2019

Evaluating the Effects of Exogenous Enzyme Supplementation on Broiler Growth

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Evaluating the Effects of Exogenous Enzyme Supplementation on Broiler Growth

Victoria E. Ayres

Thesis submitted to the Davis College of Agriculture, Natural Resources, and Design at
West Virginia University

In partial fulfillment of the requirements for the degree of

Master of Science in
Nutrition and Food Science

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Division of Animal and Nutritional Sciences

Morgantown, West Virginia
2019

Keywords: Broiler, NSPase, Phytase, Protease

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ABSTRACT

Experiments were conducted to investigate the effects of exogenous enzymes in poultry diets. In Chapter 4, a study was conducted to determine the effects of a corn-expressed recombinant carbohydrase (**AC1**) on broiler performance and digesta viscosity in high non-starch polysaccharide (**NSP**) diets through 21 days of age. One day-old Hubbard × Cobb 500 chicks were assigned to 6 dietary treatments. Each treatment consisted of 12 replicate pens of 10 birds. The positive control diet (**PC**) was a corn and soybean meal formulation. The negative control diet (**NC**) included 10% wheat and 10% corn distiller's dried grains with solubles (**DDGS**). The NC contained 100 kcal/kg less ME than the PC. Increasing inclusions of AC1 were applied to the NC to contain 50, 100, 200, and 400 U β -glucanase (**β -Glu-U**) per kg of feed. Preliminary experiments demonstrated AC1 homogeneity and stability post pelleting. Live weight gain (**LWG**) was the highest for PC fed birds from 1 to 14 d; however, birds fed NC with 400 β -Glu-U/kg also had similar LWG as the PC. Feed conversion ratio (**FCR**) from d 1 to 21 was lowest for PC fed birds; however, birds fed NC with 400 β -Glu-U/kg also had similar FCR as PC. Birds fed NC had lower LWG and higher viscosity than birds fed PC on day 14, but not on day 21. However, birds supplemented with 200 or 400 β -Glu-U/kg had similar 14 d digesta viscosity as birds fed PC. These data indicate that NSP ingredients may have a greater impact on digesta viscosity early in broiler growth and that AC1 at 200 and 400 β -Glu-U/kg produced similar results to PC.

A second study was then conducted (Chapter 2) to further investigate the effects of AC1 on dietary and intestinal viscosity and broiler performance when included in a high NSP diet. Nine hundred sixty, Hubbard x Ross 708, day-old, male broiler chicks were fed one of eight dietary treatments for 21d. Diets included a corn-soybean meal based diet (**PC₁**) and a diet of similar essential nutrient density, but with a 10% inclusion of both wheat and DDGS (**PC₂**) and a negative control (**NC**) with similar ingredients as PC₂, but with ME reduced by 125 kcal/kg.

Additional treatments had varying levels of AC1, supplying 50, 100, 200, 400, or 600 U β -Glucanase (**β -Glu-U**) per kg of feed, mixed into the NC diet. Dietary and digesta (d14) viscosity and weekly bird performance were measured. The inclusion of AC1 at 50-400 β -Glu-U/kg reduced FCR equivalent to PC₁. The results also showed that intestinal viscosity was correlated to d1-21 FCR and inversely correlated to d1-21 LWG. This study demonstrates that AC1 can reduce intestinal viscosity and improve early FCR in birds fed high viscosity diets and that an *in vitro* viscosity assay may be used to predict *in vivo* response.

In Chapter 3, a study was conducted to determine digestible amino acid concentrations and broiler performance of diets that vary in amino acid concentration and enzyme inclusion. Treatments included a PC (100% amino acid recommendations), NC (85% amino acid recommendations), and six additional diets containing commercially available enzyme supplements (Single Dose Phytase, Super Dose Phytase, Single Dose NSPase, Super Dose Phytase + Single Dose NSPase, Protease 1, and Protease 2) added to the NC based on manufacturers' recommendations. Diets were conditioned at 70°C and fed as crumbles to Hubbard x Ross 708 broiler chicks for 22d. Feeding NC with the inclusion of phytase, independent of dose or combination with the NSPase, and Protease 1 produced d22 feed conversion ratio similar to the PC. When feeding NC with the single dose NSPase, Protease 1, or Protease 2 d22 LWG was not comparable to the PC. These data suggest that the addition of phytase to an amino acid deficient diet can improve broiler performance.

Acknowledgements

This accomplishment could not have been achieved without the help of several people along the way. I would first like to thank my advisor, Dr. Joseph Moritz. I have been blessed with the opportunity to further my education with his continuous support and guidance. I would also like to thank my committee members, Dr. Robert Taylor and Dr. Janet Tou for their support throughout my research and graduate courses. Dr. Taylor, I have enjoyed learning about avian genetics and working with you throughout your project. Dr. Tou, thank you for your encouragement and kind words of advice. I would like to thank the West Virginia University Animal Science Farm staff and faculty, as well, for their assistance with our ongoing studies. I would also like to extend my gratitude to my fellow lab mates, as our area of study is quite labor intensive: Timothy Boltz, Talman Hylton, Angela Lamp, and Niles Ridgeway. Without their help, this achievement would not have been possible. Finally, I would like to thank my husband, Joshua Ayres, my parents, Richard and Janet Polentz, my sister, Alexandra Polentz, my grandparents, Ed Gayhart and Angee Polentz, and my late grandparents, Jay Polentz and Iris Sayre. I have been overwhelmed with your continuous support and encouragement; I would not be the person I am today without you.

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ABBREVIATION KEY

CHAPTER ONE

1. Phosphorous- P
2. National Research Council- NRC
3. Non-phytate phosphorous- nPP
4. Non-starch polysaccharide- NSP
5. Distillers' dried grains with solubles- DDGS
6. *Fytase* unit- FTU
7. Body weight gain- BWG
8. Feed conversion ratio- FCR
9. Feed intake- FI
10. Live weight gain- LWG
11. Pellet durability index- PDI
12. Modified pellet durability index- MPDI
13. True amino acid digestibility- TAAD

CHAPTER TWO

1. Non-starch polysaccharide- NSP
2. Distillers' dried grains with solubles- DDGS

3. Corn-produced recombinant enzyme- AC1
4. Corn-soybean meal based diet- PC_1
5. Corn-soybean meal based diet with 10% inclusion of wheat and DDGS- PC_2
6. Negative Control- NC
7. U β -Glucanase per kg of feed- β -Glu-U
8. Feed conversion ratio- FCR
9. Live weight gain- LWG
10. Anti-nutritive factor- ANF
11. Feed intake- FI

CHAPTER THREE

1. Positive Control- PC
2. Negative Control- NC
3. Non-starch polysaccharide enzyme- NSPase
4. Nitrogen- N
5. Amino acid- AA
6. Non-starch polysaccharide- NSP
7. Feed conversion ratio- FCR
8. Metabolizable energy- ME
9. Hot pellet temperature- HPT
10. Pellet durability index- PDI
11. Modified pellet durability index- MPDI
12. Gastrointestinal- GI
13. Feed intake- FI
14. Live weight gain- LWG
15. Fisher's least significant difference- LSD

16. Non-phytate phosphorous- nPP

17. Crude protein- CP

CHAPTER FOUR

1. Corn-expressed carbohydrase- AC1

2. Positive control- PC

3. Negative control- NC

4. Corn derived distillers' dried grains with solubles- DDGS

5. U β -Glucanase per kg of feed- β -Glu-U

6. Live weight gain- LWG

7. Anti-nutritional factors- ANF

8. Non-starch polysaccharide- NSP

9. Feed intake- FI

10. Feed conversion ratio- FCR

11. Fisher's least significant difference- LSD

CHAPTER ONE

LITERATURE REVIEW

I. Anti- nutritive factors

Phytate

Phosphorous (**P**) is included in poultry diets to aid in bone development and is essential for the utilization of energy. Phytic acid, *myo*-inositol hexaphosphoric acid, is an abundant compound in all seeds such as cereal grains and oilseeds. This compound also serves as the primary storage form of P in plants [1]. When P or other minerals are bound to phytic acid, the compound is known as phytin [2]. In commercial broiler diets, the majority of P is bound to phytate (*myo*- inositol hexakisphosphate, IP6). Studies report 60-70% of the P included in poultry feed is bound to phytic acid [3]. However, phytate is an anti-nutritive factor. Phosphorous becomes less available to the animal when bound to phytate, as poultry do not inherently obtain the phytase enzyme to free all of the bound P [4,5].

Phosphorous is crucial for proper skeletal growth and integrity as well as growth performance. However, because P is bound to phytate, it is often given in moderately excessive amounts than what is recommended by the National Research Council (**NRC**) to guarantee a safety margin [6]. Roberson et al. [7] found increased tibia strength and bone ash at 15 and 17 weeks of age when turkey toms were fed diets containing 145% versus 100 or 75% of the NRC recommended levels of Ca and non-phytate phosphorous (**nPP**). Along with increased tibia strength and bone ash, litter P was also increased [7].

While there are advantages for feeding P in higher quantities, the unabsorbed P is excreted in the manure and accumulates in the soil, leading to run-off. With the addition of nitrogen, these compounds can have harmful effects on the environment, causing eutrophication, which is a

detrimental condition in aquatic areas, leading to fish kills and algal blooms [4, 8]. These high levels of P are the most common cause of waterway eutrophication [9].

Non-starch polysaccharides

In the United States, the primary cereal grains used in diet formations are corn and soybeans. However, the least-cost diet formulation often requires using other grains or agricultural by-products, which may contain variable levels of anti-nutritive factors. The cell wall of cereals is composed primarily of complex carbohydrates, which are loosely termed “non-starch polysaccharides (NSPs) [10]. Corn derived distillers’ dried grains with solubles (DDGS) is a by-product of ethanol production and contains higher amounts of NSPs relative to corn [11]. Other cereal grains, such as wheat, also contain higher levels of NSPs. Annison and Choct [10] found that arabinoxylans found in wheat severely affected broilers’ growth and feed conversion efficiency. The study also found that wheat pentosans caused a general inhibition of nutrient digestion, which affected starch, fat, and protein [10]. One explanation of how pentosans elicit an anti-nutritive effect is by influencing the rate of passage of digesta. Non-starch polysaccharides increase digesta viscosity, reducing the rate of passage of digestive enzymes and do not allow mixing with gut contents [12, 13]. Other authors have also reported that the presence of NSPs can lead to increased intestinal viscosity, reduced nutrient digestibility, increased FCR, and decreased bird performance [14-17].

II. Enzyme Supplementation

According to Khattack and coauthors [18], an enzyme’s essential characteristic is to catalyze the rate of a reaction but is not themselves altered by it. Enzymes are involved in all anabolic and catabolic pathways of digestion and metabolism and are specific catalysts acting on one or limited groups of substrates [18]. Ravindran [19] reported that the potential nutritive value of ingredients

is not realized at the bird level and there is no common feed ingredient that is digested 100%. Therefore, the need to improve the digestion of these undigested substrates is the primary rationale for the use of exogenous enzymes [19]. However, enzymes can improve other aspects of the poultry industry as well. For instance, the range of feedstuffs utilized may be increased, as well as the flexibility in diet formulations [20]. Variability in the nutritive value between batches of ingredients may also be reduced and gut health can be improved [20]. As intestinal morphology improves, excreta moisture content has also been shown to decrease [21,22], as well as manure output. Animals have also been shown to have more uniformity at the time of market [19].

Phytase

A common exogenous enzyme utilized in many commercial poultry operations is known as phytase. Many animal nutritionists use exogenous phytase to enable the animal to absorb more P and reduce environmental pollution. Phytases may be defined as phosphatase enzymes able to catalyze the hydrolysis of phosphate ester bonds [2]. In order to release the P from phytate, the phytate must first be hydrolyzed into inorganic P. This dephosphorylation of phytic acid is the result of phytase activity. Phytases make up a family of enzymes that activate the release of inorganic orthophosphate from phytate in a step-wise manner, which produces five classes of intermediate products [23]. These enzymes are classified in one of two categories: 3-phytases and 6-phytases. The 3-phytases begin the dephosphorylation of phytic acid at the third carbon on the inositol ring, while 6-phytases begin dephosphorylation at the sixth carbon [24]. While the complete hydrolysis of phytic acid is possible in a lab setting, Cowieson and coauthors demonstrated that, on average, 4 phosphate moieties were removed from the inositol ring and made available for retention by the birds [25]. Maenz also notes that the full sequence of hydrolysis of phytic acid down to *myoinositol* has yet to be determined [26].

Several feed ingredients such as wheat, barley, and wheat bran are rich in phytase activity, while corn, sorghum, oats, and oilseed meals are not [23]. The addition of phytase not only improves the absorption of P, but also other minerals such as calcium, magnesium, zinc, and iron [1, 27]. Officer and Batterham [28] also found improvements of ileal digestibility of protein and essential amino acids in pigs, with the addition of phytase. Nitrogen digestibility has been shown to improve with this supplementation as well [29-31]. Shirley and Edwards found that higher levels of phytase may also improve nutrient utilization [32]. However, there is high variability in phytase data such as dosage levels and other enzyme characteristics. Therefore, there is no specific requirement to include in the diet.

One phytase unit (**FTU**) is defined as the quantity of enzyme required to liberate 1 μ mol of inorganic P/min, at pH 5.5, from an excess of 15 μ M sodium phytate at 37°C [33]. However, when calculating a dosage to include in the diet, caution should be utilized, as *in-vitro* retained activity may not always predict bird performance. Assays may also vary between companies [34].

Phytase activity was previously thought to plateau at 500 FTU in diets sufficient in total P [35, 36]. This inclusion level is in agreement with Cowieson and coauthors [25]. In this experiment, it was found that little benefits were obtained by adding phytase above 600 FTU, while significant benefits were seen with the addition of 150 or 300 FTU. However, Shirley and Edwards [32] have shown that the addition of 12,000 FTU of phytase significantly improved bird performance. Not only did this addition improve the utilization of phytate bound P, but also increased nitrogen retention by 33%. Birds given the high dose of phytase were also able to utilize an additional 200 ME/kg diet. This suggests that the inclusion of phytase at higher levels may influence more than mineral utilization. Shirley and Edwards [32] also note that this increase in nutrient utilization may be due to the ability of phytase to disrupt various interactions between

phytate, minerals, starch, and protein, thus allowing a greater utilization of all nutrients in the diet. Walk and coauthors [37] found that the supplementation of additional phytase (above 500 FTU) may improve feed efficiency in broilers by alleviating anti-nutritive factors, rather than solely releasing phytate bound P. Walk and coauthors [38] also investigated the effects of super-dosing phytase at 1,000 or 1,500 U/kg. In this experiment, they found that the super-doses of phytase lead to almost total hydrolysis of phytate, an increase in inositol concentration in the gizzard, and improved broiler performance. When the negative control was supplemented with 1,500 U/kg of phytase, both feed intake (**FI**) and body weight gain (**BWG**) increased by 0.066 kg and 0.085 kg, respectively. Consequently, feed conversion ratio (**FCR**) also improved by 0.05 points [38]. This data agrees with Cowieson and coauthors [25], as high doses of phytase improved apparent phytate P digestibility as well as total P digestibility compared to lower doses of phytase. This high inclusion of 24,000 FTU of phytase improved toe ash percentage by 3% and also improved the utilization of several nutrients more so than the lower inclusions.

Phytase is utilized by many commercial operations. Although, there is high variability in phytase data, as the phytase source may contribute to the varying characteristics and effectiveness of this enzyme. There are numerous commercially available phytases, both bacterial and fungal, which have been shown to increase FI, live weight gain (**LWG**), tibia ash percent and weight, and decrease FCR, ultimately improving bird performance [25, 38]. Research conducted by Pillai and co-authors [39] found that phytase from bacteria improves growth, bone, and carcass performance. In this experiment, phytase sourced from bacteria was more efficient than fungal phytase products at releasing phytate-bound P. Li and coauthors [40] found that when supplementing 1000 FTU of phytase sourced from bacteria, FI increased by 124 g/bird, BW gain increased by 117 g/bird, and tibia ash weight also increased by 69 mg/tibia.

As an alternative to using exogenous phytase supplementation, genetic modifications have also been explored. These modifications have been implemented in several plant seeds to increase phytate-degrading activity. In 1993, Pen and coauthors [41] engineered a phytase from *Aspergillus niger* in tobacco seeds. This transgenic seed was comparable to that of fungal phytase or the addition of P in the diet, as growth rate was improved with this inclusion. However, tobacco seeds are not typically included in commercial poultry diets and more research was required. Denbow and coauthors [42] utilized this information and compared the effects of phytase as a commercial supplement and phytase as a recombinant protein in transformed soybeans. Adding 1200 FTU phytase as transformed seeds to the basal diet provided BWG similar to that of the basal diet supplemented with 0.24% nPP. When added on an equal activity basis, phytase from transformed soybeans or commercial microbial phytase were equally effective in enhancing BWG, FI, feed efficiency, toe ash, tibia shear force, and energy [42].

While this is valuable information, one must consider the thermal stability of the enzyme prior to transforming seeds. Soybeans must undergo thermal processing before their addition to the diet, which could damage the enzyme. Corn, however, is also commonly included in poultry diets, and is not subjected to thermal processing prior to its inclusion. With this knowledge, Nyannor and coauthors [43] utilized a corn expressing phytase. These authors found that, with the addition of transformed corn, digesta cell walls degraded more rapidly and phytic acid P concentration decreased in broiler chicks. In agreement with Nyannor and cohorts, Gao and coauthors [44] found that phytase expressed in corn had comparable results with commercial microbial phytases. This is in agreement with Naynorr and Adeola [45] that a corn-based phytase was as efficacious as a microbial phytase. These authors found that the corn-based phytase fed to broiler chicks resulted in an improvement in bone mineralization, comparable to that of a microbial

expressed phytase [45]. In disagreement, Homan and Moritz [46] found that birds provided diets with a granulated bacterial phytase resulted in increased performance and tibia mineralization compared to broiler chicks fed mash diets with a transgenic phytase corn. The authors hypothesized that this difference could be due to variations in dispersion of enzyme within the diet. The transgenic phytase had a larger particle size than the granulated bacterial phytase, which the broilers would be less likely to consume, compared to the smaller more frequent particles of the granulated bacterial phytase. Also, differences may be due to the general effectiveness of the expressed enzyme, as the expression system of the granulated bacterial phytase may have been superior to the transgenic phytase corn [46].

Carbohydrase

Poultry do not naturally obtain adequate enzymes for the hydrolysis of NSPs in the cell walls of cereal grains; therefore, these NSPs often remained un-hydrolyzed and can result in low feed efficiency [18]. The negative effects associated with NSPs can be ameliorated by the supplementation of carbohydrases. These enzymes break down the NSPs, decrease intestinal viscosity, and can improve nutrient digestibility [18, 19, 47]. Similar to phytase, these NSP-degrading enzymes can be produced via microbial sources or produced through genetic modification.

Often times, enzyme “cocktails,” containing a variety of enzymes, are supplemented into diet formulations. These cocktails make substrates more available for the enzymes to degrade. Meng and coauthors [17] found that by supplementing an enzyme cocktail supplying 100 U of xylanase, 400 U of glucanase, 1,000 U of pectinase, 120 U of cellulose, 280 U of mannase, and 180 U of galactanase per kilogram of a corn based diet, broilers had an improved FCR. The authors also concluded that using an appropriate multi-carbohydrase enzyme supplement could enhance

the nutrient utilization of a corn-soybean meal based diet [17]. Similar results were also observed by Coppedge and coauthors [48]. Birds were supplemented with an enzyme cocktail during the grower phase, which supplemented 1,500 U of xylanase, 1,100 U of β -glucanase, 35 U of α -galactosidase, and 110 U of β -mannanase included in a corn and soybean meal based diet. These authors concluded that NSP-degrading enzymes had the potential to improve growth performance and processing parameters of broilers [48].

Carbohydrases are often supplemented with other enzymes such as proteases, which are necessary for protein digestion and to generate amino acids, and phytases. Many nutritionists utilize a combination of carbohydrases and proteases to improve nutrient retention and performance of chicks. Ravindran et al. [49] found that there were no negative interactions between phytase and xylanase in wheat-based diets. In fact, the inclusion of both enzymes may be advantageous. Cowieson and Adeola [50] also agreed that the combination of multiple enzymes was highly effective to improve broiler performance. Specifically, the addition of xylanase, amylase, protease and phytase can improve digestibility of nutrients and performance of birds fed a diet suboptimal in terms of Ca, P, and ME. Juanpere and coauthors [51] also examined the effects of a microbial 3-phytase and a glycosidase on corn, wheat, or barley based diets. These authors concluded that both enzymes acted independently of one another, and suggested that they may be combined in high NSP diets, without serious negative interactions. Olukosi and cohorts [52] also concluded that the combination of a phytase and an enzyme cocktail containing xylanase, amylase, and protease improved performance of chicks fed a corn-soybean meal based diet, marginally deficient in terms of energy and P.

III. Feed Manufacture

Mash

In the United States, poultry diets are typically composed of several feedstuffs such as cereal grains, soybean meal, animal byproduct meals, fats and vitamin/mineral premixes. Along with water, these ingredients provide the bird with energy and nutrients that are critical for growth, reproduction, and health. [3]. The inclusion levels of these ingredients can vary between each batch and the type of bird that is being fed, as the least cost diet formulation is utilized [53]. Along with these ingredients, other supplements, including exogenous enzymes, probiotics, prebiotics, organic acids, and phytogenics may also be included in the diet. These ingredients are then blended together using a mixer. Feed mixers typically have ribbons and/or paddles inside to evenly blend the ingredients together [54]. After mixing, the ingredients can either be left as a mash feed or they can be further processed to become pelleted feed.

Steam Conditioning

If the feed is to be pelleted, it must first be conditioned. Conditioning equipment consists of a horizontal chamber, that applies steam to the mash feed before extrusion [55]. The temperature inside the conditioner and the time that the feed is inside the chamber can vary between formulation or customer/management desire. Increasing conditioning temperature improves pellet quality by increasing the heat and moisture within the feed [56]. Loar and cohorts [57] also reported increased pellet quality with an increase of conditioning temperature. Cutlip and coauthors [58] found that when compared to low conditioning temperatures, pellet durability index (**PDI**) and modified pellet durability index (**MPDI**) increased with conditioning temperatures. While high conditioning temperatures have been shown to increase pellet quality and improve feed hygienics, other consequences should also be taken into account. Homan and Moritz [46] found that when

conditioning temperature was increased, broiler performance suffered as well as tibia mineralization. When examining various phytase products, these authors found that increased conditioning temperatures may have decreased nutrient availability, as well as denaturation of the phytase products.

Amino acids are also highly susceptible to heat. At high temperatures, the proteins in the feed could be denatured, which can be detrimental to the animals [58]. Loar and coauthors [57] found that when conditioning at 85°C and 96°C, compared to 74°C, true amino acid digestibility (TAAD) of Met, Ile, and Pro decreased by 3 to 5% ($P < 0.05$) and Lys, Val, and Leu tended to decrease by 3 to 6% ($P < 0.10$). In agreement with this study, Boney and coauthors also found that when diets were conditioned at 91°C, several amino acids had decreased digestibility [59].

Hygienizer

A second piece of equipment utilized during the pelleting process is the hygienizer. Peisker [60] notes that while pelleting does have an effect on the hygienic status of the feed, a short-term conditioner alone or in combination with a pellet press, is not sufficient for decontamination of pathogenic microorganisms. However, sufficient decontamination can be achieved with the use of a hygienizer, a horizontal retention screw or vertical shaft [60]. Instead of adding steam to improve pellet quality like the conditioner, the hygienizer maintains feed temperature for an extended time to reduce the prevalence of harmful microorganisms like *Salmonella*. This pathogen, in particular, can persist for a long period of time in a variety of materials. It can also be found in feed mills and contaminate the feed being produced [61]. By utilizing the hygienizer, *Salmonella* and other microorganisms can be decreased, making the feed more sterile. Unfortunately, the full effects of the hygienizer on feed is unknown and more research is required.

Pelleting

Pelleting can be defined as “a process that uses moisture, heat, and pressure to agglomerate smaller particles into larger particles” [62-64]. Pelleted feeds are often used in the commercial poultry industry. Steam is first added to mash feed for a certain length of time and at a specific temperature, both that can vary between operations. The mash feed is then extruded through a pellet die, which shapes the pellets. By pelleting feed, there is less ingredient segregation and, in theory, the animals receive each nutrient in every pellet [65]. Birds also expend less energy during consumption of pellets relative to consuming mash. The animal is able to utilize energy more efficiently, as they are spending less time grasping the feed, and the energy becomes available for other functions. Consequently, feed efficiency is improved, as well as growth uniformity [65]. In contrast, however, Glover and coauthors [66] found a greater coefficient of variation as birds given improved crumble/pellet percentage were also shown to have a higher within-pen variation in body weight. These authors concluded that the within-pen coefficient of variation of body weight may have partially been affected by the feeder space available. It is hypothesized that the larger, more dominant birds monopolized the feeder space access, allowing them to consume the majority of the crumbles/pellets and leaving the fines for the smaller, less-dominant birds [66].

Feed form is also an important factor to consider. Massquetto and coauthors [67] concluded that when feed form is improved, FI, weight gain, and ileal digestibility of dry matter, crude protein, and energy are also increased. Cutlip and coauthors [58] also noted that small improvements in pellet quality may also improve feed efficiency while maintaining a similar weight gain. Lilly and coauthors [68] observed similar results, as birds fed medium or high pellet quality diets consumed more feed than those fed low quality pellets. Consequently, the medium

and high pellet quality diets increased LWG and carcass weight compared to the low pellet quality diet. However, the variation of feed form had a lesser effect on FCR.

IV. Conclusions

In conclusion, anti-nutritive factors such as phytate and NSPs are found in various feedstuffs and ingredients utilized in broiler diets. By including these ingredients, however, the least cost diet formulation may be achieved. With the inclusion of phytase in the diet, broiler chickens are better able to utilize P. This is not only beneficial for the bird, as P is imperative for energy and growth, but also for the environment as the combination of P and N can lead to eutrophication of waterways. In addition to phytate, NSPs can impair the digestive system, as digesta viscosity has been shown to increase with diets containing a high concentration of NSPs. When the digesta is more viscous, nutrients become less available to the bird and are more difficult for the bird to absorb. However, these negative effects associated with NSPs can be ameliorated when carbohydrases are included in the diet. Similar to phytase, carbohydrases allow producers to achieve the least cost diet formulation, as these enzymes enable the animal to utilize more nutrients. Both enzymes are traditionally produced via microbial sources; however, there is much research to be completed concerning transgenic enzymes.

V. Future Research

While most enzymes are produced by bacterial or fungal sources, it is imperative that the poultry industry studies the use of transgenic enzymes as well. Multiple studies have shown that transgenic enzymes have similar efficacy as the traditional, microbial produced enzymes. However, prior to their use in poultry diets, they must be examined for thermal stability. Poultry feed is often pelleted in the commercial industry and is subjected to high temperatures. These temperatures could denature the enzymes, ultimately decreasing enzyme activity. Therefore, it is

imperative to perform additional analyses on transgenic enzymes when exposed to high temperatures during the pelleting process.

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CHAPTER TWO

Viscosity and growth response of broilers fed high fiber diets supplemented with a corn-produced recombinant carbohydrase

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SUMMARY Feed ingredients containing high levels of non-starch polysaccharides (**NSP**), such as wheat and corn distillers' dried grains with solubles (**DDGS**), can form viscous digesta in the gastrointestinal tract, limiting broiler performance. A study was conducted to determine efficacy of a corn-produced recombinant carbohydrase (**AC1**) on dietary and intestinal viscosity and broiler performance with a high NSP diet. Hubbard x Ross 708, day-old, male broiler chicks (n=960) were fed one of eight dietary treatments for 21d. Diets included a corn-soybean meal based diet (**PC_1**) and a diet of similar essential nutrient density, but with a 10% inclusion of both wheat and DDGS (**PC_2**) and a negative control (**NC**) with similar ingredients as PC_2, but with ME reduced by 125 kcal/kg. Additional treatments had varying levels of AC1, supplying 50, 100, 200, 400, or 600 U β -Glucanase (**β -Glu-U**) per kg of feed, mixed into the NC diet. Dietary and digesta (d14) viscosity and weekly bird performance were measured. The inclusion of wheat and DDGS increased d1-7 feed conversion ratio (**FCR**) and supplementing AC1 at 50-400 β -Glu-U/kg reduced FCR equivalent to PC_1. Intestinal viscosity correlated with d1-21 FCR and inversely correlated with d1-21 live weight gain (**LWG**). When analyzed categorically, dietary viscosity inversely correlated with d1-21 LWG ($P<0.05$). This study demonstrates that AC1 can reduce intestinal viscosity and improve early FCR in birds fed high viscosity diets and that an *in vitro* viscosity can correlate to broiler performance.

DESCRIPTION OF PROBLEM

The primary cereal grains used in diet formulations in the US poultry industry are corn and soybean meal [1-3]. However, sometimes other grains or agricultural by-products with variable levels of anti-nutritive factors (**ANF**) are used to decrease dietary costs. Corn distillers' dried grains with solubles (**DDGS**) are a by-product of ethanol production and contain low amounts of starch and higher levels of non-starch polysaccharides (**NSP**) relative to corn [4]. Other cereal grains, such as wheat, contain NSP at concentrations that could be detrimental to broiler performance [5]. The presence of NSP can lead to increased intestinal viscosity, reduced nutrient digestibility, increased feed conversion ratio (**FCR**), and decreased overall bird performance [1, 3, 6, 7]. Beta-glucan, a specific NSP found in both wheat and DDGS, is partially water soluble and contributes to the formation of a gel-like viscous layer within the gastrointestinal (**GI**) tract of the broiler [8]. Viscous digesta is known to reduce the rate of diffusion of feed substrates, digestive enzymes, and their products, thereby reducing the absorption of nutrients in the diet [9-11]. Beta-glucans may also increase microflora growth, as well as wet, sticky excreta [8, 12, 13].

To combat the negative effects associated with dietary NSP, poultry nutritionists have employed the use of carbohydrase enzymes, as poultry are not capable of adequately digesting these compounds [14-17]. Specifically, β -glucanase is a hydrolytic carbohydrase, targeting glycosidic bonds in barley, oat, or wheat based diets and has been shown to improve feed conversion efficiency, lower intestinal viscosity, and increase feed intake [18- 25].

Lowering ileal viscosity is a demonstrated mode of action for β -glucanase [17, 18, 26]. However, testing the efficacy of enzymes on reducing intestinal viscosity in feeding studies is time consuming and is associated with a high degree of variability. There are many factors that contribute to variability in broiler viscosity studies, including grain types and quality, substrate

concentrations, NSP solubility, feed preparation and processing, and bird age. Viscosity test conditions may also vary in viscometer type and operating conditions, and the amount of substrate used [9, 18, 26]. Bedford and Classen [27] reported an *in vitro* dietary viscosity assay where the resulting data had a strong correlation to *in vivo* proximal ($R^2 = 0.76$, $P < 0.001$) and distal jejunal intestinal viscosity ($R^2 = 0.66$, $P < 0.001$). Based on these results, the authors concluded that the *in vitro* viscosity assay is reliable for predicting the *in vivo* viscosity and weights of birds fed corresponding diets [27]. Other researchers [28] compared *in vitro* dietary and *in vivo* intestinal viscosity with various xylanase supplementations and found weak but significant correlations when using either 0.05 or 0.1 M HCl in the pepsin digestion phase. These authors concluded that *in vitro* viscosity may also be used for predicting an *in vivo* intestinal response to xylanase treatments [28].

Traditionally, exogenous enzymes have been microbially expressed and are formulated into a granulated, coated, or liquid dietary ingredient. However, an alternative to these traditional enzyme products are recombinant enzymes produced in transgenic grain, which provides a significant economic production advantage [29-37]. In the current study, a recombinant carbohydrase was expressed in corn grain (**AC1**, Agrivida) using methods described before [20, 21, 38, 39]. Previous research with AC1 demonstrated heat tolerance during feed pelleting [20, 21] and reduced ileal viscosity of broilers fed 200 or 400 U β -Glucanase (**β -Glu-U**) of AC1 per kg of feed [20].

The objectives of the current study were to (1) test AC1 enzyme efficacy on dietary (*in vitro*) and intestinal (*in vivo*) viscosity and subsequent animal performance with a high NSP diet and (2) determine whether *in vitro* viscosity, *in vivo* viscosity and broiler performance are correlated.

MATERIALS AND METHODS

Diet Formulations

Diets consisted of two positive controls (**PC_1** and **PC_2**), each formulated with 3,050 kcal/kg of metabolizable energy (**ME**; **Table 1**). The PC diets were formulated using ME and mineral concentrations reported in Agristat [40], and digestible amino acid concentrations based on recommendations by Tillman and Dozier [41]. The PC_1 diet was a typical corn-soybean meal based diet and PC_2 was a corn-soybean meal based diet with a 10% inclusion of wheat and a 10% inclusion of DDGS. The negative control (**NC**) was formulated with similar ingredients as PC_2 diet, except that the ME was reduced by 125 kcal/kg by decreasing soybean oil inclusion (**Table 1**). The NC diet was supplemented with AC1 to supply 50, 100, 200, 400, or 600 β -Glu-U/kg to make the other five dietary treatments. Wheat from three different sources [42-44] were acquired and tested for viscosity, and the wheat source with the highest analyzed viscosity was chosen to be included in the PC_2, NC and AC1 diets.

Wheat sample digestion for viscosity measurement

Duplicate samples of each wheat source were ground with both an air-assisted hammer mill with 5mm screen size and Wiley Mill with 1mm screen size. After all wheat had been ground, digestion was simulated and viscosity was determined using methods described by Bedford and Classen [27, 45].

Feed Manufacture and Diet Viscosity

All feed was manufactured at the West Virginia University pilot feed mill. A total of 907.18 kg of feed was batched according to the NC diet formulation (**Table 1**). The NC diet was then split into 6 allotments of 136.08 kg each. One of the 6 allotments was kept as the NC diet, and the other five were mixed with AC1 corn meal at their respective target doses. For each enzyme

diet, AC1 was premixed with a 3-kg sample of NC feed in a small paddle mixer prior to mixing with the remaining NC feed in a single-screw vertical mixer [47]. Both PC_1 and PC_2 treatments required 158.76 kg of feed per treatment. Wheat utilized in the PC_2, NC and AC1 diets was ground using a roller mill. All diets were fed in mash form and particle size was determined using a Ro-Tap Particle Size Analyzer [48] (**Table 3**). Complete feed samples for each treatment were collected post-enzyme addition for proximate analysis and β -glucanase activity (**Table 3**).

Samples from each diet were ground using a hammer mill and examined for viscosity in triplicate. Methodology of the pepsin-pancreatin digestion was similar to that used for wheat sample digestion as described above; however, to obtain an adequate amount of supernatant, the amount of the reagents/solutions utilized in the digestion series were doubled.

Live Bird Performance

A total of 960 1-day-old Hubbard x Ross 708 male chicks were obtained from a commercial hatchery [51]. On d1, the chicks were separated by weight class. Then, one bird from each weight class was selected and placed into a group of 10 total chicks, and the group was placed in 1 of 96 raised wire cages to create uniform, initial pen weights. One of the 8 diets was randomly assigned to each pen within a block. A block consisted of 8 adjacent cages; a total of 12 blocks were utilized. Chicks were housed in raised wire cages in a cross-ventilated, negative-pressure room for 21d. Two identical rooms were utilized; each containing 48 cages, creating a total of 96 cages. Standard rearing conditions were utilized throughout the study [52]. The animal performance variables measured included: d1 starting pen weight and d7, 14 and 21 bird weights, bird feed intake (**FI**), live weight gain/bird (**LWG**), mortality corrected FCR and percent mortality. All animals were reared according to protocols approved by West Virginia University Animal Care and Use Committee.

In vivo Intestinal Digesta Collection and Viscosity Measurements

On d14, four birds from each pen were euthanized via cervical dislocation. The entire digestive tract (beginning of duodenum to the ileal-cecal junction) was removed, and the digesta was squeezed out by hand into a 50mL centrifuge tube. The digesta was then centrifuged, using methodologies described by Bedford and Classen [1, 53]. To determine *in-vivo* intestinal viscosity, methodologies were similar to previous research [20, 55-58], with measurements taken at 30s or 1 min and at two different centrifugal speeds: 10 x *g* or 20 x *g*.

AC1 Enzyme Activity Assay

AC1 enzyme activity in transgenic maize grain or in manufactured feed was tested as described previously [20, 21].

Statistical Analysis

All data were analyzed in a randomized complete block design with the raised wire cage location within room determining block. The experimental unit was one pen containing 10 Hubbard x Ross 708 male chicks. The PROC GLM procedure of SAS [59] was used to analyze data by one-way ANOVA. Means were then further separated using Fisher's least significant difference (**LSD**) post hoc comparison when the ANOVA was significant ($P \leq 0.05$). Correlations between replicated control treatments (PC_1, PC_2, and NC) and the overall AC1 response (averaged by replicate, across all AC1 treatments) were determined using the PROC CORR procedure in SAS [59]. In addition, since *in vitro* dietary viscosity data was performed on feed obtained from a single batch of feed without replication of the manufacture process, these data were categorical by design and a second correlation was determined using these data and treatment mean results from live study metrics. Strength of correlation was determined using R-values and accompanying *P* values to demonstrate the significance of correlation.

RESULTS AND DISCUSSION

Wheat grown in different environments may have some level of variation in its nutritive and anti-nutritive values, therefore, broiler intestinal viscosity responses may differ when supplementing the diet with digestive enzymes. These variations are likely reflecting the differences in soluble NSP levels in wheat [60]. To ensure that increases in dietary viscosity were measurable in this study, the viscosity of three wheat varieties were examined before feed formulation. As demonstrated in **Table 2**, Source B had consistently higher viscosity compared to the other two sources; therefore, Source B wheat was chosen to be incorporated into the PC_2, NC and AC1 diets to test AC1 efficacy and intestinal viscosity response.

The *in vitro*, dietary viscosity results from the experimental treatment diets can be found in **Table 3**. *In vitro*, dietary viscosity of the formulated feed was numerically higher in diets including wheat and DDGS such as PC_2 (2.02 cP) and NC (1.87 cP), compared to a common corn-soybean meal based diet (PC_1; 1.81 cP). The authors hypothesize that the higher viscosity in PC_2 could be due to the combination of wheat, DDGS, and increased soybean oil inclusion (**Table 1**). Differences in particle size between treatments were not apparent (**Table 3**). AC1 enzyme activity recovered from each diet approximated the target dosage ± 43 U/kg shown in **Table 3**. *In vitro*, dietary viscosity was reduced with AC1 addition to the NC diet, in a step-wise manner, through 400 β -Glu-U/kg. For example, AC1 inclusion at 50 β -Glu-U/kg lowered dietary viscosity (1.82 cP) similar to the level of PC_1. As AC1 enzyme dosages increased in the diets from 200 to 600 β -Glu-U/kg, the dietary viscosity was further reduced within the range of 1.66 – 1.74 cP.

Table 4 shows the *in vivo*, intestinal viscosity response to AC1 treatments. Birds fed NC or PC_2 diets had the highest intestinal viscosity (3.06 and 3.08 cP, respectively) whereas birds

fed PC_1 had significantly lower viscosity (2.73 cP; $P < 0.05$; 10 x g for 30 s). All AC1 treatments had statistically equivalent intestinal viscosity as PC_1 ($P > 0.05$). The treatments containing 100 or 600 β -Glu-U/kg AC1 had significantly lower intestinal viscosity than the NC and PC_2 treatments ($P < 0.05$). These data demonstrate AC1 efficacy in lowering digesta viscosity; although trends toward decreased viscosity associated with the enzyme titration were not apparent. Perhaps variability of the viscosity assay and live bird model contributed to these results. In general, these results are in agreement with previously published data that demonstrated the ability of carbohydrase enzymes to reduce digesta viscosity [11, 20, 26, 61-65].

Broiler performance metrics are exhibited in **Table 5**. The addition of AC1 (50-400 β -Glu-U/kg) to the reduced ME, wheat and DDGS supplemented diet resulted in equivalent ($P > 0.05$) d1-7 FCR as the PC_1 (higher energy, corn-soybean only). From d 1-13, FCR was equivalent ($P > 0.05$) between birds fed 50 β -Glu-U/kg and the PC_1 diets. However, no AC1 dose response was observed for any growth performance variable. As reported by Steinfeldt and co-authors [65], the response to exogenous dietary enzyme addition was most notable during the first few weeks of life, which was similarly observed in the current study. Overall, from d 1-21, birds fed the corn-soybean only diet (PC_1) had the lowest FCR compared to all wheat and DDGS supplemented diets ($P < 0.001$). Unlike previous research [20, 36] that demonstrated an increase in broiler weight when AC1 was fed for 14 or 18 d, no difference in LWG ($P > 0.05$) was observed among treatments in the current study.

Using replicated pen data from the control treatments (PC_1, NC, and PC_2) and averages of all AC1 treatments (averaged by replicate; n = 12) demonstrated that *in vivo*, intestinal viscosity inversely correlated ($r = -0.387$; $P < 0.01$) with d1-21 LWG and correlated ($r = 0.326$; $P < 0.05$) with d1-21 FCR (**Table 6**). The intestinal viscosity from AC1 treatments were averaged across

doses to decrease variability associated with individual AC1 treatments prior to correlation analysis because the AC1 response observed was not dose dependent for growth performance. While the authors appreciate that use of categorical data decreases the power of correlation analysis, *in vitro*, dietary viscosity data was performed on feed obtained from a single batch of feed without replication of the manufacture process. These data were considered categorical, therefore the *in vivo* study metrics were averaged across replicate pens within each treatment for the second correlation determination (**Table 7**). The categorical data demonstrated *in vitro* dietary and *in vivo* intestinal viscosity inversely correlated ($P < 0.05$; $r = -0.725$ and -0.387 , respectively) with d1-21 LWG. There was a tendency for *in vitro* dietary viscosity to correlate ($r = 0.604$) with *in vivo* intestinal viscosity ($P = 0.11$) and inversely correlate ($r = -0.611$) with d1-13 FCR ($P = 0.11$).

In previous research [27, 28], feed assays have been explored as a more rapid and inexpensive, alternative method to live bird studies to assess viscosity differences among feed ingredients and efficacy of carbohydrases. Bedford and Classen [27] reported that dietary viscosity from two-stage simulated GI digesta can be used to predict an intestinal response to enzymes in rye-based diets. Those authors examined several amounts of substrate (complete diet) and incubation times to determine the optimum conditions for this procedure. Those authors found that the logarithmic values of the viscosities in the experimental diets correlated well with intestinal viscosity measurements and that *in vitro*, dietary viscosity was able to predict final body weights of the birds. A similar response was observed in the current study, as demonstrated by dietary and intestinal viscosity correlating (inversely) with d1-21 LWG. Murphy et al. [28] examined eighteen xylanases and detected a weak but significant relationship between *in vitro* and *in vivo* viscosity data when testing xylanases on wheat-based diets, but found no relationship between LWG and

jejunal digesta viscosity. However, unlike Bedford and Classen [27], these authors examined xylanase using wheat, alone, instead of a complete diet.

In conclusion, the addition of a highly viscous wheat variety together with DDGS increased dietary and intestinal viscosity providing a model to examine the response of exogenous enzyme supplementation. In this study, AC1 was able to decrease intestinal viscosity, comparable to that of the corn-soybean meal control treatment (PC_1). As demonstrated with this model, AC1 may be most efficacious to feed during early development, as d1-7 FCR was improved with AC1 inclusion between 50 and 400 β -Glu-U/kg. The inclusion of AC1 at 50 β -Glu-U/kg also produced d1-13 FCR comparable to that of PC_1. Utilizing control and averaged AC1 treatments, correlations were observed between intestinal viscosity and growth performance. Analysis of all treatments categorically demonstrated a correlation between *in vitro* dietary viscosity and d1-21 LWG ($P < 0.05$).

CONCLUSIONS AND APPLICATIONS

1. Descriptive diet and replicated intestinal viscosity numerically and statistically increased respectively when 10% wheat and 10% DDGS was supplemented to corn-soybean meal based diets. Inclusion of AC1 to the wheat-DDGS supplemented diet produced viscosity levels comparable to the corn-soybean meal based diet.
2. Early animal performance corresponded to viscosity response, in that addition of wheat and DDGS increase d1-7 FCR and supplementing AC1 from 50 to 400 β -Glu-U/kg to this higher viscosity diet improved FCR equivalent to the birds fed the corn-soybean meal only diet.
3. Intestinal viscosity correlated to d1-21 FCR and LWG (inversely) when comparing the control (PC_1, NC, and PC_2) and averaged AC1 treatments.

4. Analysis of all treatments categorically demonstrated a correlation between *in vitro* dietary viscosity and d1-21 LWG.

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46. TC-602 Refrigerated Bath, Brookfield Engineering Laboratories Inc., Middleboro, MA.
47. Vertical mixer, Avery Weigh-Tronix, Fairmont, MN.
48. Particle size of the crumbled feed was determined using a Ro-Tap Particle Size Analyzer [49]. One hundred grams of each crumbled diet was placed in a dust-tight enclosed series of stacked ASTM screens affixed to the Ro-Tap Particle Size Analyzer

- and shaken for 10 minutes. The screens were then separated and weighed. Particle size was then determined by subtracting the weight of the screen from the final weight of the screen and sample after shaking. The mean geometric particle size and log normal geometric standard deviation were calculated as described by McElhiney [50].
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 53. Digesta was centrifuged [54] at 12,700 x g for 5 minutes at 4°C [1]. Subsequently, 1 mL supernatant from each digesta sample was transferred into a microcentrifuge tube and placed in a 25°C water bath [TC 602] for approximately 10 minutes before viscosity measurements were obtained.
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Table 1. Diet Formulations fed for 21 d trial

Ingredients	Inclusion (%)		
	PC_1: Corn-SBM ¹	PC_2: Wheat and DDGS ²	Negative Control ³
Corn	53.02	37.83	40.24
Soybean Meal (48% CP)	37.83	33.08	32.77
Wheat	---	10	10
DDGS	---	10	10
Dicalcium Phosphate	1.79	1.71	1.70
Limestone	1.32	1.36	1.37
L-Lysine HCL	0.13	0.22	0.22
DL-Methionine	0.49	0.48	0.47
Soybean Oil	4.44	4.44	2.35
Vit/Min Premix ⁵	0.25	0.25	0.25
L-Threonine	0.30	0.31	0.31
White Salt	0.33	0.22	0.22
Sodium Bicarbonate	0.10	0.10	0.10
Calculated Nutrients ⁴			
ME (kcal/kg)	3,050	3,050	2,925
Crude Protein (%)	22.25	22.86	22.86
Calcium (%)	1.01	1.01	1.01
Available Phosphorus (%)	0.46	0.46	0.46
Sodium (%)	0.17	0.17	0.17
Dig Lysine (%)	1.20	1.20	1.20
Dig Methionine (%)	0.79	0.78	0.77
Dig Methionine and Cysteine (%)	1.08	1.09	1.08
Dig Threonine (%)	1.01	1.01	1.01
Dig Tryptophan (%)	0.24	0.24	0.24
Analyzed Nutrients			
Crude Protein (%)	22.9	21.4	21.65
Crude Fat (%)	5.34	6.37	4.81

¹Corn and soybean meal positive control diet.

²Corn and soybean meal based positive control with inclusion of 10% wheat and 10% DDGS.

³Similar to PC_2 but with ME reduced by 125 kcal/kg; corn-expressed carbohydrase replaced equal amount of corn within this dietary formulation.

⁴Metabolizable Energy and Available Phosphorus were based on Agristat values as suggested by M. Donohue. 2013. The Challenges in Feeding Broilers in Times of High and Volatile Feed Ingredient Costs: How to Cover the Costs?. 2013 Mid-Atlantic Nutrition Conference proceedings. Digestible amino acids were based on the digestible lysine value (1.2%) suggested by P. B. Tillman and W.A. Dozier. 2013. Current Amino Acid Considerations for Broilers: Requirements, Ratios, Economics. www.thepoultryfederation.com for 8 – 14 day broilers. Digestible amino acid to digestible lysine ratios followed further recommendations of this communication (minimum of 0.54 methionine, 1.02 TSAA, 0.90 threonine, 0.21 tryptophan).

⁵Supplied per kg of diet: manganese, 0.02%; zinc 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg; choline, 386mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B6, 1.38 mg; niacin, 27.56 mg; pantothenic acid, 6.61 mg; thiamine, 2.20 mg; manadione, 0.83 mg; vitamin B12, 0.01 mg; vitamin E, 16.53 IU; vitamin D3, 2133 ICU; vitamin A, 7716 IU.

Table 2. Viscosity of three wheat sources analyzed prior to diet formulation¹

Wheat Source	Mill used to Grind	Viscosity (cP) at different speeds and times			
		10 x g; 30s	10 x g; 60s	20 x g; 30s	20 x g; 60s
A	Hammer mill	2.79	2.67	2.82	2.76
	Wiley mill	3.46	3.52	3.89	3.83
B	Hammer mill	3.20	3.23	3.33	3.26
	Wiley mill	4.02	4.08	4.36	4.32
C	Hammer mill	3.01	3.04	3.46	3.38
	Wiley mill	4.22	4.00	3.69	3.64

¹Samples were examined in duplicate from three separate suppliers to determine which wheat would provide the greatest viscosity challenge within the gastrointestinal tracts of the broilers.

Table 3. Particle size, glucanase activity, and dietary (*in vitro*) viscosity in the complete diets

Diet Formulation	Particle Size ⁵ (microns)	Analyzed Glucanase Activity (β -Glu-U/kg)	<i>In vitro</i> Viscosity (cP; 10 x g for 30s)
PC_1: Corn-SBM ¹	874.68 $\pm$ 2.13	---	1.81
PC_2: Wheat and DDGS ²	900.70 $\pm$ 2.01	0.08	2.02
Negative Control (NC) ³	888.11 $\pm$ 2.05	---	1.87
NC + 50 β -Glu-U/kg ⁴	908.01 $\pm$ 2.05	27.96	1.82
NC + 100 β -Glu-U/kg	922.38 $\pm$ 2.09	72.96	1.83
NC + 200 β -Glu-U/kg	906.90 $\pm$ 2.10	157.41	1.74
NC + 400 β -Glu-U/kg	887.11 $\pm$ 2.08	392.49	1.66
NC + 600 β -Glu-U/kg	878.64 $\pm$ 2.02	599.48	1.72

¹Corn and soybean meal positive control diet.

²Corn and soybean meal based positive control with inclusion of 10% wheat and 10% DDGS.

³Similar to PC_2 but with ME reduced by 125 kcal/kg.

⁴Provided by corn-expressed carbohydrase.

⁵ Particle size determined with a Ro-Tap particle size analyzer model RX-29 Type 110V 60H2.

β -Glu-U/kg = β -Glucanase units/kg of feed

Table 4. D14 digesta viscosity of broilers fed diets with or without AC1 enzyme inclusion

Diet Formulation	Digesta Viscosity (cP)			
	10 X g		20 X g	
	30 sec	1 min	30 sec	1 min
PC_1: Corn-SBM ¹	2.73 ^c	2.72	2.85	2.80
PC_2: Wheat and DDGS ²	3.08 ^a	3.13	3.18	3.15
Negative Control (NC) ³	3.06 ^{ab}	3.18	3.03	3.00
NC + 50 β -Glu-U/kg ⁴	2.89 ^{abc}	3.03	2.93	3.02
NC + 100 β -Glu-U/kg	2.64 ^c	2.96	3.03	2.93
NC + 200 β -Glu-U/kg	2.91 ^{abc}	3.02	2.96	2.93
NC + 400 β -Glu-U/kg	2.81 ^{bc}	2.87	2.87	2.83
NC + 600 β -Glu-U/kg	2.70 ^c	2.82	2.86	2.81
Treatment P-value	0.0259	0.2963	0.1485	0.2313
Treatment SEM	0.1007	0.1373	0.0932	0.1173
Fisher's LSD ⁵	0.2774	---	---	---

¹Corn and soybean meal positive control diet.

²Corn and soybean meal based positive control with inclusion of 10% wheat and 10% DDGS.

³Similar to PC_2 but with ME reduced by 125 kcal/kg.

⁴Provided by corn-expressed carbohydrase.

⁵Fisher's least significant difference multiple comparison test (a, b, c).

^{a-c}Means with superscripts without a common letter differ significantly (P < 0.05).

AC1 = Corn-expressed carbohydrase; β -Glu-U/kg = β -Glucanase units/kg of feed

Table 5. Growth performance of broilers when fed diets with or without AC1 enzyme inclusion

Diet Formulation	Days 1-7			Days 1-13			Days 1-21		
	Bird FI (kg)	Bird LWG (kg)	FCR (kg/kg)	Bird FI (kg)	Bird LWG (kg)	FCR (kg/kg)	Bird FI (kg)	Bird LWG (kg)	FCR (kg/kg)
PC_1: Corn-SBM ¹	0.093	0.082	1.15 ^c	0.367 ^c	0.261	1.41 ^d	3.78	0.744	1.29 ^b
PC_2: Wheat and DDGS ²	0.097	0.080	1.22 ^a	0.381 ^{bc}	0.251	1.51 ^a	3.58	0.705	1.39 ^a
Negative Control (NC) ³	0.099	0.082	1.20 ^{ab}	0.387 ^{ab}	0.263	1.46 ^{abc}	3.84	0.731	1.37 ^a
NC + 50 β -Glu-U/kg ⁴	0.096	0.082	1.18 ^{abc}	0.380 ^{bc}	0.260	1.45 ^{cd}	3.90	0.732	1.36 ^a
NC + 100 β -Glu-U/kg	0.101	0.087	1.16 ^{bc}	0.389 ^{ab}	0.266	1.46 ^{bc}	3.93	0.731	1.38 ^a
NC + 200 β -Glu-U/kg	0.97	0.084	1.18 ^{bc}	0.394 ^{ab}	0.263	1.50 ^{ab}	3.84	0.730	1.38 ^a
NC + 400 β -Glu-U/kg	0.102	0.087	1.16 ^{bc}	0.400 ^a	0.271	1.46 ^{abc}	3.81	0.737	1.38 ^a
NC + 600 β -Glu-U/kg	0.098	0.082	1.20 ^{ab}	0.386 ^{ab}	0.261	1.48 ^{abc}	3.90	0.730	1.37 ^a
Treatment P-value	0.228	0.2380	0.0265	0.0180	0.1407	0.0047	0.2671	0.2566	<0.001
Treatment SEM	0.0023	0.0023	0.0169	0.0061	0.0045	0.0166	0.0959	0.0098	0.0126
Fisher's LSD ⁵	---	---	0.0476	0.0173	---	0.0469	---	---	0.0356

¹Corn and soybean meal positive control diet.

²Corn and soybean meal based positive control with inclusion of 10% wheat and 10% DDGS.

³Similar wheat and DDGS diet as PC_2 but with ME reduced by 125 kcal/kg.

⁴Provided by corn-expressed carbohydrase.

⁵Fisher's least significant difference multiple comparison test (a, b, c).

FI = Feed Intake; LWG = Live weight gain; FCR = Feed conversion ratio calculated using mortality weight; AC1 = Corn-expressed carbohydrase; β -Glu-U/kg = β -Glucanase units/kg of feed

Table 6. Pearson correlation coefficients of intestinal viscosity and performance variables¹

	Intestinal viscosity (10 x g; 30s)	Bird LWG ² d1-7	FCR ³ d1-7	Bird LWG D1-13	FCR d1-13	Bird LWG d1-21	FCR d1-21
Intestinal viscosity (10 x g; 30s)							
Correlation coefficient	1.0	0.073	-0.211	0.107	-0.265	-0.387	0.326
P-value		0.6223	0.1507	0.4707	0.0686	0.0066	0.0239
Bird LWG d1-7							
Correlation coefficient	---	1.0	-0.377	0.907	-0.120	0.107	-0.192
P-value			0.0083	<0.0001	0.4153	0.4701	0.1902
FCR d1-7							
Correlation coefficient	---	---	1.0	-0.420	0.233	0.051	0.093
P-value				0.0029	0.1109	0.7319	0.5288
Bird LWG d1-13							
Correlation coefficient	---	---	---	1.0	-0.254	0.121	-0.337
P-value					0.0815	0.4139	0.0192
FCR d1-13							
Correlation coefficient	---	---	---	---	1.0	0.128	-0.156
P-value						0.3867	0.2893
Bird LWG d1-21							
Correlation coefficient	---	---	---	---	---	1.0	-0.388
P-value							0.0064
FCR d1-21							
Correlation coefficient	---	---	---	---	---	---	1.0
P-value							

¹Using replicated pen data from the control (PC_1, NC, and PC_2) and average of all AC1 (averaged by block; n = 12) treatments.

²Live Weight Gain.

³Feed Conversion Ratio (Feed:Gain) was calculated using mortality weight.

Table 7. Pearson correlation coefficients of intestinal viscosity, dietary viscosity, and performance variables among all treatments analyzed categorically¹.

	Dietary viscosity (10 x g; 30s)	Intestinal viscosity (10 x g; 30s)	Bird LWG ² d1-7	FCR ³ d1-7	Bird LWG D1-13	FCR d1-13	Bird LWG d1-21	FCR d1-21
Diet viscosity (10 x g; 30s)								
Correlation coefficient	1.0	0.604	0.151	-0.540	0.109	-0.611	-0.725	0.035
P-value		0.1124	0.7219	0.1671	0.7967	0.1079	0.0421	0.9341
Intestinal viscosity (10 x g; 30s)								
Correlation coefficient	---	1.0	-0.238	-0.366	-0.116	-0.545	-0.615	0.277
P-value			0.5711	0.3728	0.7839	0.1628	0.1047	0.5071
Bird LWG d1-7								
Correlation coefficient	---	---	1.0	-0.780	0.935	0.086	-0.120	-0.144
P-value				0.0223	0.0007	0.8404	0.7781	0.7335
FCR d1-7								
Correlation coefficient	---	---	---	1.0	-0.774	0.134	0.435	0.068
P-value					0.0243	0.7525	0.2819	0.8720
Bird LWG d1-13								
Correlation coefficient	---	---	---	---	1.0	0.096	-0.015	-0.334
P-value						0.8214	0.9710	0.4180
FCR d1-13								
Correlation coefficient	---	---	---	---	---	1.0	0.433	-0.259
P-value							0.2841	0.5352
Bird LWG d1-21								
Correlation coefficient	---	---	---	---	---	---	1.0	-0.606
P-value								0.1109
FCR d1-21								
Correlation coefficient	---	---	---	---	---	---	---	1.0
P-value								

¹Treatment averages were used for intestinal viscosity and animal performance results.

²Live Weight Gain.

³Feed Conversion Ratio (Feed:Gain) was calculated using mortality weight.

CHAPTER THREE

Exogenous enzyme supplementation can overcome amino acid deficient diets

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SUMMARY The current study hypothesized that exogenous enzyme supplementation can overcome amino acid deficient corn and soybean based broiler starter diets. The objective of the study was to determine broiler performance of diets that vary in amino acid concentration and enzyme inclusion. Dietary treatments included a positive control (**PC**) (100% amino acid recommendations), negative control (**NC**) (85% amino acid recommendations), and six additional diets containing commercially available enzyme supplements (Single Dose Phytase, Super Dose Phytase, Single Dose NSPase, Super Dose Phytase + Single Dose NSPase, Protease 1, and Protease 2) added to the NC based on manufacturers' recommendations. Each diet was conditioned at 70°C and fed as crumbles to 12 replications of 10 male Hubbard x Ross 708 broiler chicks for 21d. Treatments were arranged in a randomized complete block design. The 15% calculated reduction in crude protein and amino acids produced expected 21d broiler live weight gain (**LWG**) differences between the PC and NC ($P<0.05$). Dietary treatments including phytase, at either level or when combined with the NSPase, had similar d7, 14, and 21 LWG compared to the PC ($P>0.05$). These data suggest that with the inclusion of the particular phytase, regardless of dose or when combined with a single dose of NSPase, improved broiler LWG in an amino acid deficient diet to an extent beyond the NSPase alone or either tested protease.

DESCRIPTION OF THE PROBLEM

Exogenous enzyme supplementation is a common nutritional practice in the U.S. poultry industry. Previous literature has stated that these enzymes can improve bird performance and aid in least cost diet formulation. Common exogenous enzymes supplemented in diets include phytase, non-starch polysaccharide enzymes (**NSPase**), and proteases.

Phytases are utilized to decrease negative effects associated with phytate bound Phosphorous (**P**) [1-3] and may also improve the absorption of protein and amino acids. Yi and co-authors [4] found that the inclusion of a microbial phytase to a corn-soybean meal based diet enhanced growth performance, as well as ileal nitrogen (**N**) and amino acid (**AA**) digestibility, and apparent N and P retention of turkey poult. The authors hypothesized that phytate has the potential of binding with protein at low and neutral pH levels, which was suggested by Cosgrove, Anderson, and Thompson [5-7]. In this case, the proteins are positively charged and can form insoluble complexes with the negatively charged phytate [8, 9]. Ultimately, the binding of proteins to phytate can alter protein structure, thus decreasing solubility, digestibility, and functionality. Therefore, when phytase frees the bound P, it may also free the binding groups of proteins bound to phytate, thereby allowing for better absorption of protein and AA.

The presence of NSPs can lead to increased intestinal viscosity, reduced nutrient digestibility, and increased feed conversion ratio (**FCR**) [10-13]. To negate the effects associated with NSPs, nutritionists may also include NSPases in the diet. These enzymes have been shown to reduce intestinal viscosity [10] and degrade cell wall polysaccharides [13]. In doing so, endogenous enzymes may have better access to their substrates, ultimately improving diet digestibility.

Variability of digestible protein and AA may also occur within the same feed ingredient [14-17]. A wide range of endogenous enzymes are produced and released in the broiler gastrointestinal tract and are able to optimize protein utilization [17-19]. However, studies have shown that a portion of the protein in the feed is passing through the gastrointestinal tract is not being utilized by the animal [16, 17]. Therefore, many nutritionists are choosing to include exogenous protease in poultry diets as well [17, 20].

Often times, supplemental enzymes are utilized in combination with one another. Such combinations are referred to as enzyme “cocktails.” These enzymes have been shown to act independently of one another and improve broiler performance [21]. For example, Cowieson and Adeola [22] found that a phytase and an enzyme cocktail composed of a xylanase, amylase, and protease can improve nutrient digestibility and broiler performance when supplemented to a diet suboptimal in terms of Ca, P, and metabolizable energy (**ME**).

The objectives of this study were to determine digestible amino acid concentrations and performance of broilers fed diets that vary in amino acid concentration and enzyme inclusion.

MATERIALS AND METHODS

Diet Formulation

Diets consisted of a positive control (**PC**), formulated with 100% of amino acid recommendations by Tillman and Dozier [23] and a negative control (**NC**), formulated with 85% of amino acid recommendations and crude protein (**CP**) relative to the PC. The NC was then supplemented with a Single Dose of Phytase, a Super Dose of Phytase, a Single Dose of NSPase, a Super Dose of Phytase + a Single Dose of NSPase, Protease 1, or Protease 2. Enzyme inclusions followed manufacturer recommendations [24]. All diets were corn-soybean meal based diets, formulated to 3,000 kcal/kg of ME (**Table 1**).

All feed was manufactured at the West Virginia University pilot feed mill. A total of 1,741.15 kg was made to create a master batch. From this master batch, 226.80 kg of feed was mixed with a premix specific for the PC diet formulation, containing additional soybean meal, soybean oil, methionine, lysine and threonine. The remaining 1514.35 kg was mixed with a premix specific for the NC diet formulation, containing additional ground corn and limestone. The NC basal diet was then divided into seven allotments of 226.780 kg each. One of the seven allotments was kept as the NC diet, and the other six were mixed via top dress with either a Single Dose of Phytase, Super Dose of Phytase, Single Dose of NSPase, Super Dose of Phytase + Single Dose of NSPase, Protease 1, or Protease 2. For each enzyme diet, the enzyme supplements were premixed with a 3 kg sample of NC feed in a small Univex paddle mixer [25]. Each batch was then mixed for 10 minutes in a one-ton single screw vertical mixer [26]. Complete feed samples for each treatment were collected post-enzyme addition, randomly throughout the batch, to generate a homogenous sample and sent to a commercial laboratory for proximate analysis [27].

Feed Manufacture

A 454 kg corn-soybean meal based diet was used prior to pelleting experimental diets to warm the steel housing of the conditioner and pellet die. All experimental treatments were steam conditioned at 70°C for 15 seconds using a California Pellet Mill Conditioner [28] with steam pressure throttled to 276 kpa prior to the Masoneilan Valve and entrance to the conditioner. Treatments were then pelleted at steady state conditions at a constant rate of 1.2 tonne/hr using a 40-horsepower California Pellet Mill [29] with a 4.76 (effective thickness) x 38.1 mm (length) pellet die without relief. Once the target temperature of 70°C was obtained, samples of each treatment were taken directly from the pellet die and allowed to cool for approximately 12 minutes [30]. The samples were then sent to a commercial laboratory for enzyme analysis [31].

Immediately after pelleting, each treatment was cooled for approximately 1.25 min on a horizontal cooler belt [32] using forced ambient air. Ambient temperature and humidity can be found in **Table 2**. After pellets were cooled, they were crumbled through a single pair roller mill [33] to achieve small, uniform crumble size among treatments. All crumbled diets were then stored in an insulated storage room, equipped with a dehumidifier to equilibrate moisture content among the treatments.

Variables measured during feed manufacture included pellet mill motor load [34] and hot pellet temperature (**HPT**) [35] (**Table 2**). For each treatment, approximately 100g of hot pellets were collected at the pellet die, as well as approximately 100g of cooled pellets from the cooler belt to determine moisture analysis (**Table 3**) [37].

Pellet quality was assessed one day following feed manufacture, using a New Holmen Portable Pellet Tester [38, 39]. Using a Pfast Tumbler [41], pellet durability index (**PDI**) and modified pellet durability index (**MPDI**) also were determined. All PDI and MPDI assays [42] were assessed in duplicate and can be found in **Table 2**. Particle size of the crumbled feed was also determined, using a Ro-Tap Particle Size Analyzer (**Table 2**) [43, 44].

Live Bird Performance

A total of nine-hundred sixty, 1-day-old Hubbard x Ross 708 male chicks were obtained from a commercial hatchery [46]. On d0, the chicks were placed into a group of 10 total chicks, weighed, and the group was then placed in 1 of 96 raised wire cages to create uniform, initial pen weights within block. One of the 8 diets was randomly assigned to each pen within a block. A block consisted of 8 adjacent cages; a total of 12 blocks were utilized. Chicks were housed in raised wire cages in a cross-ventilated, negative-pressure room for 21d. Two identical rooms were utilized; each containing 48 cages, creating a total of 96 cages. Standard rearing practices were

applied [47]. The animal performance variables measured included: d0 starting pen weight, d7, 14, and 21 pen weights, feed intake (**FI**), live weight gain/bird (**LWG**), mortality corrected FCR and percent mortality. Chicks were not deprived of feed prior to weighing. All animals were reared according to protocols approved by West Virginia University Animal Care and Use Committee.

Statistical Analysis

All data were analyzed in a randomized complete block design with the raised wire cage location within room determining block. The experimental unit was one pen containing 10 Hubbard x Ross 708 male chicks. The PROC GLM procedure of SAS [SAS 9.4] was used to analyze data by one-way ANOVA. Means were then further separated using Fisher's least significant difference (**LSD**) post hoc comparison when the ANOVA was significant ($P \leq 0.05$).

RESULTS AND DISCUSSION

In the current study, the 15% reduction in AA recommendations produced expected 7, 14, and 21d broiler LWG differences between the NC and PC dietary treatments ($P < 0.05$). Broiler performance metrics can be observed in **Table 4**. The addition of phytase to the NC, regardless of dose or combination with the NSPase, was equivalent to the PC for d0-7, d0-14, and d0-21 LWG ($P > 0.05$). When phytase was included with the NSPase, d0-7 FCR was also similar to the PC ($P > 0.05$). Overall feed intake (**FI**) for d0-21 had a similar trend, as all phytase inclusion diets were similar to the PC ($P > 0.05$).

These data are in agreement with Yi and coauthors [4], as the inclusion of phytase, independent of dose or combination with NSPase, improved growth performance of broilers fed an amino acid deficient diet. Yi and cohorts investigated the effect of a microbial phytase on protein digestibility, AA digestibility, and nitrogen retention of turkey poults fed corn-soybean meal diets. With the addition of 750 U of phytase to corn-soybean meal diets, N and ileal AA

digestibility increased, and growth performance was enhanced [4]. The current study also found that with the inclusion of phytase, d0-7, d0-14, and d0-21 LWG was similar to the PC ($P > 0.05$). Overall FI also improved with these inclusions, as treatments including phytase were similar to the PC ($P > 0.05$). Feed conversion ratio was similar between treatments including phytase and the PC as well for d0-21 ($P > 0.05$).

The current study also observed decreased d0-21 LWG and FCR when the NC was supplemented with an NSPase. This is in agreement with Barrios and coauthors [48]. While these authors found that the addition of NSPase can improve growth performance of broilers fed a corn-soybean meal based diet, it was also noted that when dietary treatments were reduced in energy and protein, the NSPase was unable to compensate, and feed efficiency decreased. Similar results were observed in the current study, as the inclusion of NSPase to a diet deficient in amino acid concentration had decreased LWG and increased FCR compared to the PC ($P < 0.05$).

The addition of Protease 1 decreased d21 FCR, similar to that of the PC as well as the NC. This is in agreement with Yan and coauthors [20]. These authors investigated the effects of a protease on growth performance and carcass traits of broilers affected by dietary CP level. These authors found that when birds were fed diets containing low (18.5%) or high (22.5%) CP, protease significantly decreased FCR on d35 and 42. Similar results were also observed in the current study, as Protease 1 had similar FCR, comparable to the PC ($P > 0.05$).

The addition of Protease 2 to the amino acid deficient diet did not improve FI, LWG, or FCR for any time point during the 21-day study, which is in agreement with Lahaye and coauthors [49]. These authors evaluated the effect of supplementation of 2 single protease enzymes (PoultryGrow 250 and a protease from *Bacillus* origin) on top of commercial pelleted corn-soybean meal based diets with reduced CP, AA, and energy levels for 35 days. These authors

found that the inclusion of PoultryGrow 250 to the CP, AA, and energy deficient diet significantly improved d28 and d35 body weight. However, there were no differences in FCR. These authors concluded that the use of the same protease used in the current study on top of a commercial pelleted diet, deficient in CP, AA, and energy has the potential to improve body weight and maintain FCR of birds. In the current study, however, the authors found no improvement in LWG or FCR. However, the current study was a 21-day study while Lahaye and coauthors evaluated various proteases for 35 days [49]. The authors hypothesize that both proteases utilized may have a greater effect on growth performance later in life.

In conclusion, the 15% reduction in CP and AA produced expected 7, 14, and 21d broiler LWG differences between the PC and NC dietary treatments. Phytase, independent of dose or combination with the NSPase, and Protease 1 produced 21d FCR similar to the PC. Finally, feeding NC with the NSPase or Protease 2 did not restore 21d LWG comparable to the PC.

CONCLUSIONS AND APPLICATIONS

1. The 15% reduction in CP and AA produced expected 7, 14 and 21d broiler LWG differences between the PC and NC.
2. Dietary treatments including phytase, at either level or when combined with the NSPase, had similar d7, 14 and 21 LWG compared to the PC, while feeding NC with the NSPase or either protease did not.

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Table 1. Diet formulation fed for 21d trial.

Ingredients	Inclusion (%)	
	Positive Control	Negative Control
Corn	53.77	62.31
Soybean Meal	37.61	30.14
Soybean Oil	3.7	2.72
Dicalcium Phosphate	1.79	1.84
Limestone	1.33	1.35
DL- Methionine	0.49	0.39
Salt	0.33	0.33
Threonine	0.30	0.25
Poultry Vitamin/Mineral Premix	0.25	0.25
Titanium dioxide	0.20	0.20
L- Lysine HCl	0.14	0.13
Sodium Bicarbonate	0.10	0.10
Calculated Nutrients		
Metabolizable Energy (Kcal/kg)	3,000	3,000
Crude Protein (%)	22.20	19.00
Calcium (%)	1.01	1.01
Phosphorous (%)	0.46	0.46
Sodium (%)	0.17	0.17
Dig. Lysine (%)	1.20	1.02
Dig. Methionine (%)	0.79	0.66
Dig. Methionine + Cystine (%)	1.08	0.92
Dig. Threonine (%)	1.01	0.86
Dig. Tryptophan (%)	0.24	0.20
Analyzed Nutrients		
Crude Fat (%)	5.27	4.82
Crude Protein (%)	20.60	18.97
Methionine (%)	0.78	0.68
Lysine (%)	1.25	1.17
Threonine (%)	0.95	0.90

¹ Metabolizable Energy and Available Phosphorus were based on Agristat values as suggested by M. Donohue. 2013. The Challenges in Feeding Broilers in Times of High and Volatile Feed Ingredient Costs: How to Cover the Costs?. 2013 Mid-Atlantic Nutrition Conference proceedings. Digestible amino acids were based on the digestible lysine value (1.2%) suggested by P. B. Tillman and W.A. Dozier. 2013. Current Amino Acid Considerations for Broilers: Requirements, Ratios, Economics. www.thepoultryfederation.com for 8 – 14 day broilers. Digestible amino acid to digestible lysine ratios followed further recommendations of this communication (minimum of 0.54 methionine, 1.02 TSAA, 0.90 threonine, 0.21 tryptophan).

²Supplied per kg of diet: manganese, 0.02%; zinc 0.02%; iron, 0.01%; copper, 0.0025%; iodine, 0.0003%; selenium, 0.00003%; folic acid, 0.69 mg; choline, 386mg; riboflavin, 6.61 mg; biotin, 0.03 mg; vitamin B6, 1.38 mg; niacin, 27.56 mg; pantothenic acid, 6.61 mg; thiamine, 2.20 mg; manadione, 0.83 mg; vitamin B12, 0.01 mg; vitamin E, 16.53 IU; vitamin D3, 2133 ICU; vitamin A, 7716 IU.

Table 2. Descriptive feed manufacture data from WVU Feed Mill on January 16, 2019. Pellets were manufactured using a 40 Horsepower California Pellet Mill and were extruded through a 4.8 x 38 mm pellet die.

Treatment	Hygienizer Temperature ⁴ (°C)	Motor Load ⁵ (%)	Hot Pellet Temperature ⁶ (°C)	PDI	MPDI	NHPT ⁷	Particle Size ⁸ (microns)
Positive Control (PC)	56	49	72.5	78	71	65	1,473
Negative Control (NC)	60	48	72.2	78	71	64	1,503
NC + Single dose Phytase	58	49	72.1	79	74	71	1,495
NC + Super dose Phytase	58	48	74.2	78	73	66	1,372
NC + Single dose NSPase	56	50	72.6	81	76	72	1,337
NC + Super dose Phytase + Single dose NSPase	57	50	72.9	80	74	69	1,459
NC + Protease 1	57	49	73.4	79	74	68	1,432
NC + Protease 2	56	49	73.7	78	73	66	1,321

¹The following metrics were recorded on day of feed manufacture: Ambient Temperature (0.56 °C); Ambient Humidity (85%); Conditioner Retention Time (15 s); Conditioning Temperature³ (70 °C).

²Production rate was maintained at 1.2 tonne/hr for all treatments.

³Conditioning temperature was measured as the reading from the conditioner temperature probe at the time of sample collection.

⁴The hygienizer was not turned on during this experiment; however, feed must run through the hygienizer for 45 seconds post conditioning and prior to pellet die extrusion based on the WVU feed manufacture system.

⁵A 100% motor load was based on FLA (full load amps) that was 47 amps based on the pellet mill motor name plate.

⁶Hot pellet temperature was determined on pellets directly following extrusion from the die. Pellets were collected into an insulated container and temperature was measured using a thermocouple thermometer and an 80PK-24 temperature probe.

⁷Measurements New Holmen Pellet Tester are where 100 g pelleted samples are subjected to air flow within a perforated chamber for 30 s.

⁸Particle size determined with a Ro-Tap particle size analyzer model RX-29 Type 110V 60H2.

Table 3. Mash and Pellet Moisture

Treatment	Mash % Moisture	Cool Pellet % Moisture	Hot Pellet % Moisture
Positive Control (PC)	12	14	16
Negative Control (NC)	12	14	14
NC + Single dose Phytase	12	14	16
NC + Super dose Phytase	13	14	16
NC + Single dose NSPase	12	14	16
NC + Super dose Phytase + Single dose NSPase	12	14	16
NC + Protease 1	12	14	16
NC + Protease 2	13	14	16

Table 4. Growth performance of broilers fed diets varying in enzyme supplementation.

Diet Formulation	Days 0-7			Days 0-14			Days 0-21		
	Bird FI ² (kg)	Bird LWG ³ (kg)	FCR ⁴ (kg/kg)	Bird FI (kg)	Bird LWG (kg)	FCR (kg/kg)	Bird FI (kg)	Bird LWG (kg)	FCR (kg/kg)
Positive Control (PC)	0.168	0.159 ^a	1.05 ^e	0.668	0.483 ^a	1.24 ^e	1.44 ^a	0.946 ^a	1.49 ^{bc}
Negative Control (NC)	0.156	0.140 ^c	1.12 ^c	0.641	0.423 ^b	1.31 ^{bcd}	1.38 ^{bcd}	0.895 ^b	1.48 ^c
NC + Single dose Phytase	0.173	0.155 ^a	1.11 ^c	0.666	0.460 ^a	1.30 ^{bcd}	1.43 ^{ab}	0.950 ^a	1.46 ^c
NC + Super dose Phytase	0.169	0.155 ^a	1.08 ^d	0.671	0.468 ^a	1.29 ^{cd}	1.42 ^{ab}	0.953 ^a	1.46 ^c
NC + Single dose NSPase	0.159	0.141 ^c	1.12 ^{bc}	0.636	0.424 ^b	1.33 ^{ab}	1.37 ^{cd}	0.588 ^{bc}	1.56 ^{ab}
NC + Super dose Phytase + Single dose NSPase	0.162	0.152 ^{ab}	1.07 ^{de}	0.664	0.463 ^a	1.28 ^d	1.42 ^{abc}	0.946 ^a	1.47 ^c
NC + Protease 1	0.162	0.142 ^{bc}	1.15 ^a	0.634	0.429 ^b	1.33 ^{bc}	1.359 ^{cd}	0.875 ^{bc}	1.52 ^{abc}
NC + Protease 2	0.163	0.141 ^{bc}	1.14 ^{ab}	0.648	0.422 ^b	1.36 ^a	1.357 ^d	0.846 ^c	1.58 ^a
Treatment P-value	0.1833	0.0010	<0.0001	0.1208	<0.0001	<0.0001	0.0147	<0.0001	0.0035
Treatment SEM	0.0045	0.0040	0.0065	0.0115	0.0084	0.0119	0.0204	0.0164	0.0254
Fisher's LSD ¹	---	0.0113	0.0183	---	0.0237	0.0336	0.0575	0.0463	0.0714

¹Fisher's least significant difference multiple comparison test (a, b, c).²Feed intake.³Live weight gain.⁴Feed conversion ratio.^{a-c}Means with superscripts without a common letter differ significantly (P < 0.05).

CHAPTER FOUR

The effect of corn-expressed carbohydrase on performance and digesta viscosity of broilers fed a high non-starch polysaccharide diet

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SUMMARY Study objectives were to determine the effects of a corn-expressed recombinant carbohydrase (**AC1**) on broiler performance and digesta viscosity in high NSP diets through 21 d of age. One day-old Hubbard x Cobb 500 chicks were assigned to six dietary treatments. Each treatment consisted of 12 replicate pens of 10 birds. The positive control diet (**PC**) was a corn and soybean meal formulation. The negative control diet (**NC**) included 10% wheat and 10% corn distiller's dried grains with solubles (**DDGS**). The NC contained 100 kcal/kg less ME than the PC. Increasing inclusions of AC1 were applied to the NC to contain 50, 100, 200, and 400 U β -Glucanase (**β -Glu-U**) per kg of feed. Preliminary experiments demonstrated AC1 homogeneity and stability post pelleting. Live weight gain (**LWG**) was the highest for PC fed birds from 1 to 14 d; however, birds fed NC with 400 β -Glu-U/kg had similar LWG as PC ($P > 0.05$). Day 1 to 21 FCR was lowest for PC fed birds; however, birds fed NC with 400 β -Glu-U/kg had similar FCR as PC ($P > 0.05$). Birds fed NC had lower LWG and higher viscosity than birds fed PC on d 14 ($P < 0.05$), but not on d 21 ($P > 0.05$). Birds fed NC with 200 or 400 β -Glu-U/kg had similar 14 d digesta viscosity as birds fed PC ($P > 0.05$). These data indicate that NSP ingredients may have a greater impact on digesta viscosity early in broiler growth and that AC1 at 200 and 400 β -Glu-U/kg produced similar results to PC.

Key words: β -glucanase, non-starch polysaccharides, performance, viscosity, wheat

DESCRIPTION OF PROBLEM

Least-cost diet formulation and geographical location may create an economic incentive to partially replace corn and soybean meal with alternative ingredients in broiler diets. However, alternative ingredients often increase the level of anti-nutritional factors (**ANF**) within the diet. Corn derived distillers dried grains with solubles (**DDGS**) contain lower amounts of starch and increased levels of non-starch polysaccharides (**NSP**) relative to corn [1]. In addition, alternative cereal grains such as wheat contain NSPs at concentrations that may be detrimental to broiler performance [2]. The NSPs are not only indigestible, but can increase digesta viscosity and therefore decrease overall digestibility of all nutrients within the diet. The specific NSP, β -glucan, found at high levels in wheat and DDGS, is partially water soluble which leads to the formation of a gel-like viscosity within the gastrointestinal tract of the broiler [3]. Viscous digesta reduces the rate of diffusion of feed substrates, digestive enzymes and their products; thereby, reducing absorption of nutrients within the diet. In addition, β -glucans can increase microflora growth as well as wet, sticky excreta [3, 4, 5].

Broilers do not produce adequate amounts of endogenous enzymes to digest NSPs. Therefore, exogenous enzymes can be added to diets for compensation [6, 7, 8, 9]. Since the early 1960's, research has been conducted on enzyme additions to poultry diets, e.g. NSP-degrading enzyme or carbohydrase, to increase nutrient digestibility and subsequent broiler performance [10, 11, 12, 13]. Among the supplemented enzymes, β -glucanase, a hydrolytic enzyme targeting beta-glycosidic bonds in barley, oat or wheat-based diets, has been shown to improve feed conversion efficiency, lower intestinal viscosity, and increase feed intake [14, 15, 16, 17, 18, 19].

Exogenous enzyme applications have historically been microbial expressed products that are presented in either a granulated, coated, or liquid form. An alternative to these traditional,

more established animal nutrition enzyme products, are recombinant enzymes produced in transgenic grain [20, 21, 22, 23, 24, 25]. A recombinant carbohydrase, with endo- β -1,4-glucanase activity, was expressed in corn grain (AC1, Agrivida) using methods described previously [26]. The enzyme in AC1 is a variant of the *Thermotoga maritima* Cel5A endoglucanase, engineered to improve its thermal stability. AC1 has multiple activities such as endo-cellulase, cellobiohydrolase (exo-cellulase), β -xylosidase, and β -1,3-glucanase, but its primary activity is endo-1,4- β -glucanase. The AC1 gene encodes a 38 kDa protein and is stable at and over 80°C, the growth temperature of *T. maritima*.

The current study investigated the effects of feeding AC1 on performance and digesta viscosity of young broilers consuming a corn/soy diet with 10% wheat and 10% DDGS.

MATERIALS AND METHODS

Preliminary Experiments

Protein Extraction for AC1 Enzyme Activity Assay. Twenty grams of ground AC1 was mixed with 100 mL of protein extraction buffer (100mM sodium phosphate, 0.01% Tween 20, pH 6.5) which was pre-warmed at 60°C. The sample and buffer mixture was placed in a temperature-control shaker [27] to shake at 250 rpm, 60°C for one hour. After one hour of protein extraction, 1 mL of the mixture was centrifuged at 16,300 x g for 10 minutes in a bench top centrifuge [28]. The resulting supernatant was saved and used to measure the AC1 enzyme activity in the samples.

AC1 Enzyme Activity Assay. β -Glucanase activity was measured using a colorimetric assay that relies on hydrolysis of a labeled, commercial substrate, azurine-cross linked barley β -glucan from Megazyme [29, 30]. Hydrolysis of this substrate by AC1 produces water-soluble dyed

fragments, and the rate of release of these dyed fragments can be related directly to enzyme activity by measuring the absorbance at 590 nm (A590).

Fifty microliters of diluted protein extract from AC1, or 100 μ L of protein extract from feed, was mixed with one tablet substrate in 450 μ L (product assay) or 400 μ L of protein extraction buffer (feed assay) in a 2 mL 96-well block [31]. A blank (500 μ L extraction buffer without protein extracts) was also included in the block. After a short vortex of the block at a low speed, the block was incubated at 80°C water bath for one hour. Enzyme reactions were stopped by adding 1 mL of 2% Tris base solution to each reaction. After centrifugation of the block at 3000 x g for 10 minutes, 100 μ L of the supernatant was removed to a new flat-bottom microplate [32] to record absorbance at 590 nm.

AC1 β -glucanase activity was calculated by subtracting the average value of the blank samples from the A590 value of each experimental sample reaction to calculate a background corrected A590 measurement. The corrected A590 measurement of each sample was divided by the sample dry weight to calculate a grain-based specific activity (A590/g of flour or A590/kg of feed). Based on previous experiments, the colorimetric activity data can be converted to the β -glucanase activity units by multiplying by 0.009 to be presented as unit/g (U/g) flour or unit/kg (U/kg) feed. The β -glucanase activity unit is defined as 1 μ mol of glucose reducing equivalents released from a substrate per minute by enzyme hydrolysis under an optimal assay condition.

Homogeneity of AC1 in Feed. Ground AC1 corn grain was mixed into a standard corn-soybean meal broiler diet. A 1,225 kg batch of feed was mixed in a vertical mixer with 4.232 kg of AC1 product that had an activity of 145 unit/g to make a diet with a target dose of 500 unit/kg diet. Ten 0.5 kg samples were collected randomly from the mixed feed and the β -glucanase activity was measured as described above.

Pelleting Stability of AC1 . To demonstrate AC1 stability in pelleted feed, 4.232 kg of ground AC1 corn meal was mixed into a 1,225 kg batch of feed to prepare diets with AC1 at a target dose of 500 unit/kg. This batch of feed was pelleted using a conditioning temperature of either 80, 85, or 90°C for 10 seconds and extruded through a 4 x 38 mm pellet die at approximately 0.91 metric ton/hr using a 40-horsepower California Pellet Mill. The steam used in the mill had an incoming pressure of 262 kPa. Pellets were cooled using a horizontal belt cooler and ambient air. Pellet conveyance post extrusion and cooling was accomplished with a series of flat bottom drag-chain conveyers and a bucket elevator. Ten 0.5 kg samples of the mash feed prior to pelleting and three 0.5 kg samples of post-pelleting feeds from each temperature treatment were collected for β -Glucanase activity analysis. The β -Glucanase activities after pelleting at the different temperatures were presented as a percentage of the original activity in the respective mash diets.

Broiler Experiment

Diet Formulations. Diets varied in ingredients, calculated ME and AC1 inclusion. The positive control diet (**PC**) was corn and soybean meal based (Table 1) and contained 3,000 kcal/kg of calculated ME. The negative control diet (**NC**) was formulated to be 100 kcal/kg calculated ME less than the PC and contained a 10% inclusion of both wheat and DDGS. Four additions of AC1, containing 50, 100, 200, and 400 U β -Glucanase (β -Glu-U) per kg of feed, were applied to the NC diet. The ME value utilized for the PC diet formulation was based on Agristat recommendations [33] and digestible amino acid values were based on recommendations by Tillman and Dozier [34].

Feed Manufacture. All feed was manufactured at the West Virginia University pilot feed mill. The PC and NC basal diets were prepared with 10 minute mixing times. The NC basal diet was then split into four allotments and then mixed with AC1 to the target dose of 50, 100, 200, or

400 β -Glu-U/kg for an additional 10 minute mixing time in a vertical single-screw mixer [35]. All diets were fed as mash feed. Complete feed samples for each treatment were collected post-enzyme addition for proximate analysis and β -glucanase activity as previously described.

Live Performance. Seven hundred twenty, 1-day-old Hubbard $\times$ Cobb 500 [36] straight-run chicks were obtained from a commercial hatchery [37]. On d 1, the chicks were weighed and allocated to 72 raised wire cages based on weight to create uniform initial pen weights. Raised wire cages were located in two identical cross-ventilated, negative-pressure rooms. The temperature for both rooms was set at 32 °C on d 1 and decreased 3 °C per week. Feed and water were provided for *ad libitum* consumption. The experimental unit consisted of one pen of 10 broilers. Treatments were arranged in a randomized complete block design with the location of the cage in the room determining the block. Each block included 6 cages with a total of 12 blocks utilized. Variables measured included feed intake (**FI**), live weight gain (**LWG**), mortality corrected feed conversion ratio (**FCR**), and digesta viscosity.

Digesta Viscosity Measurements. On d 14 and d 21, 3 birds from each pen were euthanized via cervical dislocation. The entire digestive tract was then removed and digesta was squeezed out by hand into a 50 mL centrifuge tube [38]. Digesta was centrifuged [39] at $12,700 \times g$ for 5 min at 4 °C. Using a pipette, 1 mL of supernatant was transferred into a micro centrifuge tube. Micro centrifuge tubes were placed into a 25 °C water bath [40] for approximately 10 min. A total 0.5 mL of supernatant was then placed into a Brookfield cone and plate viscometer [41] utilizing a CPE-40 cone and CPE-44Y cup. Viscosity measurements were taken at 30 s and 1 min at two speeds of $10 \times g$ and $20 \times g$. The digesta viscosity methodology used was similar to methods utilized by Lamp and coauthors [42]. All animals were reared according to protocols approved by West Virginia University Animal Care and Use Committee.

Statistical Analysis

All data were analyzed in a randomized complete block design with the raised wire cage location within room determining the block. The experimental unit was one pen containing 10 Hubbard × Cobb 500 broiler chicks. The PROC GLM procedure of SAS [43] was used to analyze data by one-way ANOVA. Means were then further separated using Fisher's least significant difference (**LSD**) post hoc comparison when the ANOVA was significant ($P \leq 0.05$).

RESULTS AND DISCUSSION

Preliminary Experiments

The results of AC1 enzyme activity measurements of the 10 samples of mixed feed are presented in Table 2. The average enzyme activity from all samples was 425 β -Glu-U/kg with a coefficient of variation (CV) of approximately 6%, indicating that ground AC1 corn grain meal was homogenously mixed in feed preparations.

The average AC1 enzyme activity measured in the mash diet prior to pelleting and in the pelleted feed samples is presented in Table 3. The results demonstrated that AC1 retains nearly 100% activity at 80 and 85°C conditioning and pelleting; and retains 90% activity after conditioning and pelleting at 90°C. The thermostability of the grain-expressed AC1 glucanase allows it to survive the heat generated during typical industry feed pelleting conditions.

Broiler Experiment

Broiler performance metrics are exhibited in Table 4. Feed Intake was similar across all treatments throughout the study ($P > 0.05$). Between d1-14, birds fed PC had an improved LWG compared to that of the NC fed birds ($P < 0.05$). A similar trend was also observed for d1-14 FCR

($P = 0.0728$), with PC being numerically lower than NC. No statistical differences were observed in d15-21 ($P = 0.0674$) and overall (d1-21; $P = 0.084$) LWG, but birds fed the PC diet tended to have the highest LWG. Overall (d1-21) FCR was significantly improved in PC vs. NC fed birds ($P < 0.01$). Birds fed 400 β -Glu-U/kg had an intermediate performance improvement, as d1-14 LWG or d1-21 FCR was similar to both PC and NC ($P > 0.05$). Throughout the duration of the study, pen mortality for a single treatment did not exceed 2.5% (data not shown).

Digesta viscosity data collected on d14 and d21 is displayed in **Table 5**. On d14, birds fed the corn/soybean diet (PC) had lower digesta viscosity (at either viscometer speed and time) than the NC group ($P < 0.05$), indicating that the addition of 10% wheat and 10% DDGS in the NC diet sufficiently increased digesta viscosity. To demonstrate the impact of exogenous feed enzymes on digesta viscosity in gastrointestinal tract, proper diet formulation is critical and can be challenging. For example, González-Ortiz *et al.* (44) reported no effect of a wheat based diet on performance or jejunal digesta viscosity despite an 18% CV in β -glucan content among the eight varieties of wheat tested in the feeding study. Loar *et al.* [45] included 8% DDGS in broiler diets during the starter phase (0-14 d) and either 0, 7.5, 15, 22.5 or 30% DDGS in the grower phase diets (14-28 d), but only measured marginal changes ($P=0.07$) in intestinal viscosity among treatments in the grower phase. Prolonged feeding of high levels of DDGS (0, 7, 14, 21 and 28%) through the finisher phase (28 to 42 d) did not increase digesta viscosity [46]. In the current study, inclusion of 10% wheat and 10% DDGS in corn/soybean diet had increased dietary NSP content and digesta viscosity demonstrating a good model to test exogenous carbohydrase enzymes such as AC1. When AC1 was added (200 and 400 β -Glu-U/kg) to the NC diet, intestinal viscosity decreased to that of the PC fed birds ($P < 0.05$) indicating AC1 is effective in lowering viscosity at a dose of 200 β -Glu-U/kg and higher during early bird development. When measured on d 21, digesta

viscosity values did not exhibit significant differences among treatments ($P > 0.05$). Past research has indicated that β -glucan and β -glucanase affect digesta viscosity more in younger birds compared to more mature birds [15]. Brenes *et al.* [19] has also found that exogenous NSP enzymes were most effective in the first weeks of life, with little or no effect observed for adult birds.

Lowering ileal viscosity is a demonstrated mode of action for β -glucanase [14, 15, 47], however, many factors including cereal type, structure, substrate concentrations, NSP solubility, and bird age can have a significant impact on intestinal viscosity as well as the response of NSP enzymes within the bird [15, 47, 48].

In the current study, 200 and 400 β -Glu-U/kg inclusion from AC1 decreased intestinal viscosity. Birds fed AC1 at the 400 β -Glu-U/kg dose had similar 14 d LWG and overall FCR as birds fed PC diet. Together, these data indicate AC1 had a positive effect on early broiler growth and feed conversion ratio that may have resulted from better access to dietary nutrients due to reduced intestinal viscosity.

CONCLUSIONS AND APPLICATIONS

1. The AC1 provided at 400 β -Glu-U/kg to a high NSP diet enabled equivalent d 14 broiler digesta viscosity, weight gain and 1 to 21 d FCR compared to a low NSP control diet formulated with 100 kcal/kg higher ME.
2. Higher NSP ingredients may have a greater impact on digesta viscosity during early broiler growth (d1-14).

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Table 1. Ingredient composition of experimental diets from 1 to 21 d

Ingredients	Inclusion (%)	
	Positive Control ¹	Negative Control
Corn	59.92	48.36
Soybean Meal	32.47	25.84
Wheat	--	10
DDGS	--	10
Dicalcium Phosphate	1.82	1.74
Limestone	1.35	1.38
L-Lysine	0.29	0.43
DL-Methionine	0.35	0.34
Soybean Oil	2.93	1.11
Vit/Min Premix	0.25	0.25
L-Threonine	0.19	0.23
White Salt	0.33	0.21
Sodium Bicarbonate	0.10	0.10
Calculated Nutrients ²		
ME (kcal/kg)	3,000	2,900
Crude Protein (%)	20.00	20.00
Calcium (%)	1.01	1.01
Available Phosphorus (%)	0.46	0.46
Dig Methionine (%)	0.63	0.62
Dig Lysine (%)	1.20	1.20
Dig Methionine and Cysteine (%)	0.90	0.90
Analyzed Nutrients		
Crude Protein (%)	20.10	19.80
Crude Fat (%)	5.55	4.20

¹The positive control diet contained 100 kcal/kg ME more than the negative control diets.

²Metabolizable Energy and Available Phosphorus were based on Agristat values as suggested by M. Donohue. 2013. The Challenges in Feeding Broilers in Times of High and Volatile Feed Ingredient Costs: How to Cover the Costs?. 2013 Mid-Atlantic Nutrition Conference proceedings. Digestible amino acids were based on the digestible lysine value (1.2%) suggested by P. B. Tillman and W.A. Dozier. 2013. Current Amino Acid Considerations for Broilers: Requirements, Ratios, Economics. www.thepoultryfederation.com for 8 – 14 day broilers. Digestible amino acid to digestible lysine ratios followed further recommendations of this communication (minimum of 0.54 methionine, 1.02 TSAA, 0.90 threonine, 0.21 tryptophan).

Table 2. AC1¹ β -glucanase activity in 10 mash diet samples to determine homogeneity.

Mash Diet Sample	β -Glucanase ² U/kg
1	411.95
2	394.10
3	471.05
4	401.30
5	433.70
6	428.15
7	448.85
8	392.00
9	451.85
10	420.20
Average	425.32
Standard Deviation	26.44
Coefficient of Variation	6.22%

¹AC1 product was mixed with feed for a target dose of 500 β -glucanase U/kg feed.

² β -glucanase activity was an average of duplicate tests on each protein extract from each feed sample.

Table 3. AC1 β -glucanase activity in feed before (mash) and after pelleting at different temperatures.

Sample / Conditioner Temperature	Activity ¹ (β -glucanase U/kg)	Standard Deviation	Recovery ² (%)
Mash ³	500.45	50.68	-
80°C Pellet	551.56	45.31	110.21
85°C Pellet	496.68	38.99	99.25
90°C Pellet	451.58	30.70	90.23

¹ β -glucanase activity was tested from duplicate protein extracts from each feed sample.

²Calculated as a percentage of the analyzed β -glucanase activity within the mash diet.

³AC1 product was mixed with feed for a target dose of 500 β -glucanase U/kg feed.

Table 4. Growth performance of Hubbard x Cobb 500 straight-run broilers fed a corn, soybean meal, wheat, and DDGS diet with various AC1 concentrations

Diet Formulation	Analyzed β -glucanase (β -Glu-U/kg)	1 to 14 d			14 to 21 d			1 to 21 d		
		Bird FI ¹ (kg)	Bird LWG ² (kg)	FCR ³ (kg/kg)	Bird FI (kg)	Bird LWG (kg)	FCR (kg/kg)	Bird FI (kg)	Bird LWG (kg)	FCR (kg/kg)
Negative Control (NC)	---	0.418	0.293 ^b	1.42	0.640	0.413	1.55	1.06	0.718	1.48 ^a
NC + 50 β -Glu-U/kg	46.1 $\pm$ 13.5	0.423	0.298 ^b	1.40	0.624	0.397	1.58	1.05	0.699	1.48 ^a
NC + 100 β -Glu-U/kg	88.6 $\pm$ 22.5	0.424	0.297 ^b	1.43	0.650	0.409	1.60	1.07	0.715	1.50 ^a
NC + 200 β -Glu-U/kg	237.5 $\pm$ 54.7	0.419	0.294 ^b	1.42	0.650	0.411	1.59	1.07	0.707	1.49 ^a
NC + 400 β -Glu-U/kg	418.8 $\pm$ 19.4	0.417	0.301 ^{ab}	1.38	0.629	0.408	1.55	1.05	0.712	1.46 ^{ab}
Positive Control ⁴	---	0.420	0.314 ^a	1.33	0.655	0.434	1.52	1.08	0.750	1.42 ^b
Treatment P-value		0.9924	0.0330	0.0728	0.2004	0.0674	0.3302	0.4862	0.0840	0.0098
Treatment SEM		0.0083	0.0046	0.0239	0.0100	0.0082	0.0271	0.0134	0.0122	0.0170
Fisher's LSD ⁵		---	0.013	---	---	0.0233	---	---	0.0345	0.0481

¹FI = Feed Intake

²LWG = Live Weight Gain

³Feed Conversion Ratio (Feed:Gain) was calculated using mortality weight.

⁴The positive control diet was corn and soybean meal based and contained 100 kcal/kg calculated ME more than the negative control diets.

⁵Fisher's least significant difference multiple comparison test (a, b, c)

^{a-b}Means, within a column, with superscripts without a common letter differ significantly (P < 0.05)

Table 5. Digesta viscosity of Hubbard x Cobb 500 straight-run broilers fed a corn, SBM, wheat, and DDGS diet with various AC1 concentrations

Diet Formulation	Analyzed β -glucanase (β -Glu-U/kg)	D14 Digesta Viscosity (cP)				D21 Digesta Viscosity (cP)			
		10 X g		20 X g		10 X g		20 X g	
		30 sec	1 min	30 sec	1 min	30 sec	1 min	30 sec	1 min
Negative Control	---	3.56 ^{ab}	3.55 ^{ab}	2.99 ^{ab}	3.01 ^{ab}	5.01	4.59	3.26	3.27
NC + 50 β -Glu-U/kg	46.1 $\pm$ 13.5	3.67 ^{ab}	3.36 ^{ab}	2.80 ^{abc}	2.85 ^{abc}	4.44	4.55	3.28	3.27
NC + 100 β -Glu-U/kg	88.6 $\pm$ 22.5	4.00 ^a	3.78 ^a	3.10 ^a	3.13 ^a	4.59	4.25	3.17	3.20
NC + 200 β -Glu-U/kg	237.5 $\pm$ 54.7	3.48 ^b	3.23 ^{bc}	2.68 ^{bc}	2.76 ^{bc}	3.57	3.51	2.85	2.90
NC + 400 β -Glu-U/kg	418.8 $\pm$ 19.4	3.43 ^b	3.20 ^{bc}	2.71 ^{bc}	2.79 ^{bc}	4.60	4.29	3.23	3.28
Positive Control ¹	---	2.94 ^c	2.86 ^c	2.55 ^c	2.60 ^c	4.22	3.95	2.85	2.85
Treatment P-value		0.0017	0.0056	0.0127	0.0157	0.1468	0.3334	0.4344	0.3870
Treatment SEM		0.163	0.163	0.113	0.108	0.3684	0.3743	0.2046	0.1927
Fisher's LSD ²		0.461	0.463	0.321	0.306	---	---	---	---

¹The positive control diet was corn and soybean meal based and contained 100 kcal/kg calculated ME more than the negative control diets.

²Fisher's least significant difference multiple comparison test (a, b, c)

^{a-c}Means, within a column, with superscripts without a common letter differ significantly (P < 0.05)

CIRRICULUM VITAE

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EDUCATION

Degree Obtained: Bachelor of Science in Agriculture, Animal Sciences; May 2017

University Attended: The Ohio State University

Undergraduate GPA: 3.2

Expected Degree: Master of Science, Animal and Food Science

Expected Graduation Date: May 2019

University Attended: West Virginia University

Current GPA: 3.43

EDUCATION HONORS/AWARDS

Scholarships/Grants Received

- Jones Hamilton Company Undergraduate Student Travel Award: July 2017
- Midwest Poultry Consortium's Center of Excellence: May 2017- August 2017
- Federal Pell Grant: 2016
- President's Affordability Grant: 2016
- Research Scholar Award: June 2016
- Robert L. Hocker Poultry Science Scholarship Fund: June 2016
- Scarlet and Gray Grant: 2016
- Ted Berry Scholarship: June 2013, 2015, 2016

Awards and Honors

- Graduation with Research Distinction: Spring 2017
 - College of Food, Agricultural, and Environmental Sciences Dean's List: Spring 2016
-

PUBLICATIONS

First Author Publications

V.E. Ayres, J.N. Broomhead, X. Li, R.M. Raab, and J.S. Moritz. 2019. Viscosity and growth response of broilers fed high fiber diets supplemented with a corn-produced recombinant carbohydrase. Submitted to Journal of Applied Poultry Research February 28, 2019.

V.E. Ayres, H. Baldwin, X. Li, H. Xu, M. Raab, J. Boney, J. Broomhead, and J.S. Moritz. 2018. Corn-expressed carbohydrase can improve performance and reduce digesta viscosity of broilers fed a high non-starch polysaccharide diet. Journal of Applied Poultry Research. 27(4): 499-506.

Abstracts

V.E. Ayres, S.A. Loop, and J.S. Moritz. 2019. Exogenous enzyme supplementation can overcome amino acid deficiency in broiler starter diets. Submitted to Poult. Sci. April 3, 2019.

K. Harding, A.E. Lamp, T. Boltz, **V.E. Ayres**, and J.S. Moritz. 2019. The effect of pelleting diets composed of large particle corn using standard conditioning and more thermally aggressive conditioning utilizing a hygienizer. Submitted to Poult. Sci. April 3, 2019.

V.E. Ayres, H. Baldwin, X. Li, H. Xu, M. Raab, J. Boney, J. Broomhead, and J.S. Moritz. 2018. Corn-expressed carbohydrase can improve performance and reduce digesta viscosity of broilers fed a high non-starch polysaccharide diet. Poult. Sci. (Accepted Abstract (132)).

V.E. Polentz, J. Griffin, S. Hutsko, M.W. Wick, and M. Cressman. 2017. The effects of diet and age on breast muscle characteristics in commercial broilers. Poult. Sci. (Accepted Abstract (74)).

Co-author Publications

H.Z. Castada, **V. Polentz**, S. Barringer, and M. Wick. 2017. Temperature- dependent Henry's Law constants of 4- alkyl branched- chain fatty acids and 3- methylindole in an oil- air matrix and analysis of volatiles in lab fat using selected ion flow tube mass spectrometry. Rapid Communications in Mass Spectrometry.

RESEARCH EXPERIENCE

National Meeting Oral Presentations

- 2018 Poultry Science Association Annual Meeting (San Antonio, TX) (Graduate Student)
 - “Corn- expressed carbohydrase can improve performance and reduce digesta viscosity of broilers fed a high non-starch polysaccharide diet.”
- 2017 Poultry Science Association (Orlando, FL) (Undergraduate Student)
 - “The effects of diet and age on breast muscle characteristics in commercial broilers.”

Graduate Teaching Assistant

- Teaching Assistant for Poultry Judging at West Virginia University: Spring 2018

- Assisted Dr. Joseph Moritz instructing classes and selected top students to compete in the National Collegiate Poultry Judging Contest.

Graduate Research Assistant

- Led a contract study with Huvepharma to determine digestible amino acid concentrations of diets that vary in amino acid concentration and enzyme inclusion: Spring 2019
- Led a contract study with Agrivida, Inc. analyzing a recombinant carbohydrase enzyme that was included at varying inclusions in high non- starch polysaccharide diets: Spring 2018
- Attended the Poultry Science Association's Annual Meeting: 2017, 2018
 - San Antonio, TX (2018)
 - Orlando, FL (2017)
- Assisted with a traveling backyard poultry demonstration in various locations throughout the state of West Virginia: 2018- present.
- Assisted with numerous West Virginia University Animal Science Farm tours: 2018-present.
- Assisted with the Poultry Festival: 2018
 - Moorefield, WV
 - Assisted with conducting an annual poultry judging competition
- Assisted with the West Virginia State FFA Poultry CDE Competition: 2017, 2018
 - Created classes and organized the competitions
- Davis College Welcome Back BBQ: 2017, 2018
 - Assisted Dr. Joseph Moritz preparing and delivering food

FIELD EXPERIENCE

- Interned with Cargill Animal Nutrition as the Poultry Additive Intern in Brookville, OH.
 - 2017
 - Conducted research investigating the effects of age and diet on the onset of woody breast and resulting breast meat quality characteristics with Dr. Cressman and Dr. Wick at The Ohio State University.
 - 2016, 2017
 - Aided in the study of "Temperature- dependent Henry's Law constants of 4- alkyl branched- chain fatty acids and 3- methylindole in an oil- air matrix and analysis of volatiles in lab fat using selected ion flow tube mass spectrometry" at The Ohio State University.
 - 2016
 - Assist graduate students with research investigating poultry muscle biology and immunology at The Ohio State University.
 - 2015-2017
-

SKILLS

- Efficient in Microsoft Word, Microsoft Excel, and SAS 9.4
- Feed Manufacture
- Diet Formulation
- California Pellet Mill operation
- Poultry processing
- Tibia extraction
- Ileum extraction
- Poultry vaccination experience
- Brookfield cone and plate viscometer
- Caecectomy surgery
- RNA isolation
- cDNA synthesis
- qRT-PCR
- Nanodrop
- Agarose gel electrophoresis
- Histology preparation including sample fixing, embedding, slicing, and staining with hematoxylin and eosin
- Meullenet-Owens Razor Shear Force Analysis
- Selected Ion Flow Tube Mass Spectroscopy (SIFT-MS)