source and phytase in the diets of broiler chickens. Poult. Sci. 91:2255-2263. http://dx.doi.org/10.3382/ps.2012-02224.

Wen, J., K.A. Livingston, and M. E. Persia. 2019. Effect of high concentrations of dietary vitamin D3 on pullet and laying hen performance, skeleton health, eggshell quality and yolk vitamin D3 content when fed to W36 laying hens from day of hatch until 68 weeks of age. Poult. Sci. http://dx.doi.org/10.3382/ps/pez386.

Zhang, B., J. V. Caldas, and C. N. Coon. 2017. Effect of dietary calcium intake and limestone solubility on eggshell quality and bone parameters for aged laying hens. Int. J. Poult. Sci. 16:132-138. DOI: 10.3923/ijps.2017.132.138.

Table 4.1. Composition of experimental diets supplemented with various sources and concentrations of calcium fed to Ross 708 broiler chicks from 0 to 14 days of age.

	Limestone					Aragonite			
Ingredient	0.35%	0.43%	0.51%	0.59%	0.67%	0.43%	0.51%	0.59%	0.67%
Corn	59.06	59.06	59.06	59.06	59.06	59.06	59.06	59.06	59.06
DDGS[1]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Soybean meal (48% CP)	32.83	32.83	32.83	32.83	32.83	32.83	32.83	32.83	32.83
Soy oil	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06	1.06
Sodium chloride	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Sodium bicarbonate	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
DL-Met (99%)	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
L-Lys•HCl (78.5%)	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.23
L-Thr (98.5%)	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Limestone	0.00	0.20	0.40	0.59	0.79	---	---	---	---
Aragonite	---	---	---	---	---	0.20	0.40	0.59	0.79
Acid insoluble ash	0.79	0.59	0.39	0.20	0.00	0.59	0.39	0.20	0.00
Monocalcium phosphorous	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67	1.67
Choline chloride 60%	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin/mineral premix[2]	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Calculated nutrients									
Crude protein	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0	22.0
ME (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000	3,000
Calcium	0.35	0.43	0.51	0.59	0.67	0.43	0.51	0.59	0.67
Available phosphorous	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Crude fat	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.04
Digestible SAA	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
Digestible Lys	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Digestible Trp	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Digestible Thr	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Digestible Ile	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Analyzed nutrients									
Crude protein	21.68	21.68	21.68	21.68	21.68	21.68	21.68	21.68	21.68
Crude fat	2.67	2.67	2.67	2.67	2.67	2.67	2.67	2.67	2.67
Calcium	0.45	0.53	0.56	0.71	0.86	0.55	0.61	0.79	0.87
Total phosphorous	0.71	0.76	0.81	0.74	0.80	0.73	0.72	0.83	0.72

[1] Dried distiller's grain with solubles (DDGS).

[2] Provided per kg of diet: vitamin A – 9,900 IU; vitamin D3 – 3,300 ICU; vitamin E – 13 IU; vitamin B12 – 0.01 mg; vitamin B2 – 5.8 mg; menadione – 0.5 mg; choline – 265 mg; selenium – 0.30 mg; iodine – 0.7 mg; thiamine HCl – 0.5 mg; niacinamide – 30 mg; pantothenic acid – 8.2 mg; Fe - 50 mg; Zn – 60 mg; Cu – 7 mg; Mn - 125 mg.

Table 4.2. Composition of experimental diets varying in calcium source, concentration, and particle size fed to Hy-Line W-36 laying hens from 28 to 56 weeks of age.

Ingredient	60% LS[1]	80% LS[1]	100% LS[1]	60% FArg[2]	80% FArg[2]	100% FArg[2]
Corn	51.51	51.51	51.51	51.51	51.51	51.51
DDGS[3]	12.00	12.00	12.00	12.00	12.00	12.00
Poultry biproduct meal	5.00	5.00	5.00	5.00	5.00	5.00
Soybean meal (48% CP)	16.10	16.10	16.10	16.10	16.10	16.10
Soy oil	3.44	3.44	3.44	3.44	3.44	3.44
Sodium chloride	0.14	0.14	0.14	0.14	0.14	0.14
Sodium bicarbonate	0.20	0.20	0.20	0.20	0.20	0.20
DL-Met (99%)	0.16	0.16	0.16	0.16	0.16	0.16
Limestone, large	3.67	4.44	4.83	---	---	---
Limestone, small	3.67	4.44	4.83	---	---	---
Aragonite, small	---	---	---	7.33	8.88	9.65
Acid insoluble ash	2.32	0.77	---	2.32	0.77	---
Monocalcium phosphorus	1.33	1.33	1.33	1.33	1.33	1.33
Choline chloride (60%)	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin/mineral premix[4]	0.38	0.38	0.38	0.38	0.38	0.38
Calculated nutrients						
Crude protein	17.90	17.90	17.90	17.90	17.90	17.90
ME (kcal/kg)	2,880	2,880	2,880	2,880	2,880	2,880
Calcium	2.46	3.28	4.10	2.46	3.28	4.10
Total phosphorous						
Available phosphorous	0.47	0.47	0.47	0.47	0.47	0.47
Crude fat	7.26	7.26	7.26	7.26	7.26	7.26
Digestible SAA	0.69	0.69	0.69	0.69	0.69	0.69
Digestible Lys	0.76	0.76	0.76	0.76	0.76	0.76
Digestible Trp	0.16	0.16	0.16	0.16	0.16	0.16
Digestible Thr	0.60	0.60	0.60	0.60	0.60	0.60
Digestible Ile	0.65	0.65	0.65	0.65	0.65	0.65
Analyzed nutrients						
Crude protein	17.92	17.92	17.92	17.92	17.92	17.92
Crude fat	4.43	4.43	4.43	4.43	4.43	4.43
Calcium	2.63	3.20	3.93	2.98	3.37	4.12
Total phosphorous	0.78	0.78	0.78	0.78	0.78	0.78

[1] 60 (2.46%), 80 (3.28%), or 100% (4.10%) of the breeder recommended calcium provided as a 50/50 blend of fine and coarse limestone.
[2] 60 (2.46%), 80 (3.28%), or 100% (4.10%) of the breeder recommended calcium provided as fine aragonite.
[3] Dried distiller's grains with solubles (DDGS).
[4] Provided per kg of diet: vitamin A – 9,900 IU; vitamin D3 – 3,300 ICU; vitamin E – 13 IU; vitamin B12 – 0.01 mg; vitamin B2 – 5.8 mg; menadione – 0.5 mg; choline – 265 mg; selenium – 0.30 mg; iodine – 0.7 mg; thiamine HCl – 0.5 mg; niacinamide – 30 mg; pantothenic acid – 8.2 mg; Fe - 50 mg; Zn – 60 mg; Cu – 7 mg; Mn - 125 mg.

Table 4.3. Effects of various concentrations of limestone and Aragonite fed to broilers from 0 to 14 d of age on body weight (BW), mortality corrected feed conversion ratio (FCRm), and tibia ash.[1]

Dietary Ca	Ca Source	BW	FCRm[2]	Tibia Ash	
(%)		(g/bd)	(g/g)	(%)	(mg/bd)
0.35	Basal	392	1.282	40.5^{f}	302^{e}
0.43	Limestone	398	1.314	41.1^{def}	331^{cd}
0.51	Limestone	403	1.278	41.8^{cdef}	330^{cd}
0.59	Limestone	403	1.298	42.7^{bc}	353^{abc}
0.67	Limestone	414	1.257	42.5^{cd}	370^{a}
0.43	Aragonite	406	1.262	41.0^{ef}	322de
0.51	Aragonite	396	1.283	42.1^{cde}	335^{bcd}
0.59	Aragonite	414	1.247	44.2^{a}	362^{ab}
0.67	Aragonite	412	1.247	44.0^{ab}	361^{ab}
Pooled SEM		10	0.0204	0.51	10
P-value		0.76	0.27	≤ 0.01	≤ 0.01

[1] Initial body weight = 40.4 g/chick (P = 0.53; SEM = 0.39).
[2] A single mortality occurred on d 5 in treatment 1 replicate 6 and no other mortality were observed. Therefore, mortality data were not analyzed for differences, but this value was used to correct FCR.
[a-f] Means within a column that do not share a common superscript differ ($P \leq 0.10$).

Table 4.4. Effect of various sources and concentrations of calcium on hen day egg production (HDEP), egg weight (EW), egg mass (EM), feed intake (FI), and feed efficiency (FE) of Hy-Line W-36 hens from 28 to 56 weeks of age.[1,2]

Source	Con[4]	HDEP	EW	EM	FI	FE
		(%)	(g)	(g/d)	(g/bird)	(g egg/kg)
Limestone	2.46	91.8^{ab}	61.2^{a}	56.1^{a}	96.4	582^{ab}
Limestone	3.28	92.9^{a}	60.3^{b}	55.9^{ab}	96.1	582^{ab}
Limestone	4.10	90.8^{b}	60.4^{b}	54.7^{b}	96.3	568^{b}
Fine Arg[3]	2.46	92.2^{ab}	60.2^{b}	55.4^{ab}	96.1	577^{ab}
Fine Arg	3.28	91.8^{ab}	61.1^{a}	56.0^{ab}	96.1	583^{a}
Fine Arg	4.10	92.3^{ab}	61.0^{a}	56.2^{a}	96.3	584^{a}
Pooled SEM		0.50	0.21	0.33	0.11	3
Limestone	---	91.8	60.6	55.6	96.3	577
Fine Arg	---	92.1	60.8	55.9	96.2	581
Pooled SEM		0.29	0.12	0.19	0.06	2
---	2.46	92.0	60.7	55.8	96.3	580
---	3.28	92.4	60.7	56.0	96.1	582
---	4.10	91.5	60.7	55.5	96.3	576
Pooled SEM		0.36	0.15	0.24	0.08	2
P-value						
Source		0.52	0.36	0.27	0.20	≤ 0.01
Con		0.27	0.97	0.34	0.21	0.22
Wk		≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Source x Con		0.03	≤ 0.01	≤ 0.01	0.19	≤ 0.01

[1] Data are means of 10 groups of three Hy-Line W-36 laying hens.

[2] Initial body weight: 1,540 g/bird (P = 0.88; SEM = 23) and initial egg production: 91.0 % (P = 0.77; SEM = 2.1).

[3] Fine aragonite.

[4] Concentration of calcium in the diet: 60 (2.46%), 80 (3.28), or 100% (4.10%) of the breeder recommendation.

[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.10$).

Table 4.5. Effect of various sources and concentrations of calcium on shell thickness, relative shell weight, breaking force, and specific gravity of Hy-Line W-36 hens from 28 to 56 weeks of age.[1]

Source	Con[3]	Shell Thickness	Relative Shell Weight	Breaking Force	Specific Gravity
		(mm)	(%)	(g)	
Limestone	---	0.393[a]	9.6[a]	4465[a]	1.082[a]
Fine Arg[2]	---	0.386[b]	9.5[b]	4323[b]	1.081[b]
Pooled SEM		0.0016	0.03	35	0.0002
---	2.46	0.385	9.5	4402	1.081
---	3.28	0.391	9.6	4451	1.082
---	4.10	0.391	9.6	4329	1.082
Pooled SEM		0.0020	0.04	43	0.0002
Limestone	2.46	0.390	9.6	4489	1.082
Limestone	3.28	0.394	9.7	4526	1.082
Limestone	4.10	0.395	9.6	4379	1.082
Fine Arg[2]	2.46	0.381	9.5	4315	1.081
Fine Arg	3.28	0.388	9.6	4375	1.082
Fine Arg	4.10	0.388	9.6	4279	1.082
Pooled SEM		0.0028	0.06	61	0.0003
P-value					
Source		≤ 0.01	0.04	≤ 0.01	≤ 0.01
Con		0.06	0.19	0.13	0.23
Wk		≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Source x Con		0.92	0.82	0.83	0.77

[1] Data are means of 10 groups of three Hy-Line W-36 laying hens from 28 to 56 weeks of age.
[2] Fine aragonite.
[3] Concentration of calcium in the diet: 60 (2.46%), 80 (3.28), or 100% (4.10%) of the breeder recommendation.
[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.10$).

Table 4.6. Effect of various sources and concentrations of calcium on body weight (BW) and tibia ash (percent and mg/tibia) of Hy-Line W-36 hens from 28 to 56 weeks of age.[1]

Source	Con[3]	BW	Tibia Ash	Tibia Ash
		(g/bird)	(%)	(mg/tibia)
Limestone	2.46	1,498[b]	53.8[b]	2643
Limestone	3.28	1,540[ab]	54.6[ab]	2691
Limestone	4.10	1,543[ab]	55.4[a]	2718
Fine Arg[2]	2.46	1,517[b]	55.1[ab]	2759
Fine Arg	3.28	1,527[b]	54.9[ab]	2705
Fine Arg	4.10	1,565[a]	54.9[ab]	2703
Pooled SEM		9	0.37	47
Limestone	---	1,527	54.6	2684
Fine Arg[2]	---	1,537	55.0	2723
Pooled SEM		5	0.21	27
---	2.46	1508	54.5	2701
---	3.28	1534	54.8	2698
---	4.10	1554	55.2	2711
Pooled SEM		6	0.26	33
P-value				
Source		0.16	0.24	0.32
Con		≤ 0.01	0.17	0.96
Week		≤ 0.01	---	---
Source x Con		0.08	0.07	0.35

[1] Data are means of 10 groups of three Hy-Line W-36 laying hens at 56 weeks of age.
[2] Fine aragonite.
[3] Concentration of calcium in the diet: 60 (2.46%), 80 (3.28), or 100% (4.10%) of the breeder recommendation.
[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.10$).

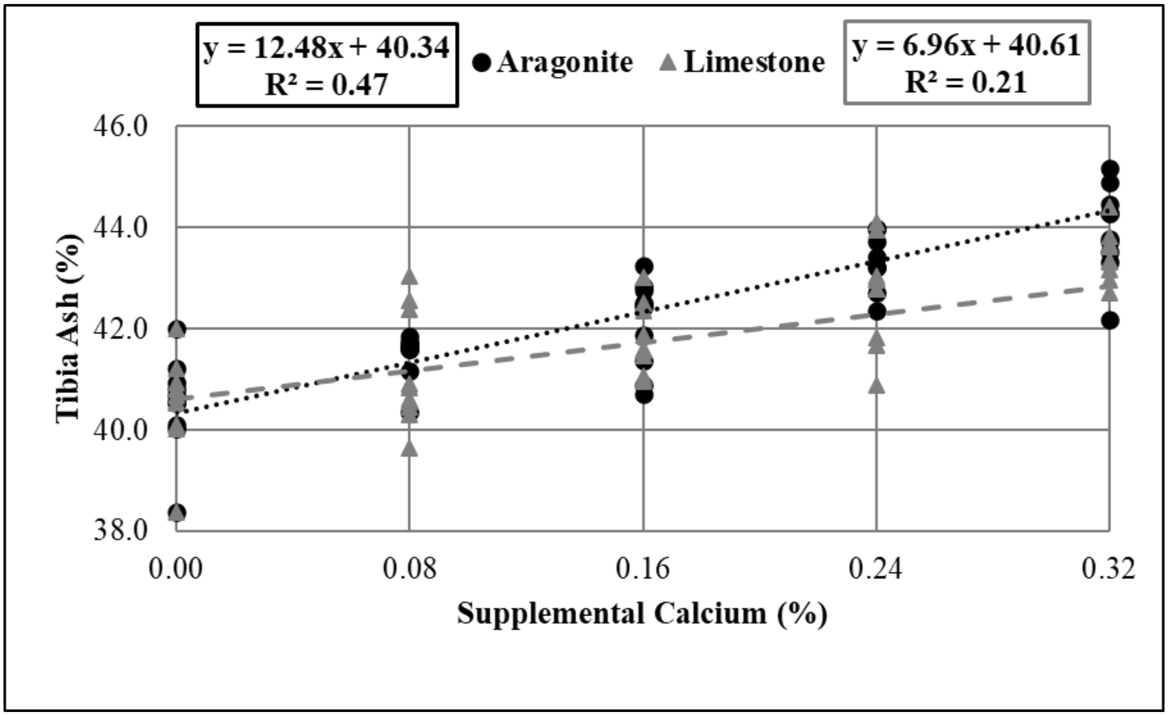

Figure 4.1: Effects of various concentrations of supplemental fine limestone and aragonite (0, 0.08, 0.16, 0.24, or 0.32%) when included in a basal diet containing 0.35% calcium fed to broilers from 0 to 14 days of age on tibia ash percent.

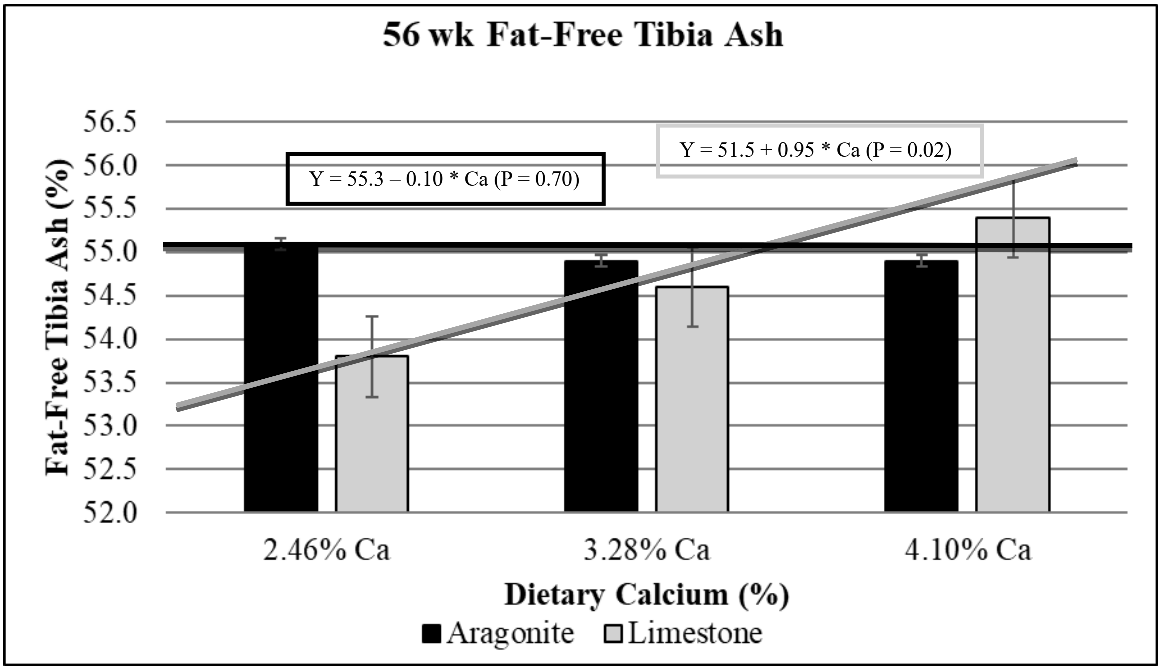

Figure 4.2: Linear analysis of 60 (2.46%), 80 (3.28%), or 100% (4.10%) of Hy-Line Breeder recommended calcium in the form of 50:50 fine and coarse limestone and fine aragonite on fat-free tibia ash percent of 56-week-old Hy-Line W-36 laying hens.

CHAPTER 5:

Evaluation of *Bacillus*-based direct fed microbials on the performance and eggshell quality of first cycle laying hens from 55 to 75 weeks of age

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SUMMARY

An experiment was conducted to determine the effects of various direct fed microbials (DFM) on 55-75 wk old laying hens using performance, egg quality, and abdominal fat pad weights. A total of 144 Hy-Line W-36 laying hens were fed experimental diets with no DFM (NC), Novela® ECL (ECL), Novela® (NOV), or Amnil® (AMN). No differences in hen day egg production were noted, but the inclusion of ECL resulted in the highest egg weight (EW), followed by AMN then NOV and the NC as the lowest ($P \leq 0.01$). The increased EW of the ECL and AMN treated birds increased egg mass ($P \leq 0.01$) and resulted in a 7- and 9-point improvement in FCR, respectively ($P \leq 0.01$). Both the ECL and AMN increased egg production energy compared to the NC and NOV treated hens ($P \leq 0.05$). Body weights increased in birds fed NOV with all other treatments similar to the NC ($P \leq 0.01$). Hens fed NOV resulted in a 26% increase in abdominal fat pad weight compared to the NC and other treatments ($P = 0.01$). Both ECL and AMN improved performance including FCR and egg production energy while NOV increased BW and body composition as stored energy.

DESCRIPTION OF PROBLEM

Direct fed microbials (DFM) are live microorganisms that can confer a benefit to the host by improving gastrointestinal health and enhancing nutrient digestibility in a variety of ways including secretion of enzymes or antimicrobial substances (Elshaghabee et al., 2017). There are a variety of bacterial species that are used as DFM such as *Lactobacillus* spp. and *Bifidobacterium* spp., but the most common are *Bacillus* spp. (Ray et al., 2022). *Bacillus* spp. have an advantage because they are spore forming facultative anaerobes that are resistant to the low pH conditions encountered in the proventriculus and ventriculus allowing the DFM to travel further down the intestinal tract and act on the lower part of the intestines (Elshaghabee et al., 2017; Grant et al., 2018). Ingested spores germinate in the intestinal tract and can work through various modes of action including competitive exclusion (Patterson and Burkholder, 2003; Knap et al., 2011), secretion of digestive enzymes (Mazotto et al., 2011; Elshaghabee et al., 2017), and volatile fatty acid production (Chichlowski et al., 2007).

Hy-Line W-36 laying hens responded with reduced body weight and abdominal fat pad weights before loss of egg production when moderate reductions in dietary energy were introduced over shorter time periods (Murugesan and Persia, 2013; Lyons et al., 2023). Although DFM have been utilized in both research and commercial conditions, little information has been reported regarding the effects of DFM on laying hen energy utilization. Hy-Line Brown laying hens were fed diets containing 2,550 vs 2,650 kcal/kg from 32 to 47 wk resulting in decreased egg weight, egg mass, and worsened FCR ($P \leq 0.05$) in hens fed lower energy diets (Mikulski et al., 2020). However, supplementation with DFM reversed these effects resulting in performance and abdominal fat pad weights similar to the higher energy diet. These results suggest that DFM may alter energy utilization in the laying hen. Direct enzyme production in the small intestine of the hens or VFA production in the lower intestine are direct methods of increased energy utilization with DFM treatment. Additionally, competitive exclusion of pathogens within the gastrointestinal tract of the bird can decrease the energetic needs of the immune system.

Outside of hen energy metabolism, improvements in eggshell quality have been demonstrated in laying hens supplemented with DFM. Specific gravity, eggshell thickness, and relative eggshell weight were increased when Hy-Line Brown laying hens were fed DFM supplemented diets from 23 to 46 wk (Mikulski et al., 2012). Hy-Line Brown laying hens that were fed diets supplemented with *P. acidilactici* resulted in increased shell thickness and shell weight ($P < 0.05$; Mikulski et al., 2020). Lohmann Brown laying hens demonstrated improvements in both eggshell thickness and breaking strength ($P < 0.05$) when fed diets supplemented with *B. subtilis* from 18 to 42 wk (Sobczak and Kozlowski, 2015).

The first objective of the current experiment was to determine how DFM affect the performance and energy partitioning of first cycle Hy-Line W-36 laying hens from 55 to 75 wk of age. The second objective was to determine eggshell quality effects of feeding DFM to laying hens from 55 to 75 weeks of age.

MATERIALS AND METHODS

Experimental design

All animal procedures were approved by the Virginia Tech Animal Care and Use Committee. A total of 144 Hy-Line W-36 laying hens were housed in cages (464 cm^2/bird) with 12 replicate cages per treatment and three birds per cage. Egg production was calculated for the two-week period prior to initiation of the experiment. When hens reached 55 weeks of age, they were weighed and allotted to experimental diets to ensure no statistical difference in initial egg production or BW. The experiment was a randomized complete block design with the experimental unit being one cage of three birds. One cage was left empty between treatments within a block to minimize cross contamination among treatment groups. Additionally, only the top and bottom rows of the three-tier A frame battery cage were used to reduce the possibility of cross-contamination. Experimental diets were randomly assigned to cages within established blocks across the rows. Each experimental unit received one of four dietary treatments: control with no DFM (**NC**), control + Novela ECL® (**ECL**; 250 ppm), control + Novela® (**NOV**; 250 ppm), control + Amnil® (**AMN**; 500 ppm; Table 5.1). The guaranteed microbial count for each

DFM was as follows: ECL - 1.47 x 10^9 CFU/g of *B. subtilis* and *B. pumilus*, NOV – 2.94 x 10^8 CFU/g of *B. subtilis*, and AMN – 1.48 x 10^8 CFU/g of *B. subtilis* and *B. licheniformis* (United Animal Health, Sheridan, IN). A basal diet was formulated according to the Hy-Line breeder recommendations and treatment diets were generated by adding the DFM on top of the basal diet. Hens were control-fed 101 g of feed daily from 55 to 75 weeks to align with breeder guide recommendations (Hy-Line Management Guide, 2020) and water was provided ad libitum throughout the experiment. The lighting program was maintained at 16L:8D for the duration of the experiment.

Laying hen performance and production

Body weight of the hens was measured at 55 wk and then every four wk throughout the experimental period. Hen-day egg production (**HDEP**), egg weight (**EW**), egg mass (**EM**), feed intake (**FI**), and FCR were calculated every two wk over the 20-week experimental period. Eggs were collected and weighed daily to determine HDEP and EW. Egg mass was calculated by multiplying HDEP × EW. Feed intake was determined by weighing the remaining feed in the respective bucket for each cage at the end of the two-week period and subtracting it from the weight of all feed added. Old feed was removed, and the new diet was weighed and added to the respective bucket for each cage. Feed conversion ratio was calculated based on total cage production or consumption and is expressed on a g/g basis (EM/g of feed consumed) and on a per dozen eggs basis (kg of feed consumed x 12/total number of eggs produced).

External egg quality

Eggs were collected over a five-day period every two wk throughout the experiment for eggshell quality analysis. All collected eggs were refrigerated for one wk prior to analysis. One egg per cage per day over the first 3 days of collection was used to determine relative shell weight (**RSW**) after drying and shell thickness (**ST**) was measured using a digital caliper (Visbrite, Guangdong, China). One egg per cage per day over the first three days was used to determine eggshell breaking force (**BF**) using an Instron Universal Testing Machine Model 1011 (Instron Corp, Canton, MA). All remaining eggs were used for egg specific gravity (**SG**) determination to measure the density of the eggshell relative to saltwater concentrations ranging from 1.060 to 1.100 (Wen et al., 2019).

Abdominal fat pad weights and egg production energy

All birds were individually weighed and euthanized on wk 75 for abdominal fat pad weight determination. Abdominal fat pad weights were expressed on a total g basis and as a percentage of the final BW. Abdominal fat pads were reported on a cage basis. Egg production energy (kcal/day) was calculated to determine the amount of energy that is excreted via egg production per day using the following formula:

Egg Prod (kcal/day) = $\left(EMg/d \times \left(\frac{EWg-SWg}{EWg}\right) \times GE\ kcal\right) \times \left(\frac{DM\%}{100}\right)$

EM: egg mass (g/d)

EW: egg weight (g)

SW: shell weight (g)

GE: gross energy of egg solids (kcal)

DM: dry matter of the egg solids

Statistical analysis

Performance and egg quality data were analyzed using ANOVA in JMP Pro 16.0. Repeated measures were applied to use replication over the two-week feeding period for performance measurements and every four weeks for external eggshell quality data ($P \leq 0.05$). Means were further separated using student's t-test when $P \leq 0.05$. Abdominal fat pad data were analyzed using ANOVA in JMP Pro 16.0 and means were further separated using student's t-test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Laying hen performance and production

Initial egg production and body weights were not different among treatments and averaged 85.3% ($P = 0.98$) and 1,456 g/bird ($P = 0.74$), respectively. Hen day egg production from 55 to 75 wk was not different among DFM treated hens and ranged from 85.3 to 87.1% ($P = 0.35$; Table 5.2). In previous experiments with white leghorns DFM treatments were able to increase egg production ($P \leq 0.05$), but in those experiments ad libitum FI was employed, and the increased egg production was seen with increased FI ($P \leq 0.05$; Nahashon et al., 1994; DeLeon et al., 2023) above what is common in commercial production. In the current experiment, ECL supplementation increased EW compared to NOV and the control hens whereas AMN was intermediate ($P \leq 0.01$). Egg mass was highest in birds supplemented with ECL and AMN compared to both the control and NOV fed birds ($P \leq 0.01$). This response has been consistent with previous reports that demonstrated increased EW and EM when laying hens were fed diets containing DFM even when provided ad libitum feed (Nahashon et al., 1994; DeLeon et al., 2023). As expected with the controlled feeding, FI was not different among treatments ($P = 0.26$) and ranged from 100.8 to 101.5 g per bird/d consistent with common commercial FI (Hy-Line Management Guide, 2020). As expected, hens supplemented with ECL and AMN showed improved FCR on a g/g basis with the increased EW and EM ($P \leq 0.01$), however the lack of ability to increase FI may have limited the total number of eggs produced and precluded differences in FCR when reported on a kg/dozen eggs produced basis ($P = 0.26$). Overall, the ECL treated birds had a 7-point improvement in FCR whereas AMN demonstrated a 9-point improvement, indicating that when birds were limited to similar feed consumption patterns the DFM supplementation was able increase the productive use of the feed towards additional EM production. When overall egg production performance is compared, these differences in FCR are equivalent to the increased egg production noted previously as the increased FI in these cases either resulted in similar FCR or even worsened the FCR noted with DFM feeding (Nahashon et al., 1994; DeLeon et al., 2023). It is interesting to note that when FI is controlled and represented at a more industry relevant consumption rate, FCR tends to be a more sensitive response criteria than egg production, but when hens are allowed to over consume feed, egg production seems to be more sensitive at the expense of FCR.

Egg production energy, hen body weight, and hen abdominal fat pad weight

Egg production energy accounts for energy leaving the hen's body as egg production per day. Therefore, egg production energy is a method to account for energy being partitioned towards a productive use rather than storage in the egg for later use. Egg production energy increased in the ECL and AMN fed birds compared to the control whereas NOV was intermediate ($P = 0.05$; Table 5.3). This is a first attempt to better quantify energy output to an egg to better track wholistic energy utilization and destination within the laying hen model. The increased energy in the egg is related to the increased EM, especially any increased egg yolk that is rich in lipid material (Wang et al., 2023).

Hen body weights and abdominal fat pad weights were increased over time with NOV supplemented birds ($P \leq 0.01$), representing storage as an alternative use for excess metabolic energy (Table 5.3). When fat pad weights were put on a relative basis to hen BW, the NOV fed birds had 23% more abdominal fat pad compared to the control hens and 26 to 28% more abdominal fat compared to the ECL and AMN supplemented hens, respectively. When both performance and body storage are examined together, it appears that all three DFM supplements evaluated are altering energy metabolism. When evaluating energy metabolism, the balance of the system equates energy intake to maintenance energy requirements, productive energy output and energy storage (Murugesan et al., 2013) so overall discussion should focus on all three mechanisms and not isolate one out of context. Limited research has been conducted that has considered energy metabolism responses to DFM treatment of laying hens. One report in this area using Hy-Line Brown laying hens fed on a lower energy plain (2,550 to 2,650 kcal/kg) than the current experiment from 32 to 47 wk of age (Mikulski et al., 2020). Despite the differences in bird size, FI and dietary energy, DFM supplementation was able to increase egg production, egg weight, egg mass, and decrease FCR while maintaining both hen body weight and abdominal fat pad weight when similar amounts of dietary energy were consumed. This response is consistent with the responses demonstrated by ECL and AMN in the current experiment showing that DFM can alter energy metabolism in the laying hen. Potential modes of action for altered energy metabolism could be increased intestinal VFA production from nondigested feed material (Callaway et al., 2017) and increased digestive enzyme production (Mazotto et al., 2011; Elshaghabee et al., 2017). These modes of action would increase the amount of energy absorbed by the hen and can be captured via AMEn quantification (Neijat et al., 2019). Alternative modes of DFM action include either an increased intestinal health and antimicrobial action such as competitive exclusion to reduce pathogenic challenge (Forte et al., 2016). This increased intestinal health and/or reduced pathogenic challenge can reduce the hen's daily maintenance requirements allowing more energy for productive or storage purposes. These data indicate that all DFM were altering energy utilization within the laying hen. Birds consuming ECL and AMN were diverting energy down a productive pathway into the egg and NOV was increasing the stored energy as demonstrated by the increased abdominal fat pad weights.

External egg quality

Evaluating the effects of a DFM on eggshell quality are difficult when those DFM result in an increase in egg weight as egg weights have been negatively correlated to eggshell breaking measurements (Hamilton, 1978). The flat ($P > 0.05$) or reduced ($P \leq 0.05$) results attributed to ECL for RSW, ST, SG and BF were unsurprising. Limited conclusions can be derived due to the 2g difference in EW between the ECL supplemented hens and those fed the control diet. To a more limited extent, the same response was noted with AMN supplementation as the 1g increase in EW could have been reflected in the lack of response ($P > 0.05$) or reduced ($P \leq 0.05$) response to RSW, ST and SG. Unlike ECL, AMN was able to slightly increase ($P \leq 0.05$) shell BF. The lack of a response to AMN on the mineral side of eggshell quality (RSW, ST and SG) with an improvement in shell BF, might suggest that AMN is altering the protein fraction of the eggshell not the mineral component. Hen supplementation with NOV resulted in flat responses to mineral based eggshell quality measurements including RSW, ST and SG, but once again increased shell BF ($P \leq 0.05$). Once again, this positive response in shell BF was noted without differences in EW between the NOV and control eggs. The literature supports increased eggshell quality with DFM supplementation including increased RSW, SG, ST and egg breaking strength in laying hens (Mikulski et al., 2012; Mikulski et al., 2020; Sobczak and Kozlowski, 2015).

CONCLUSIONS AND APPLICATIONS

1. All DFM altered energy status of the laying hen which reduced the amount of energy needed for maintenance, allowing the bird to partition energy towards egg production energy and storage.
2. Supplementation of Novela ECL® and Amnil® improved the productive performance of laying hens while Novela® increased the stored energy of the laying hens.
3. Novela® and Amnil® increased egg breaking force, suggesting improved eggshell quality when egg weight was not drastically increased.

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DISCLOSURES

We wish to confirm that there are no known conflicts of interest associated with this publication. Brian Dirks and Hector Leyva-Jimenez are employed by United Animal Health but were not involved in data collection or analysis.

REFERENCES

Callaway, T. R., T. S. Edrington, J. A. Byrd, D. J. Nisbet, and S. C. Ricke. 2017. Use of direct-fed microbials in layer hen production – Performance response and *Salmonella* control. 301-322. https://doi.org/10.1016/B978-0-12-802582-6.00015-X.

Chichlowski, M. J. Croom, B. W. McBride, G. B. Havenstein, and M. D. Koci. 2007. Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: A brief review of current knowledge. Int. J. Poult. Sci. 10:694-704.

DeLeon, C., M. L. Wiersema, M. Araba, T. Lohrmann, and D. A. Koltes. 2023. Effects of a commercial *Bacillus subtilis* supplementation on late lay hen performance, egg quality, and microbial communities. J. Appl. Poult. Res. 32:100361. https://doi.org/10.1016/j.japr.2023.100361.

Elshaghabee, F. M. F., N. Rokana, R. D. Gulhane, C. Sharma, and H. Panwar. 2017. Bacillus as potential probiotics: Status, concerns, and future perspectives. Front. Microbiol. 8:1490. doi: 10.3389/fmicb.2017.01490.

Forte, C., G. Acuti, E. Manuali, P. Casagrande Proietti, S. Pavone, M. Trabalza-Marinucci, L. Moscati, A. Onofri. C. Lorenzetti, and M. P. Franciosini. 2016. Effects of two different probiotics on microflora, morphology, and morphometry of gut in organic laying hens. Poult. Sci. 95:2528-2535. http://dx.doi.org/10.3382/ps/pew164.

Grant, A., C. G. Gay, and H. S. Lillehoj. 2018. *Bacillus* spp. As direct-fed microbial antibiotic alternatives to enhance growth, immunity, and gut health in poultry. Avian Pathol. 47:339-351. DOI: 10.1080/03079457.2018.1464117.

Hamilton, R.M. G. 1978. Observations on the Changes in Physical Characteristics that Influence Egg Shell Quality in Ten Strains of White Leghorns. Poult. Sci. 57:1192-1197. https://doi.org/10.3382/ps.0571192.

Knap, I., A. B. Kehlet, M. Bennedsen, G. F. Mathis, C. L. Hofacre, B. S. Lumpkins, M. M. Jensen, M. Raun, and A. Lay. 2011. *Bacillus subtilis* (DSM17299) significantly reduces *Salmonella* in broilers. Poult. Sci. 90:1690-1694. doi: 10.3382/ps.2010-01056.

Lyons, A. M., C. A. Granghelli, and M. E. Persia. 2023. Estimating energy utilization in laying hens: what are the best response criteria?. J. Appl. Poult. Res. 32:100357. https://doi.org/10.1016/j.japr.2023.100357.

Mazotto, A. M., R. R. Rodrigues-Coelho, S. M. Lage-Cedrola, M. F. de Lima, S. Couri, E. Paraguai de Souza, and A. B. Vermelho. 2011. Keratinase production by three Bacillus spp. using feather meal and whole feather as substrate in a submerged fermentation. Enz. Res. 4:523780. http://dx.doi.org/10.4061/2011/523780.

Mikulski, D., J. Jankowski, J. Naczmanski, M. Mikulska, and V. Demey. 2012. Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol, and fatty acid profile in laying hens. Poult. Sci. 91:2691-2700. http://dx.doi.org/ 10.3382/ps.2012-02370.

Mikulski, D., J. Jankowski, M. Mikulska, and V. Demey. 2020. Effects of dietary probiotic (*Pediococcus acidilactici*) supplementation on productive performance, egg quality, and body composition in laying hens fed diets varying in energy density. Poult. Sci. 99:2275-2285. https://doi.org/10.1016/j.psj.2019.11.046.

Murugesan, G. R. and M. E. Persia. 2013. Validation of the effects of small differences in dietary metabolizable energy and feed restriction in first-cycle laying hens. Poult. Sci. 92:1238-1243. http://dx.doi.org/10.3382/ps.2012-02719.

Nahashon, S. N., H. S. Nakaue, and L. W. Mirosh. 1994. Production variables and nutrient retention in single comb white leghorn laying hen pullets fed diets supplemented with direct-fed microbials. Poult. Sci. 73:1699-1711.

Neijat, M., R. B. Shirley, J. Barton, P. Thiery, A. Welsher, and E. Kiarie. 2019. Effect of dietary supplementation of Bacillus subtilis DSM29784 on hen performance, egg quality indices, and apparent retention of dietary components in laying hens form 19 to 48 weeks of age. Poult. Sci. 98:5622-5635. http://dx.doi.org/10.3382/ps/pez324.

Patterson, J. A. and K. M. Burkholder. 2003. Application of prebiotics and probiotics in poultry production. Poult. Sci. 82:627-631.

Ray, B. C., S. D. Chowdhury, S. C. Das, B. Dey, A. Khatun, B. C. Roy, and M. A. Siddik. 2022. Comparative effects of feeding single- and multi- strain probiotics to commercial layers on the productive performance and egg quality indices. J. Appl. Poult. Res. 31:100257. https://doi.org/10.1016/j.japr.2022.100257.

Sobczak, A. and K. Kozlowski. 2015. The effect of a probiotic preparation containing Bacillus subtilis ATCC PTA-6737 on egg production and physiological parameters of laying hens. Ann. Anim. Sci. 15:711-723. DOI: 10.1515/aoas-2015-0040.

Wang Y, W. Luo, B. Wang, D. Wu, J. Wang, and F. Geng. 2023. Research Note: Changes in chicken egg yolk metabolome during its formation. Poult Sci. 102:103154. Doi: 10.1016/j.psj.2023.103154.

Wen, J., K.A. Livingston, and M. E. Persia. 2019. Effect of high concentrations of dietary vitamin D3 on pullet and laying hen performance, skeleton health, eggshell quality and yolk vitamin D3 content when fed to W36 laying hens from day of hatch until 68 weeks of age. Poult. Sci. http://dx.doi.org/10.3382/ps/pez386.

Table 5.1. Composition of the basal diet supplemented with various direct fed microbials fed to Hy-Line W-36 laying hens from 55 to 75 weeks of age.[1]

Ingredient	Basal diet (%)
Corn	54.62
Dried Distillers Grains with Solubles	15.00
Soybean meal, (48% CP)	11.88
Poultry byproduct meal	5.00
Soy oil	1.74
Sodium Chloride	0.17
Sodium Bicarbonate	0.20
DL-Met (99%)	0.15
L-Lys•HCl (78.5%)	0.09
Limestone, small	3.96
Limestone, large	5.93
Monocalcium phosphate	0.79
Choline chloride (60%)	0.10
Vitamin/mineral premix[2]	0.38
Calculated nutrients	
Crude protein	17.09
ME (kcal/kg)	2,800
Calcium	4.10
Total phosphorous	0.58
Non Phytate Phosphorus	0.37
Crude Fat	5.98
Digestible Met + Cys	0.67
Digestible Lys	0.74
Digestible Trp	0.15
Digestible Thr	0.57
Digestible Arg	0.89
Digestible Ile	0.61
Digestible Leu	1.34
Digestible Val	0.73
Analyzed nutrients	
Crude protein	16.94
Crude fat	3.66
Crude fiber	2.68

[1] Experimental diets were generated by adding 0.025% Novela ECL®, 0.025% Novela® and 0.050% Amnil® on top of the basal diet.
[2] Provided per kg of diet: vitamin A – 9,900 IU; vitamin D3 – 3,300 ICU; vitamin E – 13 IU; vitamin B12 – 0.01 mg; vitamin B2 – 5.8 mg; menadione – 0.5 mg; choline – 265 mg; selenium – 0.30 mg; iodine – 0.7 mg; thiamine HCl – 0.5 mg; niacinamide – 30 mg; pantothenic acid – 8.2 mg; Fe - 50 mg; Zn – 60 mg; Cu – 7 mg; Mn - 125 mg.

Table 5.2. Effects of Novela ECL® (ECL), Novela® (NOV) and Amnil® (AMN) on hen day egg production (HDEP), egg weight (EW), egg mass (EM), average daily feed intake (ADFI), and feed conversion ratio (FCR) measured every 2 weeks and body weight (BW) measured every 4 weeks in Hy-Line W-36 laying hens from 55 to 75 weeks of age.[1,2]

	HDEP	EW	EM	ADFI	FCR		BW
	(%)	(g)	(g/bird)	(g/bird)	(g/g)	(kg/doz eggs)	(kg/bird)
Control	85.3	61.6^{c}	52.5^{b}	100.8	1.950^{a}	1.432	1.553^{b}
ECL	86.0	63.7^{a}	54.7^{a}	101.5	1.862^{b}	1.411	1.547^{b}
NOV	85.9	61.4^{c}	52.7^{b}	100.8	1.938^{a}	1.418	1.589^{a}
AMN	87.1	62.8^{b}	54.7^{a}	101.0	1.860^{b}	1.390	1.548^{b}
SEM	0.72	0.18	0.44	0.28	0.0199	0.0150	0.008
Trt	0.35	≤ 0.01	≤ 0.01	0.26	≤ 0.01	0.26	≤ 0.01
Time	0.02	≤ 0.01	≤ 0.01	0.95	≤ 0.01	≤ 0.01	≤ 0.01
Trt x Time	0.99	0.99	0.99	0.97	0.99	0.99	0.99

[1] Mean egg production the two weeks prior to the initiation of the experiment: 85.3% (P = 0.98; SEM = 2.1); initial body weight: 1.456 kg (P = 0.74; SEM = 0.024).
[2] Over the duration of the experiment, there were two mortality total, one in each of NOV and AMN.
[a-c] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

Table 5.3. Effects of Novela ECL® (ECL), Novela® (NOV) and Amnil® (AMN) on abdominal fat pad weight, and egg production energy of Hy-Line W-36 laying hens from 55-75 weeks of age.[1]

	Abdominal Fat Pad Weight		Egg Production Energy
	(total g)	(relative to BW)	(kcal/d)
Control	47.5[b]	3.0[b]	82.1[b]
ECL	46.0[b]	2.9[b]	86.9[a]
NOV	63.9[a]	3.9[a]	82.9[ab]
AME	44.7[b]	2.8[b]	87.8[a]
Pooled SEM	4.5	0.2	1.65
Trt	0.02	0.01	0.05

[1] Over the duration of the experiment, there were two mortality total, one each in the NOV and AMN treatments.

[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

Table 5.4. Effects of Novela ECL® (ECL), Novela® (NOV) and Amnil® (AMN) on relative shell weight, shell thickness, eggshell breaking force and specific gravity of egg produced by Hy-Line W-36 laying hens from 55 to 75 weeks of age.[1]

	Relative shell weight (%)	Shell thickness (mm)	Breaking force (kg f)	Specific gravity
Control	9.7[a]	0.359	3.54[c]	1.080[a]
ECL	9.6[b]	0.363	3.55[c]	1.079[b]
NOV	9.8[a]	0.355	3.89[a]	1.081[a]
AMN	9.5[b]	0.352	3.70[b]	1.079[b]
Pooled SEM	NA	0.005	0.05	0.0003
Trt	≤ 0.01	0.35	≤ 0.01	≤ 0.01
Time	≤ 0.01	0.01	≤ 0.01	≤ 0.01
Trt x Time	0.91	0.48	0.98	0.99

[1] External egg quality was analyzed using repeated measures over 2-week periods.
[a-c] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

CHAPTER 6:

The effects of corn particle size on 21 to 42 d hen poult performance and nutrient digestibility when challenged with a 15 x coccidial vaccine on 28 days of age

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SUMMARY

An experiment was conducted to determine the effects of corn particle size (CPS) on turkey hen poults from D21 to 42 that were exposed to a coccidial challenge on D28. Response criteria included growth performance, intestinal permeability (IP), nutrient digestibility, and litter moisture. A total of 1,600 Hybrid turkey hen poults were sourced from a local hatchery and raised until 21 d of age when 1,472 poults were allotted to floor pens from D21 to 42 to ensure similar BW among treatments. The experiment was a 2 x 2 factorial (pelleted and crumbled starter 2 diets made with 597 vs 1,049 µm corn and a 15x dose of coccidiosis vaccine administered at 28 d vs no vaccine administration) with 16 replicate pens of 23 poults per treatment. Pellet quality and mill operations were measured during feed manufacture. Body weight gain (BWG), feed intake, and FCR were measured from D21 to 42. Nutrient digestibility and IP were measured on D35. Litter moisture was measured on D21, 28, 35, and 42. Data were analyzed using JMP Pro 18.0 ($P \leq 0.05$) with means separated using Student's t-test. Pellet quality was not influenced by CPS ($P > 0.05$) although the coarse corn had a higher pellet mill load ($P < 0.01$). There were no interactions between CPS and coccidial challenge from 21 to 42 d. Hen poults fed coarse corn resulted in reduced FI and BWG ($P \leq 0.01$) with no difference in FCR ($P = 0.60$). Hen poults challenged with coccidiosis on D 28 had reduced performance for all parameters ($P \leq 0.01$). Day 35 litter moisture was increased with the challenge ($P = 0.02$) and the challenged birds had reduced crude fat digestibility ($P = 0.02$). Unlike previous data, larger particle corn (1,049 µm) reduced overall poult performance and suggests caution should be used to move to larger particle size corn with starter poults. Additionally, a single dose of 15x coccidial vaccine was shown to reduce poultry performance and alter the digestion of nutrients.

DESCRIPTION OF PROBLEM

Coarse grinding of feed ingredients has been reported to reduce the costs associated with feed manufacture and electricity use (Flores et al., 2021; Lyons et al., 2022). There have been multiple reports conducted to determine how ingredient PS affects broiler performance, health, and nutrient digestibility but results have not been consistent depending on the PS used (Nir et al., 1994; Amerah et al., 2007; Amerah et al., 2008; Rubio et al., 2020). In theory, the larger corn PS is retained in the broiler gizzard for a longer time resulting in stimulation of gizzard function including increased exposure to a low pH environment and additional grinding (Pacheco et al., 2013). This larger particle size approach has also been shown to promote energy and nutrient utilization in broiler chickens (Gabriel et al., 2003; Naderinejad et al., 2016). A lower pH within the gizzard can potentially increase broiler health by preventing harmful bacteria from entering the lower portion of the intestinal tract at higher concentrations (Engberg et al., 2004; Bjerrum et al., 2005). Although this system has been well researched from a broiler standpoint, there has been limited work on PS in turkey diets (Charbeneau and Roberson, 2004; Favero et al., 2009; Lyons et al., 2024). Previous work using 28d turkey hen poults reported a linear increase in nutrient retention when poults were fed corn ranging in PS from 606 to 1,094 µm (Charbeneau and Roberson, 2004). A larger corn PS (964 vs 581 µm) reduced bird performance over the starter 1 phase but was able to maintain it over the starter 2 phase, suggesting that a larger corn PS may be used to reduce feed grinding costs and support turkey performance (Lyons et al., 2024). These reports in broiler chickens potentially suggest that feeding a larger corn PS will stimulate the gizzard in turkey poults after the starter 1 phase and can have a beneficial effect on performance, intestinal health and nutrient digestibility in turkeys.

Coccidiosis presents major economic losses of upwards of $14.4 billion yearly to the poultry industry due to reduced bird performance and increased mortality (Adhikari et al., 2020; Blake et al., 2020). In 2024, coccidiosis ranked 16th out of 38 health issues surveyed in the US turkey industry and poses a threat to producers (Clark and Chiaia, 2024). Seven major species of *Eimeria* have been reported in turkeys although four are highly pathogenic and pose an economic threat to the turkey industry; *E. adenoeides*, *E. meleagrimitis*, *E. gallopavonis*, and *E. dispersa* (Milbradt et al., 2014). Previous strategies to mitigate coccidiosis in turkeys include vaccinations and anticoccidial drugs, however with the transition to no antibiotics in the poultry industry, alternative methods to control or reduce coccidiosis should be investigated.

Therefore, the first objective of the current experiment was to determine the effects of corn particle size on feed mill efficiency and pellet quality. The second objective was to determine the effects of corn particle size on 21 to 42 d old turkey hen poults challenged with a 15x coccidial vaccine challenge using performance, intestinal permeability, fecal oocysts shedding, nutrient digestibility, and litter dry matter.

MATERIALS AND METHODS

Corn and diet manufacture

The fine and coarse corn particle sizes (**CPS**) were manufactured from a single batch of whole corn to ensure similar nutritional quality. All corn was ground at West Virginia University using a hammermill fitted with a 6.35 mm screen or a 4.76 mm screen resulting in the coarse (**CGC**) and fine corn (**FGC**), respectively. The fine corn was ground twice with the 4.76 mm screen.

Diets only varied in the CPS and were otherwise nutritionally similar. Diet formulations were based on the Hybrid Turkey breeder recommendations and digestible amino acids were adjusted according to industry practices at the time of the experiment. Titanium dioxide was added into the experimental diets at 0.3% as an indigestible marker. All feed was manufactured at the West Virginia University pilot feed mill. Feed manufacture was replicated three times and randomized within each replicate to reduce treatment effects based on run order. A California Pellet Mill conditioner (CPM; Crawfordsville, IN; Model C181NF6.5H), CPM hygenizer, 40 horsepower pellet mill (Master Model Pellet Mill), and 4.5 x 38 mm pellet die were used to manufacture feed. The conditioning temperature was maintained at 80℃ for 30 s. Although the hygenizer was not used, there is no way to bypass it at the pilot feed mill. A portion of the soybean oil was added at the mixer (2.0%) whereas the remainder of the soybean oil was added post pelleting and crumbling using a horizontal double shaft paddle ribbon mixer.

Pellet production rate and hot pellet temperature (**HPT**) were recorded in duplicate after steady state production was achieved in each replicate. Pellet production rate was measured by collecting and weighing all pellets extruded from the pellet die in a 60 s period. Hot pellet temperature was measured using an insulated container and an 80 PK-24 temperature probe with a thermocouple (Fluke 51 II, Everette, WA). The range of the pellet mill motor load throughout manufacture was recorded for each replicate.

Particle size and pellet quality analyses

Particle size of the corn and mash diet samples was analyzed using ASABE method S319.4 (ASABE, 2022). Dry sieving of the corn and mash samples was conducted using a Ro-Tap shaker (WS Tyler Ro-Tap RX-29 Sieve Shaker) fitted with a set of sieves ranging from 37 μm to 4,760 μm. No dispersing agent was used and individual sieves were weighed to determine average PS after 10 min.

Pellets were collected from the cooling deck to determine pellet quality 24 h post manufacture using pellet durability index (PDI), modified PDI (MPDI), and New Holmen Pellet Tester (NHPT) with 30 s of forced air. All pellet samples were sieved using a No. 5 W. S. Tyler testing sieve (Mentor, OH) to obtain pure pellets for analysis. Both PDI and MPDI were analyzed in duplicate whereas NHPT was analyzed in triplicate for each manufacturing replicate.

Animal Experiment

All animal procedures used in this experiment were approved by the Virginia Tech Animal Care and Use Committee. The experimental period from 21 to 42 d of age was arranged as a 2 x 2 factorial with CPS (crumbled starter 2 diets manufactured with 597 or 1,049 μm corn) and coccidial challenge (15x dose coccidiosis vaccine challenge provided on D28 vs. no coccidiosis vaccine challenge) as the main effects. The experiment was set up in a randomized complete block design with the experimental unit being the pen. From 0 to 21 d, a total of 1,600 turkey hen poults were raised on clean litter and provided a common crumbled starter 1 diet (Table 6.1) containing 597 μm corn. On D 21, 1,472 Hybrid turkey hens were weighed and allocated to floor pens (4' x 8') with 16 replicate pens of 23 poults per pen per treatment to ensure similar BW among treatments. The experimental period began on D21 and turkey poults were provided *ad libitum* access to crumbled experimental starter 2 diets varying in CPS and water, over the 21 to 42 d period (Table 1). On D28, the poults challenged with the coccidial vaccine were orally gavaged with a 15x dose of a live coccidial vaccine containing sporulated oocysts of *E. adenoeides*, *E. meleagrimitis*, and *E. gallopavonis*. Temperature and lighting were adjusted according to breeder recommendations and bird age (Hybrid Turkey Management Guide, 2021).

Bird performance and gizzard weights

Feed intake (**FI**) was reported from D21-42 and was calculated by subtracting the weight of the remaining feed at the end of the period from the amount of feed added over the duration of the starter 2 feeding phase. Initial BW was measured on D21 and final BW was determined on D42. All birds were weighed on a pen basis and then divided by the number of birds in each pen to get BW/bird. Body weight gain (**BWG**) was calculated on a pen basis by subtracting the initial BW from the final BW. Turkeys that died, were sampled, or euthanized were weighed and added to the pen BWG to correct the feed conversion ratio. The mortality weight gain was calculated by subtracting initial BW/bird from the mortality weight/bird. Mortality corrected feed conversion ratio (**FCRm**) was then calculated and reported over the 21-42 d period on a pen basis with the following formula:

$$\text{FCRm} = \text{FI/pen} / (\text{mortality weight gain/pen} + \text{BWG/pen})$$

On D35 and 42, 3 poults per pen were selected, weighed and euthanized before gizzards were collected, clean of excess fat and digesta content before being weighed with the koilin lining intact. Gizzard weights were reported on a relative basis to BW. The sampled birds were weighed and added to the pen BWG as mortality weight gain to correct the feed conversion ratio for mortality.

Intestinal permeability and nutrient digestibility

One bird per pen was used on D35 to measure intestinal permeability using FITC-dextran (**FITC-d**) following the methodologies of Vuong and others (2021). Briefly, a bird was orally gavaged with 8.32 mg/kg BW of FITC-d dissolved in purified water and 1 ml of blood was drawn from the brachial vein of the same bird 1 hour after gavage. Blood was allowed to clot at room temperature for 4 hours and then centrifuged with the serum removed via pipettor. Serum samples were diluted 1:5 in 0.9% saline to a total volume of 100 µl in a 96 well flat bottom plate. Samples were analyzed using a multi-mode plate reader (Infinite M200 Pro, Tecan, Morrisville, NC) at excitation wavelength of 485 nm and emission wavelength of 528 nm. A standard curve was generated by adding a known concentration of FITC-d to serum from turkeys that had not been gavaged with FITC-d.

On D35, 6 birds per pen were euthanized via cervical dislocation for ileal content collection. Ileal contents were collected from Meckel's diverticulum to 2 cm above the ileal-cecal junction. The collected contents from the 6 hens were pooled and frozen at -20°C until analysis. Ileal contents were dried in a 55°C oven for 24h (Freas 645, Thermo Electron Corporation, Marietta, OH). Dried ileal contents and feed samples were then ground using a coffee grinder. Crude fat of the ileal content was determined in duplicate using approximately 1.0 g of dried ileal content and feed using ether extraction (Thiex et al., 2006). The crude protein content of dried ileal and feed were measured in duplicate using the Elementar Vario EL cube (Elementar Americas Inc., Ronkonkoma, NY). Gross energy of approximately 0.3 g of ileal content and 0.5 g of feed were measured in duplicate using a Parr 6400 bomb calorimeter using benzoic acid as the calibration standard (Parr 6400 Calorimetric, Parr Instrument Company, Moline, IL). Titanium (Ti) analysis was completed to determine the apparent ileal digestibility (**AID**; Leone, 1973). Approximately 0.3 g of dried ileal content and 0.5 g of dried feed were ashed at 580°C (Thermo Fisher Scientific, Asheville, NC) for 12 h in duplicate for the ileal content and quadruplicate for the feed. Samples were transferred to Pyrex screw cap tubes with 0.8 g of anhydrous sodium sulfate and digested in 5 mL of sulfuric acid for 72 h at 135°C. After digestion, samples were diluted to 50 mL and then analyzed using a colorimetric method which involved addition of 15 µL of hydrogen peroxide to 100 µL of diluted sample. Plates were gently shaken for 30 minutes to allow color development before reading in a microplate reader (Infinite M200 Pro, Tecan, Morrisville, NC). The Ti values within the ileal and feed content were determined by multiplying the weight of the sample by the dilution factor and the final Ti concentration in the solution. The following equation was used to determine apparent ileal crude fat (AIDf), energy (AIDe), and protein digestibility (AIDp).

$$AID\% = 100 - \left(\frac{Nutrient\ component\ in\ ileal}{Nutrient\ component\ in\ diet} * \frac{Ti\ component\ in\ diet}{Ti\ component\ in\ ileal}\right) * 100$$

Litter moisture content

Litter samples were collected from all pens on D21, 28, 35, and 42 to determine litter moisture content using the general methods of Sizmaz et al. (2022). Briefly, litter was collected from the center of the pen and the center of each quadrant when the pen is divided into 4 equal squares and combined into one composite litter sample. All samples were analyzed in duplicate and dried in an oven for 24 h at 100°C (Thermo Fisher Scientific, Model 645, Asheville, NC), then re-weighed to determine litter moisture using the following equation:

$$100 - (Dry\ wt\ of\ litter\ (g) / Wet\ wt\ of\ litter\ (g)\ \times 100)$$

Oocyst shedding

On 5 to 7 d post infection (33 to 35 d of age), fresh excreta samples were collected and pooled by pen to generate one composite sample over the three d period. Oocysts were counted in quadruplicate using the McMaster counting chamber (McMaster; JA Whitlock & Company, New South Wales, Australia) under a microscope (Olympus CX21-FS1, Tokyo, Japan). The following formula was used to calculate the total number of oocysts:

$$Total\ oocysts/g\ of\ excreta = \left(\frac{oocysts\ counted}{chamber\ volume}\right) x\ dilution\ factor\ x\ \left(\frac{composite\ sample}{g\ of\ excreta\ used}\right)$$

Statistical analysis

Feed manufacture and pellet quality data were analyzed using ANOVA in JMP Pro 18 with each of the three manufacture runs as the experimental unit. All performance data, litter moisture, and nutrient digestibility were analyzed as a 2 x 2 factorial with CPS and coccidial challenge as main effects in JMP Pro 18 ($P \leq 0.05$). If significance was found, means were separated using Student's t-test (Sarsour and Persia, 2022). The pen was used as the experimental unit for analysis for performance data and poults collected from within each pen were used for the experimental unit for other measurements. Percentage data were transformed using arcsine for analysis (Fritzlen et al., 2024).

RESULTS AND DISCUSSION

Corn and diet particle size

The particle distribution of the corn used to manufacture the starter 2 diets is shown in Figure 6.1A. Particle size of the corn was 597 µm for the FGC and 1,049 µm for the CGC. With the 1,049 µm corn inclusion, the PS of the mash diet was 965 µm, increased in comparison to the PS of 775 µm for the mash diet generated from FGC inclusion (Figure 6.1B). These results are similar to previous work demonstrating a mash PS of 736 µm for FGC inclusion (581 µm) whereas CGC inclusion (964 µm) increased the mash PS to 879 µm (Lyons et al., 2024). Particle size of the mash diets differed in comparison to the CPS due to other ingredient PS within the diet.

Pellet quality analyses and feed mill efficiency

There were no differences in HPT or production rate between diets containing fine and coarse corn ($P > 0.05$; Table 6.2). This is in agreement with previous work demonstrating no difference in production rate when corn ground to 595 or 876 µm was pelleted (Martin, 1985). Mill load was increased in the diet containing the 1,049 µm corn ($P < 0.01$). The diet with the coarse corn used more power to push the coarser particles through the pellet die, possibly due to the size of the pellet diameter which could affect the degree of grinding that occurs (Svihus et al., 2024). An increase in mill load would mean greater energy consumption at the pellet mill and a potential increase in feed costs so the pellet diameter should be considered with ingredient PS.

Although there was a 190 µm difference in particle size between the FGC and CGC mash diets, there were no differences in pellet quality parameters including PDI, MPDI, and NHPT ($P > 0.05$; Table 6.2). This is in agreement with previous work that demonstrated no pellet quality differences in starter 2 turkey diets that contained 581 and 964 µm corn (Lyons et al., 2024). As the particle size of a mash diet increases, the degree of grinding during the pelleting process increases due to the frictional forces within the pellet die (Bonilla et al., 2022). A lack of difference in pellet quality may be attributed to the idea that the coarse corn was ground to a greater extent than the fine corn in the pellet die.

Oocyst shedding

Oocyst counts were used as a method to validate coccidial infection. There was no interaction between CPS and coccidia challenge or a CPS main effect. As expected with the orally gavaged 15x dose of coccidiosis vaccine on D28, a main effect occurred where the challenged birds had a higher number of oocysts on d 5 to 7 post vaccination ($P \leq 0.01$; Table 6.3). Birds that were not challenged had minimal oocysts in the excreta. A similar response of increased fecal oocyst counts on d 7 post inoculation ($P = 0.02$) was reported when Nicholas turkey poults were inoculated with 30 or 90 oocysts per bird on d of hatch compared to a control not inoculated with oocysts (Yeboah et al., 2025).

Bird performance and gizzard weights

Initial BW was not different at D21 and was 0.57 kg/bird ($P = 0.96$). There were no interactions throughout the 21-42d experiment for any of the performance data so only the main effects are discussed. Birds that consumed the CGC had a lower FI (46.4 vs 48.0 kg/pen) over the 21 to 42 d period ($P \leq 0.01$; Table 6.4) compared to those consuming the FGC. Body weight followed a similar pattern with the birds consuming the 1,049 μm corn having a lower BW (2.09 vs 2.14 kg/bird; $P \leq 0.01$). Although there were differences in FI and BW, FCRm was not different from 21 to 42d ($P \leq 0.01$). Hybrid converter turkeys demonstrated a linear decrease in BWG over the 0-7d and 0-15d period when fed diets containing 606, 701, 832, or 1,094 μm corn (Charbeneau and Roberson, 2004). These data indicate that coarse corn is detrimental during early growth in turkeys (starter 1 phase). Recently published work reported no differences in BW, FI, or FCR by D42 when turkeys were fed either 587 or 964 μm corn during the starter 2 phase (Lyons et al., 2024). However, turkeys in the Lyons et al., 2024 experiment were fed pelleted diets with the CGC being 964 μm whereas birds in the current experiment were fed pelleted and crumbled diets with 1,049 μm corn, an 8% increase in CPS. Particle sizes that are too large may have adverse effects on turkey performance, particularly during early stages of growth. Birds have a natural preference for larger particles but this preference increases with age (Portella et al., 1988; Nir et al., 1994). An 8% increase in the CGC during the starter 2 phase (21 to 42 d) may have been too large, thus explaining the negative effects on FI and BW unlike what was reported in previous turkey data.

The coccidiosis challenge introduced on D28 reduced BW and FI over the 21-42d period (Table 6.4). Body weight on D42 was reduced 10.7% (2.00 vs 2.24 kg/bird) when turkeys were challenged with the coccidiosis vaccine ($P \leq 0.01$). With the reduction in both FI and BW, there was a 12-point increase in FCRm (1.790 vs 1.673 kg/kg) for birds challenged with coccidiosis ($P \leq 0.01$). Current research is in line with previous work demonstrating the negative effects that coccidiosis has on turkey performance (Milbradt et al., 2014; Yeboah et al., 2025). Recent published work administered coccidia oocysts to poults on the day of hatch and reported a reduction in BW for poults given the oocysts ($P < 0.05$; Yeboah et al., 2025).

There was no difference in relative gizzard weights for birds consuming the 597 or 1,049 µm corn ($P > 0.05$). Turkeys that consumed either a crumbled or micropelleted diet containing 380 µm corn had a decreased gizzard weight compared to those consuming 806 µm corn in the diet ($P < 0.01$; Favero et al., 2009). The FGC in the current experiment was 597 µm, approximately 200 µm larger than the FGC in the research conducted by Favero et al., 2009. Broilers that were fed 700 and 912 µm corn in a mash diet had a higher relative gizzard weight compared to those fed 492 µm corn ($P < 0.05$; Mtei et al., 2019). An increase in relative gizzard weight was reported when 50% coarse corn (1,362 µm) was included in a pelleted broiler diet compared to 100% fine corn (294 µm; $P < 0.05$; Xu et al., 2015). Since there was a lack of difference in pellet quality between the varying CPS in the current experiment, there may not have been a large enough PS difference to elicit a response in the gizzard. There is also the potential that CPS in a pelleted turkey diet may not affect the gizzard to the extent that it does in a broiler diet due to a slower growth rate in turkeys and a lower FI. Turkeys that were provided the coccidiosis vaccine on D28 demonstrated a reduced relative gizzard weights on D35 ($P \leq 0.01$) and D42 ($P = 0.04$; Table 6.3). The reduction in relative gizzard weight with a coccidial challenge is the first report in turkeys and *Eimeria meleagrimitis* in the coccidial vaccine used affects the duodenum and upper jejunum, also possibly resulting in damage to the gizzard.

Intestinal health and nutrient digestibility

There were no interactions between CPS or coccidiosis challenge for any of the intestinal permeability or nutrient digestibility data. There was no main effect for intestinal permeability regarding CPS or coccidiosis challenge ($P > 0.05$; Table 6.5). Broilers had a higher concentration of FITC-d 5 to 6 days post infection when given a 2×10^5 dose of *E. maxima* oocysts (Schneider et al., 2019). Similarly intestinal permeability was determined on d 3, 5, 6, 7, and 9 post vaccination after broilers were gavaged with a mixed *Eimeria* vaccine and differences were only found on d 5, 6, and 7 ($P < 0.01$; Teng et al., 2020). The timing of intestinal permeability measurements should be considered since the timing of the coccidiosis challenge and d of sampling likely affect intestinal permeability.

There was a CPS main effect for AIDe, but there were no differences for AIDf or AIDp (Table 5). An increase in apparent metabolizable energy digestibility was reported when broilers were fed 651 or 796 µm corn in a pelleted diet compared to 490 µm corn (Naderinejad et al., 2016). A longer retention time in the gizzard allows for increased enzymatic digestion which would solubilize the larger particle corn at a slower rate and increase the digestibility compared to smaller particles. Previous work also reported an increase in crude fat digestibility with turkeys consuming 606 or 806 µm corn in a crumbled diet compared to those fed 380 µm corn ($P \leq 0.01$; Favero et al., 2012). The PS of the FGC used in the Favero et al., 2012 experiment was approximately 200 µm smaller than the fine corn used in the current experiment and therefore it is difficult to compare across experiments with varying PS. There was a challenge main effect for AIDf with the challenged birds having reduced digestibility ($P = 0.02$). Coccidiosis has also been shown to reduce nutrient transport expression and thus a reduction in nutrient digestibility (Su et al., 2015). Past research has reported upwards of a 19% reduction in ileal digestibility of energy when broilers were challenged with graded levels of *E. maxima*, *E. tenella*, and *E. acervulina* (Persia et al. 2006; Teng et al., 2020), although data on nutrient digestibility in turkeys infected with coccidiosis has not been reported to the authors knowledge. Of the three *Eimeria* strains in the live coccidiosis vaccine used, *E. meleagrimitis* has been reported to affect the duodenum and upper jejunum where fat digestion would occur. The other two *Eimeria* strains, *E. adenoeides* and *E. gallopavonis*, primarily affect the lower small intestine where protein absorption occurs and the ceca. *Eimeria meleagrimitis* typically has a higher prevalence in the turkey industry (Chapman, 2008) and may be more infectious to poults compared to other strains, thus indicating why AIDf was decreased in birds given the 15x coccidiosis vaccine challenge.

Litter moisture content

There was no interaction between CPS and coccidiosis challenge or a main effect for CPS. Litter moisture was different on D35 with the turkeys that were challenged with coccidiosis having a higher litter moisture content ($P = 0.02$; Table 6.6). There were no differences on D21, 28, or 42 for litter moisture content. The increased moisture reported with the challenged birds on D35 coincides with peak oocyst shedding that typically occurs around D5-7 post infection in turkeys (Chapman, 2008). Prevalence of coccidiosis increases the incidence of diarrhea in birds which means increased water content and a likely increase in litter moisture (Collett, 2012).

CONCLUSIONS AND APPLICATIONS

1. Pellet quality was not different for turkey starter 2 diets containing 597 or 1,049 µm corn although mill load was higher when the 1,049 µm corn was pelleted, indicating that there is a potential influence between pellet die diameter and particle size.
2. Inclusion of coarse corn (1,049 µm) decreased poult performance over the 21 to 42 day starter 2 period.
3. A turkey coccidiosis model was validated when turkeys gavaged with a 15x dose of a live coccidial vaccine on day 28 demonstrated reduced feed intake, body weight, and feed conversion ratio.
4. A 15x dose of the live coccidial vaccine on day 28 reduced apparent ileal crude fat digestibility.

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DISCLOSURES

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

REFERENCES

Adhikari, P., A. Kiess, R. Adhikari, and R. Jha. 2020. An approach to alternative strategies to control avian coccidiosis and necrotic enteritis. J. Appl. Poult. Res. 29:515-534. https://doi.org/10.1016/j.japr.2019.11.005.

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

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. Poult. Sci. 87:2320-2328. doi:10.3382/ps.2008-00149.

Bjerrum, L., K. Pedersen, and R. M. Engberg. 2005. The influence of whole wheat feeding on salmonella infection and gut flora composition in broilers. Avian Dis. 49:9-15. https://doi.org/10.1637/7223-061504R.

Chapman. H. D. 2008. Coccidiosis in the turkey. Avian. Pathol. 37:205-223. https://doi.org/10.1080/03079450802050689.

Clark, S. R. and L. Chiaia. 2024. Current Health Issues Facing the US Turkey Industry (HVP.PO.100324.1). Proceedings 128th Annual Mtg of the USAHA, Virtual; Committee on Poultry and Other Avian Species. Pending Publication. Presented Sep 30, 2024.

Engberg, R. M., M. S. Hedemann, S. Steenfeldt, and B. B. Jensen. 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. Poult. Sci. 83:925-938.

Ferket, P. R. and A. G. Gernat. 2006. Factors that affect feed intake of meat birds: A review. Int. J. Poult. Sci. 5(10):905-911. DOI: 10.3923/ijps.2006.905.911.

Flores, K. R., A. Fahrenholz, P. R. Ferket, T. J. Biggs, and J. L. Grimes. 2021. Effect of methionine chelated Zn and Mn and corn particle size on Large White male turkey live performance and carcass yields. Poult. Sci. 100:101444. https://doi.org/10.1016/j.psj.2021.101444.

Fritzlen, C. J., J. J. Maurer, W. Chen, Z. Tan, H. Liu, X. Peng, Z. Qin, Y. Peng. and M. E. Persia. 2024. Effects of dietary enteric-released or pure benzoic acid on Ross 708 male broilers. J. Appl. Poult. Res. 33:100468. https://doi.org/10.1016/j.japr.2024.100468.

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

Hendrix Genetics. Technical guide for Hybrid Turkeys commercial products. 2021.

Leone, J. L. 1973. Collaborative study of the quantitative determination of titanium dioxide in cheese. J. Assoc. Anal. Chem. 56:535-537.

Lyons, A. M., P. H. Patterson, and J. W. Boney. 2022. Hammermill screen selection for soybean processing: soybean meal particle size and pullet performance effects. J. Appl. Poult. Res. 31:100262. https://doi.org/10.1016/j.japr.2022.100262.

Lyons, A. M., J. S. Moritz, J. W. Boney, and M. E. Persia. 2024. Evaluation of corn particle size on the growth performance, intestinal permeability, pellet quality, and litter moisture of turkey hens raised without antibiotics. J. Appl. Poult. Res. 33:100420. https://doi.org/10.1016/j.japr.2024.100420.

Martin, S. A. 1985. Comparison of hammermill and roller mill grinding and the effect of grain particle size on mixing and pelleting. Kansas State Univ., Manhattan MS Thesis.

Mathis, G. F., B. Lumpkins, H. M. Cervantes, S. H. Fitz-Coy, M. C. Jenkins, M. K. Jones, K. R. Price, and R. A. Dalloul. 2024. Coccidiosis in poultry: Disease mechanisms, control strategies, and future directions. Poult. Sci. 104:104663. https://doi.org/10.1016/j.psj.2024.104663.

Milbradt, E. L., A. A. Mendes, J. G. Ferreira, I. Almeida Paz, M. B. Martins, C. Sanfelice, B. C. Fernandes, and A. S. Okamoto. 2014. Use of live oocyst vaccine in the control of turkey coccidiosis: Effect on performance and intestinal morphology. J. Appl. Poult. Res. 23:204-211. http://dx.doi.org/ 10.3382/japr.2013-00877.

Naderinejad, S., F. Zaefarian, M. R. Abdollahi, A. Hassanabadi, H. Kermanshahi, and V. Ravindran. 2016. Influence of feed form and particle size on performance, nutrient utilisation, and gastrointestinal tract development and morphometry in broiler starters fed maize-based diets. Anim. Feed Sci. Tech. 215:92-104. https://doi.org/10.1016/j.anifeedsci.2016.02.012.

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

Pacheco, W. J., C. R. Stark, P. R. Ferkert, and J. Brake. 2013. Evaluation of soybean meal source and particle size on broiler performance, nutrient digestibility, and gizzard development. Poult. Sci. 92:2914-2922.

Persia, M. E., E. L. Young, P. L. Utterback, and C. M. Parsons. 2006. Effects of dietary ingredients and Eimeria acervulina infection on chick performance, apparent metabolizable energy, and amino acid digestibility. Poult. Sci. 85:48-55.

Portella, F. J., L. J. Caston, and S. Leeson. 1988b. Apparent feed particle size preference by broilers. Can. J. Anim. Sci. 68:923-930.

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

Rueda, M. S., S. Bonilla, C. de Souza, J. D. Starkey, C. W. Starkey, L. Mejia, and W. J. Pacheco. 2024. Evaluation of particle size and feed form on performance, carcass characteristics, nutrient digestibility, and gastrointestinal tract development of broilers at 39 d of age. Poult. Sci. 103:103437. https://doi.org/10.1016/j.psj.2024.103437.

Sarsour, A. H. and M. E. Persia. 2022. Effects of sulfur amnio acid supplementation on broiler chickens exposed to acute and chronic cyclic heat stress. Poult. Sci. 101:101952. https://doi.org/10.1016/j.psj.2022.101952.

Schneiders, G., J. Foutz, M. C. Milfort, A. Ghareeb, U. G. Sorhue, J. N. Richter, A. L. Fuller, S. M. Williams, R. Rekaya, and S. E. Aggrey. 2019. Ontogeny of intestinal permeability in chickens infected with Eimeria maxima: implications for intestinal health. J. Adv. Parasitol. 6:41-50.

Sizmaz, O, N. W. Barrett, J. Lewis, H. Yakout, and M. E. Persia. 2022. Effect of various concentration of butyric acid on growth performance, intestinal lesion scores, and body composition of broilers raised on used litter. J. Appl. Poult. Res. 31:100296. https://doi.org/10.1016/j.japr.2022.100296.

Su. S., K. B. Miska, R. H. Fetterer, M. C. Jenkins, and E. A. Wong. 2015. Expression of digestive enzymes and nutrient transporters in *Eimeria*-challenged broilers. Exp. Parasit. 150:13-21. http://dx.doi.org/10.1016/j.exppara.2015.01.003.

Svihus, B., C. G. Storkas, M. K. Neteland, S. E. O. Reierstad, S. Dhakal, and H. Hetland. 2024. Effect of grinding and pellet dimensions on performance, digestive tract functionality and feeding behvaior of broiler chickens fed diets based on wheat and maize. J. Appl. Poult. Res. 33:100413. https://doi.org/10.1016/j.japr.2024.100413.

Teng, P-Y., S. Yadav, F. L. de Souza Castro, Y. H. Tompkins, A. L. Fuller, and W. K. Kim. 2020. Graded *Eimeria* challenge linearly regulated growth performance, dynamic change of gastrointestinal permeability, apparent ileal digestibility, intestinal morphology, and tight junctions of broiler chickens. Poult. Sci. 99:4203-4216. https://doi.org/10.1016/j.psj.2020.04.031.

Thiex, N. J., S. Anderson, and B. Gildemeister. 2006. Chapter 4—Crude fat in feeds, cereal grains, and forages. Pages 40−42 in Official Methods of Analysis of AOAC International. 18th ed. AOAC International, Arlington, VA.

Vuong, C. N., G. J. Mullenix, M. T. Kidd, W. G. Bottje, B. M. Hargis, and G. Tellez-Isaias. 2021. Research Note: Modified serum fluorescein isothiocyanate dextran (FITC-d) assay procedure to determine intestinal permeability in poultry fed diets high in natural or synthetic pigments. Poult. Sci. 100:101138. https://doi.org/10.1016/j.psj.2021.101138.

Xu, Y. 2014. Interaction of dietary coarse corn with litter conditions on broiler live performance and gastrointestinal tract function. Dissertation. NC State Univ. Raleigh, NC.

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

Table 6.1. Composition of experimental diets fed to Hybrid turkey hen poults from 0-42 days of age.[1]

Ingredient	Starter 1	Starter 2[2]
	(%)	
Corn	42.80	40.74
Soybean meal (48% CP)	38.14	42.73
Poultry by-product meal	10.00	5.00
Soybean oil	4.24	6.58
Monocalcium phosphate	1.53	1.55
Limestone	1.73	1.79
Vitamin and mineral premix[3]	0.38	0.38
DL-Met (99%)	0.39	0.39
L-Lys•HCl (78.5%)	0.40	0.36
L-Thr (98.5%)	0.09	0.09
Sodium chloride	0.06	0.12
Sodium bicarbonate	0.24	0.27
Phytase	0.01	0.01
Calculated nutrient content		
Crude protein	27.4 (27.1)	26.0 (25.5)
ME (kcal/kg)	3,000	3,102
Calcium	1.49	1.38
Available phosphorous	0.76	0.69
Crude fat	7.91 (6.37)	9.63 (8.33)
Crude fiber	2.47 (2.17)	2.54 (2.32)
Digestible Lys	1.66	1.59
Digestible Met	0.75	0.73
Digestible SAA	1.08	1.05
Digestible Thr	0.96	0.93
Digestible Trp	0.28	0.28
Digestible Ile	0.98	0.97
Sodium	0.17	0.18
Chloride	0.20	0.20

[1] Values presented within ( ) are analyzed values

[2] Diets varied in corn particle size for starter 2; fine (597 µm) and coarse (1,049 µm).

[3] Provided per kg of diet: vitamin A – 9,900 IU; vitamin D3 – 3,300 ICU; vitamin E – 13 IU; vitamin B12 – 0.01 mg; vitamin B2 – 5.8 mg; menadione – 0.5 mg; choline – 265 mg; selenium – 0.30 mg; iodine – 0.7 mg; thiamine HCl – 0.5 mg; niacinamide – 30 mg; pantothenic acid – 8.2 mg; Fe - 50 mg; Zn – 60 mg; Cu – 7 mg; Mn - 125 mg.

Table 6.2. Effects of fine and coarse particle corn on hot pellet temperature (HPT), production rate, pellet mill load, New Holmen Pellet Tester (NHPT), pellet durability index (PDI), and modified pellet durability index (MPDI).

Treatment	Corn	Mash	HPT[1]	Production Rate[2]	Pellet Mill Load	NHPT[3]	PDI[4]	MPDI[5]
	(µm)	(µm)	(˚C)	(Mt/h)[6]	(% of FLA)[7]	(%)	(%)	(%)
Fine corn	597	775	83.4	0.965	43.5[b]	90.2	91.6	89.6
Coarse corn	1,049	965	83.8	0.976	45.8[a]	89.1	90.4	87.8
Pooled SEM			0.28	0.007	NA	NA	NA	NA
P-value			0.41	0.34	< 0.01	0.28	0.16	0.23

[1] Hot pellet temperature was measured 2 times throughout feed manufacture once steady state production was achieved using a 80PK-24 temperature probe and insulated thermos.
[2] Production rate was measured 2 times throughout feed manufacture by feed collection directly from the pellet die for a 60 second period once steady state production was achieved.
[3] 100 g of sifted pellets were placed into the chamber, 30 seconds of forced air was applied, and remaining pellets were weighed.
[4] 500 g of sifted pellets were placed into the P:Fost tumbler box (Gamet Manufacturing Inc., Saint Paul, MN) and tumbled for 10 minutes at 53 RPM, after which remaining pellets were weighed.
[5] Similar to pellet durability index but modified by adding five 13 mm hexagonal nuts to the sample before tumbling.
[6] Metric tons per hour.
[7] Motor average was relative to 100% of the full load amps of 47 amps for the 40 HP motor of the pellet mill.
[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

Table 6.3. Effect of corn particle size (CPS) and coccidial challenge on oocyst shedding of Hybrid turkey hen poults from 21 to 42 days of age

CPS[1]	Challenge[2]	Oocyst Shedding D5-7
		(oocysts/g)
597 µm	---	80,983
1,049 µm	---	75,976
Pooled SEM		31,712
---	Yes	156,912[a]
---	None	46[b]
Pooled SEM		31,712
597 µm	None	55
597 µm	Yes	161,910
1,049 µm	None	37
1,049 µm	Yes	151,915
Pooled SEM		44,847
CPS		0.91
Challenge		≤ 0.01
CPS x Challenge		0.91

[1] Corn particle size.
[2] Poults were given a 15x dose of a turkey coccidiosis vaccine on day 28 containing *E. adenoeides*, *E. meleagrimitis*, and *E. gallopavonis*.
[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

Table 6.4. Effect of corn particle size (CPS) and coccidial challenge on the feed intake (FI), body weight (BW), body weight gain (BWG), mortality corrected feed conversion ratio (FCRm), and relative gizzard weight (RGW) of Hybrid turkey hen poults from 21-42 days of age.[1]

CPS[2]	Challenge[3]	D21 to 42 FI	D42 BW	D21 to 42 FCRm	D35 RGW	D42 RGW
		(kg)	(kg/bird)	(kg/kg)	(%)	(%)
597 µm	---	48.0[a]	2.14[a]	1.728	2.22	1.70
1,049 µm	---	46.4[b]	2.09[b]	1.736	2.20	1.75
Pooled SEM		0.34	0.01	0.0111	NA	NA
---	Yes	45.4[b]	2.00[b]	1.790[a]	2.15[b]	1.69[b]
---	None	49.0[a]	2.24[a]	1.673[b]	2.27[a]	1.75[a]
Pooled SEM		0.34	0.01	0.0111	NA	NA
597 µm	None	50.0	2.26	1.679	2.30	1.72
597 µm	Yes	46.0	2.03	1.776	2.14	1.67
1,049 µm	None	48.1	2.21	1.667	2.24	1.78
1,049 µm	Yes	44.8	1.97	1.805	2.16	1.71
Pooled SEM		0.48	0.02	0.0157	NA	NA
CPS		≤ 0.01	≤ 0.01	0.60	0.70	0.10
Challenge		≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01	0.04
CPS x Challenge		0.48	0.76	0.20	0.31	0.74

[1] Initial BW at day 21 was 0.57 kg/bird (P = 0.96; SEM = 0.005).
[2] Corn particle size.
[3] Poults were given a 15x dose of a turkey coccidiosis vaccine on day 28 containing *E. adenoeides*, *E. meleagrimitis*, and *E. gallopavonis*.
[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

Table 6.5. Effect of corn particle size (CPS) and coccidial challenge on gut permeability (Serum FITC-dextran), crude fat and protein digestibility at 35 days of age.

CPS[1]	Challenge[2]	Serum FITC-D	Crude Fat	Energy	Protein
		(ng/ml)	(%)	(%)	(%)
597 µm	---	290	93.1	71.7[b]	78.7
1,049 µm	---	276	93.6	73.6[a]	79.0
Pooled SEM		7.3	NA	NA	NA
---	Yes	291	92.9[b]	72.6	78.9
---	None	275	93.8[a]	72.8	78.8
Pooled SEM		7.3	NA	NA	NA
597 µm	None	279	93.3	72.0	78.8
597 µm	Yes	300	92.9	71.5	78.5
1,049 µm	None	272	94.4	73.5	78.7
1,049 µm	Yes	281	92.8	73.7	79.3
Pooled SEM		10	NA	NA	NA
CPS		0.20	0.21	< 0.01	0.60
Challenge		0.14	0.02	0.73	0.86
CPS x Challenge		0.54	0.11	0.45	0.54

[1] Corn particle size.

[2] Poults were given a 15x dose of a turkey coccidiosis vaccine on day 28 containing *E. adenoeides*, *E. meleagrimitis*, and *E. gallopavonis*.

[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

Table 6.6. Effect of corn particle size (CPS) and coccidial challenge on litter moisture of Hybrid turkey hen poults from 21 to 42 days of age

		Litter Moisture Content			
CPS[1]	Challenge[2]	D21	D28	D35	D42
		(%)	(%)	(%)	(%)
597 µm	---	22.1	18.9	22.1	28.9
1,049 µm	---	21.9	19.8	23.5	29.6
Pooled SEM		NA	NA	NA	NA
---	Yes	22.6	20.4	24.4[a]	30.0
---	None	21.3	18.3	21.3[b]	28.5
Pooled SEM		NA	NA	NA	NA
597 µm	None	22.1	18.1	21.3	29.0
597 µm	Yes	22.0	19.6	23.0	28.9
1,049 µm	None	20.5	18.6	21.3	28.1
1,049 µm	Yes	23.3	21.1	25.7	31.0
Pooled SEM		NA	NA	NA	NA
CPS		0.86	0.41	0.29	0.65
Challenge		0.39	0.08	0.02	0.25
CPS x Challenge		0.32	0.66	0.27	0.25

[1] Corn particle size.
[2] Poults were given a 15x dose of a turkey coccidiosis vaccine on day 28 containing *E. adenoeides*, *E. meleagrimitis*, and *E. gallopavonis*.
[a-b] Means within a column that do not share a common superscript differ ($P \leq 0.05$).

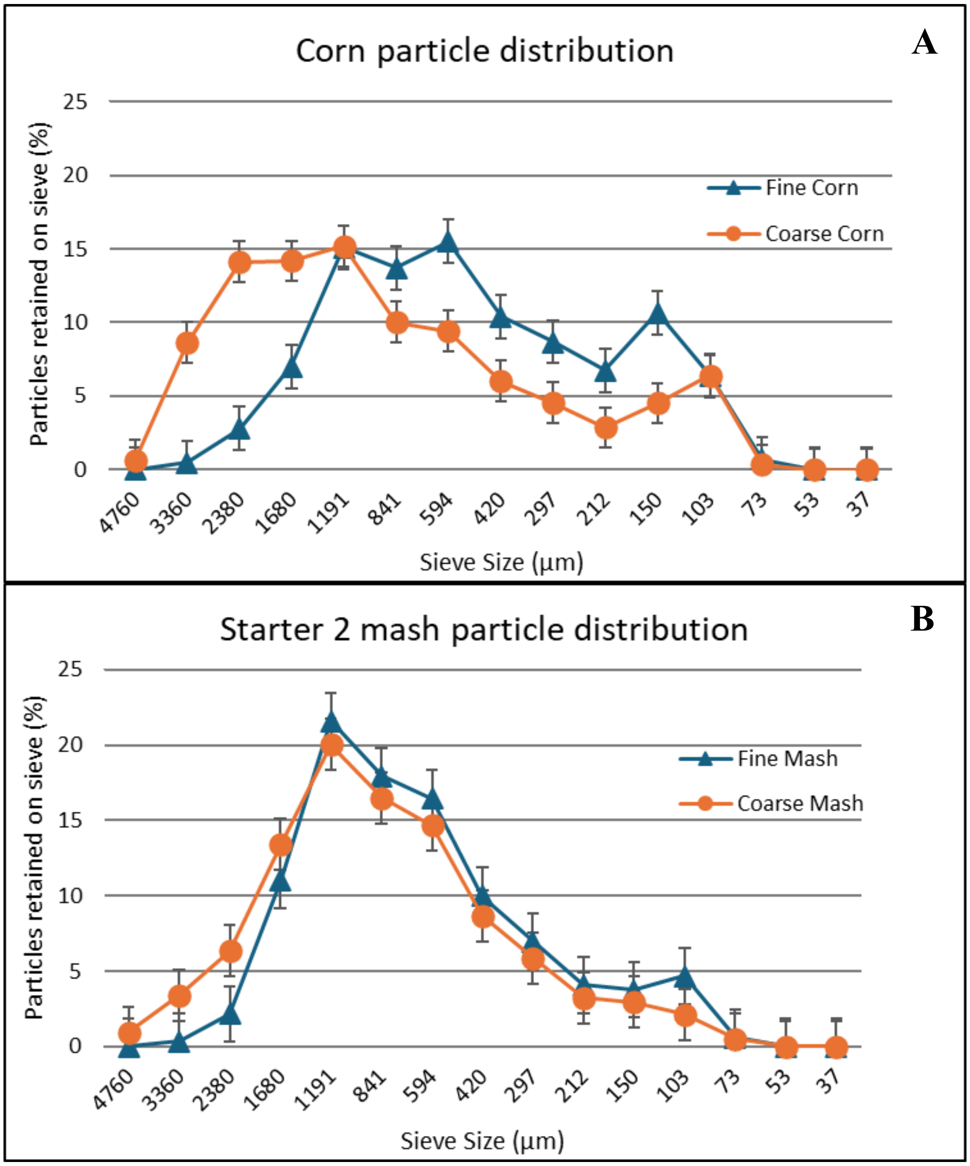

Figure 6.1. Particle distribution of corn (A) and starter 2 mash feed (B) before pelleting. The particle size of the fine corn was 597 µm and the coarse corn was 1,049 µm. Particle size of the mash feed containing fine was 775 µm whereas coarse corn inclusion was 965 µm.

CHAPTER 7:

Conclusions and Future Directions

The objective of this dissertation was to investigate optimal corn particle size in turkey diets, alternative calcium sources for laying hens, and feed additives to improve turkey and laying hen health and energy metabolism. This research aimed to provide insight into how alternative feed ingredients and varying ingredient particle sizes can utilized in poultry production to decrease feed costs while improving bird health and maintaining performance.

Inclusion of small (581 µm) and large (964 µm) particle corn in turkey starter 2 diets did not result in differences in pellet quality, possibly due to the small difference in mash diet particle size (736 to 879 µm). Large particle corn (964 µm) reduced turkey hen performance over the starter 1 phase compared to small particle corn (581 µm). However, there were no differences in turkey performance during starter 2, indicating that a larger particle corn may be included after the starter 1 phase to maintain performance and potentially reduce corn grinding costs and overall feed milling costs. Litter dry matter and intestinal permeability were not different at 42 days. A larger range of corn particle sizes should be considered to determine an optimal corn particle size (500 to 1,000 µm) for pellet quality and turkey performance. Energy usage at the feed mill to grind the corn could be measured to validate a lower energy usage with grinding the larger particle corn. Additionally, histology samples could be taken from the jejunum to measure villus height and crypt depth to determine if a larger corn particle size impacts nutrient digestibility through the enhanced mechanical digestion that has previously been reported with larger particles (Nir et al., 1994a; Amerah et al., 2008).

Finely ground aragonite was able to maintain both egg production and tibia ash when formulated to 2.46% dietary Ca, validating an increased bioavailability of fine ground aragonite compared to limestone. However, sole inclusion of the fine aragonite did not maintain eggshell quality, indicating the need for large particle Ca in the diet, which is particularly important over periods of darkness, when the hen is not consuming feed and eggshell calcification is occurring (Scott et al., 1971). Further investigation into the combination of a large particle Ca and fine aragonite is needed to validate if lower concentrations of the fine aragonite can be used with the addition of large particle Ca. Additionally, varying combinations of fine aragonite and coarse limestone could be included in the diet at different concentrations to determine if the dietary calcium level could be reduced further. Reducing dietary calcium could potentially reduce overall feed costs in the laying hen industry.

Furthermore, inclusion of all direct fed microbials (Novela ECL®, Novela®, and Amnil®) altered energy metabolism and partitioning in laying hens, reducing the energy that is necessary for maintenance. A reduction in maintenance energy would allow for additional energy to be utilized for egg production or storage. The inclusion of both Novela ECL® and Amnil® partitioned energy towards productive performance as shown through the increase in egg production energy. The relative abdominal fat pad weight was increased with supplementation of Novela®, indicating an increase in stored energy of laying hens. Improved eggshell quality was noted with supplementation of Novela® and Amnil® through an increase in breaking force. Direct fed microbials have previously been reported to secrete enzymes and antimicrobial substances to enhance nutrient digestibility and support improved intestinal health (Elshaghabee et al., 2017) where bacterial counts of the small intestine and ceca would determine which DFM is enhancing digestibility and a more direct mode of action for improved intestinal health.

Additionally, the pH of the small intestine could be measured since a mode of action of DFM is to secrete lactic acid (Neal-McKinney et al., 2012) which decreases the pH, thus limiting pathogenic bacteria.

A larger particle corn may be detrimental in turkey starter 2 diets and a finely ground corn should be considered to improve performance. The mill load was higher in turkey diets manufactured with coarse corn (1,049 µm) compared to those manufactured with fine corn (597 µm), but pellet quality was not different between particle sizes. Inclusion of coarse corn over the starter 2 period resulted in reduced body weight and feed intake with an increase in apparent ileal energy digestibility. A turkey coccidiosis model was validated when turkeys receiving a 15x oral dose of a coccidiosis vaccine at 28 days of age had decreased performance compared to those not receiving the vaccine as well as a reduction in fat digestibility. The inclusion of a larger corn particle size was not able to effectively ameliorate a coccidiosis challenge in turkeys. A wider range of corn particle sizes (500, 700, 800, 1,000 µm) could be used to determine a more optimal particle size for feed milling efficiency and turkey performance. Additionally, the pH of the gizzard and proventriculus could be measured to determine if the larger particles have a longer retention time and decrease the pH, potentially altering gut microbial populations and improving overall gastrointestinal health.

This dissertation emphasizes the importance of ingredient particle size to maximize turkey and laying hen performance as well as supplementation of direct fed microbials to improve gastrointestinal health in laying hens. A focus on the dietary factors to improve poultry performance and health can help to maintain or maximize turkey and laying hen production while also emphasizing the importance of sustainable practices in the poultry industry.

REFERENCES

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. 2008. Influence of feed particle size on the performance, energy utilization, digestive tract development, and digesta parameters of broiler starters fed wheat- and corn-based diets. Poult. Sci. 87:2320-2328. doi:10.3382/ps.2008-00149.

Elshaghabee, F. M. F., N. Rokana, R. D. Gulhane, C. Sharma, and H. Panwar. 2017. Bacillus as potential probiotics: Status, concerns, and future perspectives. Front. Microbiol. 8:1490. doi:10.3389/fmicb.2017.01490.

Neal-McKinney, J.M., Lu, X., Duong, T., Larson, C.L., Call, D.R., Shah, D.H., Konkel, M.E., 2012. Production of organic acids by probiotic lactobacilli can be used to reduce pathogen load in poultry. PLoS One 7.

Nir, I., R. Hillel, G. Shefet, and Z. Nitsan. 1994. Effect of grain particle size on performance. 2. Grain texture interactions. Poult. Sci. 73:781-791.

Scott, M. L., S. J. Hull, and P. A. Mullenhoff. 1971. The calcium requirements of laying hens and effects of dietary oyster shell upon egg shell quality. Poult. Sci. 50:1055-1063. https://doi.org/10.3382/ps.0501055