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Research Article
Effects of Increasing Concentrations of Maize-Expressed Non-Starch Carbohydrase Enzyme on Broiler Growth Performance and Ileal Nutrient Digestibility

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Abstract

Background and Objective: An experiment (phase 1) was conducted to evaluate the retained activity of a maize-derived, recombinant carbohydrase enzyme (AC1) when exposed to heat during the pelleting process and homogeneity when mixed into mash diet. A second experiment (phase 2) was conducted to evaluate the effects of increasing AC1 concentrations on male broiler growth performance and ileal nutrient digestibility. **Materials and Methods:** A total of 728 broilers were randomly assigned to seven dietary treatments with each treatment consisting of 13 replicates with eight Cobb 500 male broilers. The experimental design included a positive control (PC), a reduced energy negative control (NC, comprising the PC less 132 kcal kg^{-1}) diet and five additional treatments with increasing dose of AC1 (5, 50, 100, 250 and 500 U glucanase kg^{-1}) added to the NC diet. Birds were fed a starter ration for the duration of the study (16 d). Average body weight (BW), mortality adjusted feed conversion ratio (FCR) and feed consumption (FC) were determined on 16 d of age. At the conclusion of the experiment, all birds from each replicate pen were necropsied and ileal contents pooled within replicate to determine ileal digestibility of energy (IDE) and nitrogen (IDN). **Results:** In Phase 1, the recovery of ß-glucanase activity in the pellets (in relation to mash feed) were 111, 83 and 82% when pelleted at 80, 85 and 90°C, respectively. The coefficient of variation of glucanase recovered when AC1 was mixed into feed and was less than 10%. In Phase 2, the inclusion of AC1 at 100 and 250 U glucanase kg^{-1} increased ($p<0.01$) BW compared to both the PC and NC fed broilers. This elevation in BW was related to an increase ($p<0.01$) in FC. As expected, reducing energy in the NC diet decreased ($p<0.01$) IDE value of the feed as compared to the PC diet. The addition of AC1 to supply ≥100 U glucanase kg^{-1} increased ($p<0.01$) IDE compared to the NC diet and restored IDE equivalent ($p>0.05$) to the PC diet. Regression analysis confirmed linear and quadratic increases in IDE and IDN with the addition of AC1. **Conclusion:** These data demonstrate the thermal stability of maize-derived, recombinant AC1 and that increasing levels positively influences nutrient digestibility leading to significant improvements in broiler performance.

Key words: ß-Glucanase, maize-derived carbohydrase, ileal nutrient digestibility, ileal digestibility of energy, ileal digestibility of nitrogen, growth performance, broilers

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the United States, the majority of broiler diets are corn and soybean meal (SBM) based, comprising a minimum of 70% of the total diet. Corn and SBM provide a large portion of energy and protein to the diet; however, these ingredients contain a vast array of indigestible components that not only represent a potential source of nutrients but also anti-nutrient properties that can hinder further digestion of nutrients important for animal performance. Non-starch polysaccharides (NSP) represent a portion of the indigestible components found in cereal grains and legumes such as corn and SBM. In corn/soy diets approximately 400-450 kcal kg^{-1} of energy is indigestible to broilers in the form of fat, protein and starch[1]. This large amount of undigested energy stems from the broilers inability to endogenously produce the enzymes responsible for hydrolyzing and digesting ingredients that comprise poultry diets[1]. The concentration of NSP in corn and SBM can be as high as 7 and 27%, respectively[2]. Non-starch polysaccharides primarily function as structural constituents of the cell wall, inhibiting enzyme access to the macro-nutrient components of plant based ingredients[3]. This inhibition results in a loss of nutrient digestibility that negatively influences feed efficiency and profitability. Additionally, excess soluble NSP within the lumen of the gut can form viscous gels, decrease passage rate and impede particle movement across the intestinal wall[3,4].

The inclusion of exogenous NSP degrading enzymes (NSPase) may be utilized by nutritionists to alleviate the negative effects of corn and SBM NSP components to improve broiler performance and profit[5]. In cereal grain, the aleurone layer and the endosperm contain the highest concentration of NSP that are responsible for encapsulating the nutrient rich portion of the grain[6], which can hinder nutrient digestibility[5,7]. Gutierrez *et al*.[8] reported that corn bran (outer portion of corn grain) with solubles contained 17.1% of total NSP, of which 5.7% were in the form of glucose residues within the NSP. These authors also reported reduced oil distillers dried grains to contain 25.0% total NSP, of which 8.3% represent glucose residues. Prior research has shown total combined value of cellulose and glucose was 2.8 and 6.5% of corn and SBM (dry matter basis), respectively[6,9]. While these are modest values compared to other grain sources and only increase the viscosity of fecal material minimally, the encapsulated nutrients could be made accessible with enzyme supplementation[10].

With the vast array of anti-nutritive components found in common macro-ingredients in U.S. broiler diets, the addition of NSPase's may be justifiable through improvements in cost and performance. Glucanase inclusion, alone or in combination with other NSPase's, has been shown to improve the breakdown and digestion of the anti-nutritive components in grains and legumes including cellulose and glucans[10-13]. The objective of the current study was to evaluate and establish the heat stability of maize-derived, recombinant carbohydrase enzyme (AC1) and evaluate its dose-dependent impact on animal performance and nutrient digestibility in a reduced energy diet during a 16-day feeding period.

MATERIALS AND METHODS

Phase 1: Thermal stability and in feed homogeneity of AC1: This study investigated the pellet stability of a maize-derived, recombinant carbohydrase enzyme, referred to as AC1 herein. The enzyme is similar to the *Thermotoga maritima* Cel5A endoglucanase, with several additional mutations introduced to improve its thermal stability. AC1 was produced in maize grain using methods described previously[14]. AC1 has multiple activities including cellobiohydrolase, β-1, 3-glucanase, β-xylosidase and cellulase but its primary activity is endo-1, 4-β-glucanase. The AC1 gene encodes a ~38 kDa protein and is stable over 80°C, the common growth temperature of *T. maritima*. AC1 was delivered and used as a coarse ground corn meal in these studies.

AC1 preparation and enzyme assay: Maize grain (AC1) was milled in a knife mill (Retsch SM100, Haan, Germany) before conducting the enzyme activity assay or being mixed with feed. The feed material containing AC1 was also milled by the knife mill before measuring enzyme activity.

For protein extraction from AC1 grain or feed, 20 g of ground material was mixed with 100 mL of pre-warmed (60°C) protein extraction buffer (100 mM sodium phosphate, 0.01% Tween 20, pH 6.5). The sample and buffer mixture was placed in a temperature-control shaker (New Brunswick, Model: Innova 43, Enfield, CT, USA) to shake at 250 rpm, 60°C for one hour. After one hour protein extraction, 1 mL of the mixture was centrifuged at 16,300×g for 10 min in a bench top centrifuge (Labnet, Spectrafuge 24D, Edison, NJ, USA). The supernatant was used to measure the enzyme activity.

AC1 β-glucanase activity is measured using a colorimetric assay that relies on hydrolysis of a labeled, commercial substrate, azurine-cross linked barley β-glucan (AZCL-Beta-Glucan; Megazyme, Wicklow, Ireland). Hydrolysis of this substrate produces water soluble dyed fragments and the rate of release of these dyed fragments is directly related to enzyme activity by measuring the absorbance at 590 nm (A590; Megazyme[15]).

Fifty microliters of diluted protein extract from AC1 grain, or 100 µL of protein extract from feed, was mixed with one tablet substrate in 450 µL (grain assay) or 400 µL (feed assay) of protein extraction buffer in a 2 mL 96-well block (Corning, Inc Costar, Corning, NY, USA). A blank (500 µL extraction buffer mixed with substrate but without protein extract) was also included in the block. After a short vortex of the block at a low speed, the block was incubated in an 80°C water bath for one hour. The enzyme reaction was stopped by adding with 1 mL of 2% Tris base solution. After centrifugation of the block at 3000×g for 10 min, 100 µL of the supernatant was transferred to a new flat-bottom microplate (Corning, Inc- Costar, Corning, NY, USA) to record absorbance at 590 nm.

Beta-glucanase activity was calculated by subtracting an average of the blank A590 value from sample reaction value and then the corrected A590 of each sample was divided by the sample dry weight to calculate the activity on a dry weight basis as A590 g^{-1} of flour or A590 kg^{-1} of feed. The colorimetric activity data can be converted to the ß-glucanase activity units by multiplying by a factor of 0.009 to be presented as unit g^{-1} (U g^{-1}) for AC1 grain or then unit kg^{-1} (U kg^{-1}) for feed analysis.

Thermal stability of AC1 protein extracts: The ß-glucanase activity in (1) Protein extracts from three independent AC1 product batches, (2) AC1 protein purified from AC1-expressing grain or (3) *Escherichia coli* and (4) A thermostable endo-glucanase (Sigma-Aldrich, St. Louis, MO, USA) were tested over a range of temperatures to determine the thermal stability of the enzymes. Protein extracts prepared from each of the AC1 products, the purified AC1 proteins and the endo-glucanase were diluted using extraction buffer. Four-hundred microliters of diluted protein was placed in a Thermo-Shaker MSC-100 (Allsheng, Hangzhou, Zhejiang, China) at temperatures of 50, 60, 70, 80, 90, 95 and 100°C. Heat treatment at each temperature was carried out for 5 min with shaking at 1000 rpm. The temperature of sample wells was monitored using a Dual Channel Digital Thermometer (Fisher Scientific, Hampton, NH, USA). After heat treatment, the protein samples were allowed to cool on ice and further diluted in extraction buffer prior to measuring the enzyme activity.

Homogeneity of AC1 in feed: Ground AC1 corn meal was mixed into a corn-SBM broiler diet at the inclusion rate of 0.06% for a target dose of 100 U β-glucanase kg^{-1}. The mixer used was a Scott's single screw ribbon mixer (Scott Equipment Co., New Prague, MN, USA) with 100 cubic feet of mixing capacity. Ten mash feed samples (0.5 kg per sample) were randomly collected and two protein extracts were conducted for each feed sample. The enzyme activity was measured (in duplicate) from each extracted sample and then average β-glucanase activity per feed sample was used to determine the homogeneity of AC1 in the feed.

Pelleting stability of AC1: To demonstrate AC1 enzyme stability in pelleted feed, AC1 (114 g) was mixed into 454 kg broiler feed for a target dose of 100 U β-glucanase kg^{-1} feed. Three batches of mash feed (136 kg each) were pelleted at 80, 85, or 90°C in a California Pelleting Mill, Master model (California Pellet Mill Co., Crawfordsville, IN, USA), with pelletizer flow rate of 1 t h^{-1} and steam pressure of 20 PSI. Feed conditioning was 15 sec and pellet temperature was measured upon exit from the conditioner, prior to pelleting. Pellets (11/64 inch diameter) were cooled to room temperature (25°C) prior to bagging. Ten 0.5 kg samples of the mash feed (prior to pelleting) and five 0.5 kg samples of pelleted feed per temperature were collected for β-glucanase activity analysis. The AC1 recovery (β-glucanase activity) in pelleted feed, at different temperatures, is calculated as a percentage of the analyzed β-glucanase activity within the mash diet.

Phase 2: Animal feeding trial

Experimental diets: The experimental design consisted of seven dietary treatments. Each treatment consisted of 13 replicates containing eight Cobb 500 males each, for a total of 728 male broilers. A standard U.S. corn-SBM diet containing 5.00% distiller's dried grains with solubles (DDGS) was formulated and manufactured to be used as a positive control (PC), in which nutrient recommendations according to the age and breed of animal were met (Table 1). Furthermore, a negative control (NC) diet was formulated with a 132 kcal kg^{-1} reduction in energy compared to the PC diet (Table 1). Five additional dietary treatments contained increasing levels of AC1 at 5, 50, 100, 250 and 500 U glucanase kg^{-1} added to the NC. Birds were fed a starter ration in the form of a crumble for the duration of the study (d 1-16) that contained titanium as an indigestible marker at 0.50%.

Animals and management practices: On day of hatch, 728 Cobb 500 male broilers were wing-banded and allocated to battery pens and treatments based on initial body weight to ensure statistically equivalent weights across treatments. Chicks were housed in environmentally controlled houses and age appropriate heat and ventilation program was followed throughout the trial. All birds had *ad libitum* access to feed

Table 1: Dietary formulations for experimental diets

Ingredients	Positive control	Negative control
Corn	53.54	56.80
Soybean meal (48%)	33.88	33.29
Low oil DDGS	5.00	5.00
DL-methionine (98%)	0.29	0.29
Lysine HCl	0.20	0.21
L-threonine	0.08	0.08
Soy oil	3.29	0.59
Limestone	1.54	1.54
Monocalcium phosphate	0.90	0.89
Sodium chloride	0.44	0.44
Trace minerals[1]	0.05	0.05
Vitamin premix[2]	0.25	0.25
Phytase[3]	0.01	0.01
Salinomycin[4]	0.05	0.05
Titanium dioxide[5]	0.50	0.50
Calculated nutrient content (%)		
Protein	22.72	22.72
Calcium	0.92	0.92
Available phosphorus	0.45	0.45
AME poultry (kcal kg^{-1})	3076	2944
Av. methionine	0.60	0.60
Av. lysine	1.20	1.20
Av. TSAA	0.90	0.90
Av. tryptophan	0.23	0.23
Av. threonine	0.80	0.80
Av. arginine	1.32	1.31
DEB[6]	214.23	213.10
Sodium	0.20	0.20
Analyzed nutrient content (%)		
Protein	21.9	21.5
Crude fat	4.61	3.04
Crude fiber	5.1	3.90
Ash (%)	5.66	5.80

[1]Trace mineral premix added at this rate yields Manganese: 149.6 mg, Zinc: 125.1 mg, Iron: 16.5 mg, Copper: 1.7 mg, Iodine: 1.05 mg, Selenium: 0.25 mg, a minimum of 6.27 mg calcium and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil, [2]Vitamin premix added at this rate yields: Vitamin A: 11,023 IU, Vitamin D_3: 3,858 IU, Vitamin E: 46 IU, B_{12}: 0.0165 mg, Riboflavin: 5.845 mg, Niacin: 45.93 mg, d-pantothenic acid: 20.21 mg, Choline: 477.67 mg, Menadione: 1.47 mg, Folic acid: 1.75 mg, Pyridoxine 7.17 mg, Thiamine: 2.94 mg, Biotin per kg diet: 0.55 mg. The carrier is ground rice hulls, [3]OptiPhos 2000 PF. -Huvepharma Inc. -Peachtree City, GA, [4] SACOX 60 yields 60 g of Salinomycin Sodium per pound of SACOX-Huvepharma Inc. -Peachtree City, GA, [5]Titanium dioxide -99.5% min. -Alfa Aesar-Ward Hill, MA, [6]DEB: dietary electrolyte balance per milliequivalent value for dietary electrolyte balance. Calculated from dietary sodium, potassium and chloride concentration (DEB, mEq kg^{-1} of diet: Na+K+Cl−, mEq kg^{-1} of diet), calculated values

and water. Chicks were subjected to continuous lighting, which consisted of 24 h of light at 2 foot candles. Average body weight (BW), mortality adjusted feed conversion ratio (FCR) and feed consumption (FC) were determined on day 16, post-hatch. Animal care was provided in accordance with a protocol approved by Texas A&M Institutional Animal Care and Use Committee (IACUC).

Ileal digestible energy (IDE)/ileal digestible nitrogen (IDN): On day 16, all broilers were euthanized by CO_2 asphixiation, ileal (section posterior to Meckel's diverticulum and anterior 3cm of the ileo-cecal junction) contents were drained, collected and pooled per replicate pen. Pooled samples were freeze dried via a Lyophilizer (FreeZone® Freeze Dry Systems, Labconco, Kansas City, MO, USA). Gross energy of feed and ileal content was determined using a Parr 6400 Bomb Calorimeter (Parr Instrument Co., Moline, IL, USA). Nitrogen content was determined using an Elementar Rapid N Cube (Elementar, Ronkonkoma, NY, USA). Titanium concentration was determined following a protocol outlined by Short *et al.*[16].

Statistical analysis: All data were analyzed via a one-way analysis of variance (ANOVA) for a complete randomized block design using the general linear model (GLM) procedure (SPSS V22.0). Treatment means were deemed significantly different at $p \leq 0.05$ and seperated using Duncan's Multiple Range Test. Regression analysis was conducted to determine the impact of increasing levels of AC1 inclusion in the NC on ileal digestibility of energy and nitrogen.

RESULTS

Phase 1: The percent of β-glucanase activity recovered at different temperatures, relative to the activity measured at room temperature (25°C), is presented in Fig. 1 for AC1 grain, purified AC1 and a reference endo-glucanase. AC1 in aqueous extract from three batches demonstrated recovery of 100% activity when heat-treated at temperatures from 50-100°C, relative to its untreated activity at 25°C. AC1 protein purified from grain or *E. coli* maintained more than 82% activity when heat-treated between 60-100°C. The reference thermostable endo-glucanase kept 100% at 70°C relative to its activity at 25°C but maintained only 25.9% activity at 80°C and then lost activity completely at 90, 95 and 100°C.

With an inclusion rate of 560 g per metric ton of feed in the homogeneity study, the AC1 β-glucanase recovery in the mash feed was 97% related to the target dose (Table 2). The average β-glucanase activity from 10 randomly collected mash feed samples was 97 U kg^{-1} feed and the coefficient of variation (CV) was 9.98%. These data demonstrate that AC1 is homogeneously distributed in corn-SBM based feeds.

AC1 β-glucanase activity from mash and pelleted (80, 85 and 90°C) feed samples for the pellet stability trial is shown in Table 3. Enzyme recovery in pelleted feed, calculated as percent of mash feed activity, was more than 80% at all conditioning temperatures.

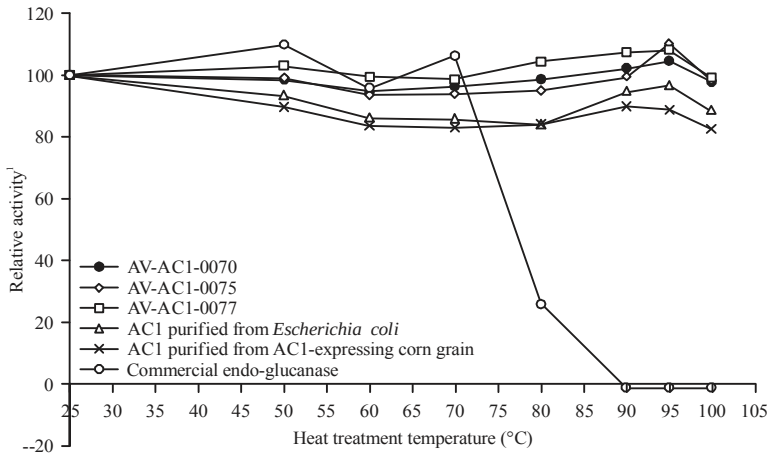

Fig. 1: Relative glucanase activities at different temperatures of three representative AC1 product batches, AC1 protein purified from AC1-expressing grains and *Escherichia coli*, as well as a reference thermostable endo-glucanase

[1]Glucanase recovered at temperature denoted on X-axis as a percentage of that recovered at 25°C

Table 2: AC1 β-glucanase activity from 10 mash[1] diet samples from the homogeneity test

	β-glucanase (U kg^{-1})		
Sample no.	Extract 1*	Extract 2*	Average
1	99.41	106.91	103.16
2	108.09	111.16	109.62
3	106.56	88.17	97.37
4	107.14	105.78	106.46
5	114.12	93.04	103.58
6	96.05	62.62	79.33
7	85.50	82.20	83.85
8	105.41	93.78	99.59
9	106.22	88.69	97.45
10	83.00	100.94	91.97
Overall average activity			97.24
Overall standard deviation			9.70
Coefficient of variation (%)			9.98

[1]AC1 product was mixed with feed for a target dose of 100 β-glucanase U kg^{-1} feed, *β-glucanase activity was an average of duplicate tests on each protein extract from each feed sample

Table 3: AC1 β-glucanase activity from before (mash) and after pelleting at different temperatures

Sample/conditioner temperature	No. of samples	Average activity[1] (β-glucanase U kg^{-1})	Standard deviation	Recovery[2] (%)
Mash[3]	10	72.21	18.80	-
80°C pellet	5	80.01	13.57	110.8
85°C pellet	5	60.17	8.85	83.3
90°C pellet	5	59.00	11.54	81.7

[1]β-glucanase activity was tested from duplicate protein extracts from each feed sample, [2]Calculated as a percentage of the analyzed β-glucanase activity within the mash diet, [3]AC1 product was mixed with feed for a target dose of 100 β-glucanase U kg^{-1} feed

Phase 2: AC1 β-glucanase recovery values from feed samples (mash and crumble) are shown in Fig. 2. β-Glucanase activities in the feed samples of treatment 1 (PC), 2 (NC) and treatment 3 (NC+5 U glucanase kg^{-1}) were not different from each other and represent the background of the measurement from feed samples. β-Glucanase activities in mash and crumble diets were similar to or above the expected target dose.

Performance: The energy reduction (-132 kcal kg^{-1}) in the NC did not negatively impact BW compared to the PC. The inclusion of AC1 in the NC diet at 100 and 250 U β-Glucanase kg^{-1} increased BW ($p<0.01$) compared to both the PC and NC fed broilers (Table 4). The elevations in BW observed in the treatments containing 100 and 250 U β-glucanase kg^{-1} were related to increases in FC

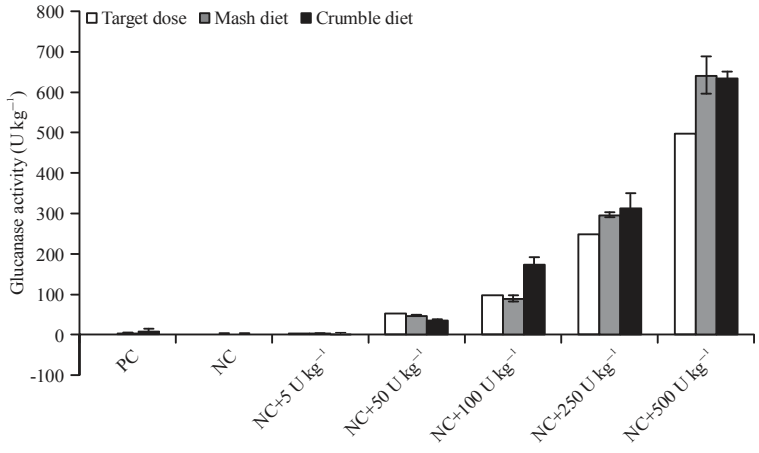

Fig. 2: AC1 β-glucanase activity from mash and pelleted (crumble) diets manufactured for the feeding trial

Table 4: Average Body weight (BW), feed consumption (FC), feed conversion ratio (FCR), ileal digestible energy (IDE), ileal digestible nitrogen (IDN) and ileal digestible energy coefficient (IDEC) for male broilers fed various AC1 concentrations

Treatments	BW (g)	FC (g/bird/day)	FCR (feed:gain)	IDE (kcal kg^{-1})	IDN (%)	IDEC (%)
Positive control	572.3^{b}	42.0^{b}	1.186	2933.3^{a}	0.7641	0.672^{a}
Negative control	570.9^{b}	41.7^{b}	1.203	2780.9^{c}	0.7529	0.656^{c}
NC+5 β-glucanase U kg^{-1}	587.3^{ab}	44.0^{a}	1.209	2802.3^{c}	0.7495	0.661^{c}
NC+50 β-glucanase U kg^{-1}	585.4^{ab}	44.0^{a}	1.219	2846.8^{bc}	0.7701	0.672^{bc}
NC+100 β-glucanase U kg^{-1}	592.9^{a}	43.7^{a}	1.205	2940.5^{a}	0.7783	0.694^{a}
NC+250 β-glucanase U kg^{-1}	592.8^{a}	44.2^{a}	1.213	2894.5^{ab}	0.7808	0.683^{ab}
NC+500 β-glucanase U kg^{-1}	582.9^{ab}	43.7^{a}	1.222	2955.8^{a}	0.7756	0.697^{a}
ANOVA						
p-value	0.016	<0.001	0.167	<0.001	0.114	<0.001
Pooled CV	3.8	4.7	3.3	3.5	4.0	3.5
Regression						
	BW	FC	FCR	IDE	IDN	
Linear						
p-value	0.59	0.263	0.279	<0.001	0.035	
Quadratic						
p-value	0.065	0.136	0.551	<0.001	0.007	

$^{a-c}$Means within a column with different superscripts differ at p<0.05

(p<0.01, Table 4). Even though differences (p<0.01) between treatments were observed in both BW and FC, no differences (p>0.05) in FCR were observed amongst treatments.

Digestibility: The reduction of energy in the NC diet decreased (p<0.01) IDE value of the feed as compared to the PC diet (Table 4). The addition of AC1 >100 U β-glucanase kg^{-1} increased (p<0.01) IDE compared to the NC diet and restored IDE to levels similar (p>0.05) to the PC diet (Table 4). No significant differences (p>0.05) in IDN were observed between treatments, though increasing levels of AC1, up to 250 U β-glucanase kg^{-1}, numerically increased IDN values (Table 4). Regression analysis confirmed linear and quadratic increases in ileal digestibility of energy and nitrogen (p<0.05) with the addition of AC1 at increasing levels (Table 4). These data demonstrate that increasing levels of maize-derived recombinant AC1 positively influences nutrient digestibility leading to significant improvements in broiler performance.

DISCUSSION

Thermostability and homogeneity: Thermostability of enzymes in pelleted animal feed, such as broiler and turkey feed, is a concern for poultry producers. Conditioning of broiler feed prior to pelleting is typically between 80 -90°C in the US. Enzymes can heat denature and become less effective at higher pelleting temperatures, which is why enzymes are often coated or engineered to improve their thermostability[17]. AC1 grain demonstrated intrinsic thermostability by recovering over 80% of glucanase activity following pelleting at 90°C. Even when the enzyme is extracted from the AC1 maize, the thermostability remained high (>80% recovery) at temperatures up to 100°C.

Feed additives dosed at low inclusion rates should be homogeneously mixed into feed to reduce variability in animal performance. The feed industry considers a mixing coefficient of variation (CV) of <10% an adequate level of mixing when

determining mixer uniformity utilizing a marker in the feed[18]. When measuring diet homogeneity, the CV of AC1 activity recovered from feed (n = 10 samples) was less than 10%.

Performance: The use of a carbohydrase in commercial broiler diets is common place in wheat and barley because of the high level of substrate present in these two cereal grains; however less information is present about the impact in corn based diets. In the current study utilizing a corn based diet, reducing energy level (-132 kcal kg^{-1}) in the NC did not significantly impact body weight compared to the PC. The lack of separation may be attributed to the rearing environment and short duration, as this experiment was conducted in battery units and only went through the starter phase. Regardless, the inclusion of AC1, supplying 100 and 250 U β-glucanase kg^{-1}, to the NC was sufficient to impact feed consumption leading to an increase in BW compared to both control diets. Differences in FC were not observed between the PC and NC which contradicts previous literature stating that broilers consume feed to meet their energy requirement[19-21]. Again this may be attributed to rearing environment and short experimental period, however, the addition of the enzyme did increase feed consumption rate. This FC increase may have been caused by the increase in body weight leading to a further increase in maintenance requirements. Bi and Chung[22] reported an increase in BW through d 21 and 38 with supplementation of an enzyme cocktail containing amylase, glucanase and xylanase to a corn based diet with a 3% reduction in ME compared to the NC (with no enzyme supplementation) and equivalent to the PC (adequate in ME). However, no differences in FC or FCR were observed by Bi and Chung[22] at any point during the trial. Cowieson *et al.*[12] reported a significant improvement in BW gain and FCR with the inclusion of glucanase at 15,000 and 30,000 U kg^{-1} respectively to a reduced energy (110 kcal kg^{-1}) diet through d 21. However, no differences in 42 d BW gain were observed amongst treatments[12].

The current study, along with the findings of Bi and Chung[22], demonstrated that feeding ß-glucanase at lower levels (0-500 U kg^{-1}) did not impact FCR, however it is not necessarily anticipated that supplementation will impact both BW and FCR. Contrary to the current experiment, Newman and Newman[23] reported the inclusion of ß-glucanase at 100 U kg^{-1} in a corn based diet exhibited no improvement in 21 d BW or FCR compared to the NC corn based diet. However, ß-glucanase inclusion in a wheat based diet (absent of corn) increased BW compared to the NC wheat based diet. These results agree with findings of previous literature, that increasing the level of substrate (with anti-nutritional properties) increases response from enzyme addition, with more notable improvements in digestion and absorption of dietary nutrients[4]. In a three study experiment conducted by West *et al.*[24] evaluating reduced energy corn-soybean meal diets with varying amino acid concentrations, with or without the inclusion of a commercial NSPase consisting of xylanase and ß-glucanase, reported no differences in BW gain among treatments during all growth periods.

Early improvements in BW gain (d 21 and earlier, as observed in current study) with the inclusion of β-glucanase may be due to the lack of endogenous enzyme production and microbial activity within the gastrointestinal tract of young birds.

Digestibility: Proper digestion of food is imperative for efficient absorption of nutrients into the body. Stefanello *et al.*[3] reported an NSPase (xylanase) inclusion of 50 and 100 FXU kg^{-1} improved IDE compared to the NC (absent of enzyme inclusion), however the inclusion of 150 and 200 FXU kg^{-1} produced intermediate results. These data suggest a threshold for enzyme activity in relation to substrate available in the diet, as increasing NSPase inclusion did not statistically improve IDE compared to the NC. Cowieson *et al.*[12] reported improvements in nitrogen digestibility with 30,000 BU kg^{-1} inclusion of glucanase and improvements in IDE with a 15,000 and 30,000 BU kg^{-1} inclusion compared to the NC without the enzyme. With the combination of xylanase and glucanase above 8,000 XU kg^{-1} and/or 15,000 BU kg^{-1}, respectively, IDE increased to similar levels as the PC. Similarly, in the current trial, the inclusion of AC1 to supply 100, 250 and 500 U β-glucanase kg^{-1} increased IDE when compared to the NC and reached similar levels to that of the PC. The linear and quadratic increase in IDE with increasing AC1 dose may be associated with substrates in the diet which are not fully hydrolyzed at lower inclusion levels. The current experiment did not exhibit any significant changes in nitrogen digestibility compared to controls, however increasing AC1 resulted in linear and quadratic improvements in IDN values. Additional studies should be conducted to investigate the potential impact of AC1 on crude protein digestibility in diets varying in substrate concentration.

Histological samples were not evaluated in the current study, however Karimi and Zhandi[25] reported improvements in duodenal and ileal villi length with addition of ß-glucanase or ß-mannanase in diets containing barley, soybean meal and corn. The exact mechanism for the improvements in gut morphology with the inclusion of ß-glucanase is unclear;

however the enzymes ability to reduce digesta viscosity may have a beneficial effect on the intestinal morphology. These improvements in intestinal morphology (increasing the surface area for the major structures) were responsible for increased absorption of nutrients[26].

CONCLUSION

The inclusion of AC1 at or above 100 U β-glucanase kg^{-1} increased BW, FC and IDE compared to the NC and were similar to or above that of the PC. These data suggest that improvements in digestibility with increasing levels of AC1 could lead to further improvements in growth and performance. Based on its activity profile, AC1 is capable of hydrolyzing and ameliorating some of the anti-nutritive components commonly found in broiler diets, therefore, improving digestion and absorption, ultimately improving performance.

SIGNIFICANCE STATEMENT

The current study evaluated the heat stability of a carbohydrase enzyme (AC1) and its effect on broiler growth performance and nutrient digestibility. The result will help individuals better understand the efficacy of the AC1 enzyme derived from maize, on broiler performance. Providing the framework for its use in commercial broiler production and future research.

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