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Research Article
Effect of Corn-Expressed Glucanase on Mineral Digestibility and Apparent Metabolizable Energy in Broiler Diets

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Abstract

Background and Objective: Poultry diets are often formulated with corn-derived distillers dried grains and cereal grains. These grains contain higher levels of non-starch polysaccharides (NSPs) which are not digestible by broilers and can increase intestinal viscosity. β-glucan is partially water soluble which in turn causes a gel-like viscosity in the gastrointestinal tract of broilers. This reduces the diffusion rates of substrates and enzymes which leads to reduced nutrient absorption. The addition of glucanase enzymes has been shown to degrade plant cell walls releasing nutrients from grain endosperm while providing a probiotic effect. As a result, glucanases can improve broiler performance by allowing NSPs to be digested and absorbed. This experiment was conducted to evaluate the effects of different inclusion rates of corn-expressed glucanase (AC1) on apparent ileal digestibility (AID) and apparent metabolizable energy (AME). **Materials and Methods:** Broilers were fed a diet with corn-expressed glucanase enzyme (AC1) at different inclusion rates: Positive Control (PC, no enzyme balanced diet), Negative Control (NC, -100 kcal kg^{-1} ME reduction from PC diet) and NC diets with corn-expressed glucanase added at different inclusion rates, with AC1 added at either 0.18 kg t^{-1}, 0.35 kg t^{-1}, 0.50 kg t^{-1}, 0.75 kg t^{-1}, or 2.00 kg t^{-1}. At day 28 and 42, intestinal contents were collected from the ileum of 48 birds/trt. On d28, samples were analyzed for ileal digestibility and on day 42 samples were analyzed for AME and AME corrected for nitrogen. **Results:** Ileal digestibility was improved or equal to the PC and better than the NC in broilers fed AC1 at rates 0.35 kg t^{-1} and higher for Ca and P digestibility ($p<0.05$). Both AME and AMEn were improved in broilers fed AC1 at rates of 0.35 kg t^{-1} and higher when compared to the NC. The AMEn was equal in the PC and AC1 fed at 2.00 kg t^{-1}. **Conclusion:** Feeding AC1 at rates greater than 0.35 kg t^{-1} increases calcium and phosphorus digestibility and can return AME/AMEn to normal levels in diets with -100 kcal kg^{-1} ME reductions. This could lead to improved feed efficiency and bone strength in broilers which could lead to improved performance and welfare.

Key words: Apparent metabolizable energy, broiler diet, broiler, corn-expressed enzyme, glucanase, growth performance

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Glucanase has been shown to improve the nutritive value of non-starch polysaccharides included in poultry diets by reducing antinutritional effects[1,2]. Studies have shown that glucanase supplementation can improve AME[3,4]. It is uncertain as to which mechanism increases AME content. In a study conducted by Rutherfurd *et al.*[5], it was shown that feeding a corn-soy based diet supplemented with xylanase, amylase and β-glucanase led to increases in AME (2,829 kcal kg^{-1}) in diets that were supplemented with enzymes when compared to unsupplemented diets (2,766 kcal kg^{-1}). Similar results were seen in a study by Meng and Slominski[3] when feeding a corn-soybean diet supplemented with 400 U kg^{-1} of glucanase, they saw a 2.4% increase in dietary AME. Similarly, Rutherfurd *et al.*[5] saw that feeding a corn-based diet supplemented with 140 fungal β-glucanase units kg^{-1} led to a 2.3% increase in AME. These results indicate that, although the mechanism for which AME content is improved is unknown, the supplementation of glucanase in diets can improve AME.

It is well understood that glucanase can improve ileal digestibility by reducing intestinal viscosity which allows for better mineral absorption. In an experiment by Perttilä *et al.*[6], when evaluating apparent ileal digestibility of broilers while feeding a semi-purified soyabean meal basal diet or a mixture of the basal diet and barley (50:50 on dry matter basis) supplemented with β-glucanase, AID of amino acids was improved in dried barley. Leslie *et al.*[7] found that feeding a corn-soybean diet supplemented with 500 units of glucanase kg^{-1} improved ileal-digestibility at all ages relative to diets that lacked the glucanase. Throughout the 23-day trial, diets supplemented with glucanase had a higher IDE (3,210 kcal kg^{-1}) than all other diets. It is hypothesized that these results are due to an increase in amylase access to starch granules within the cells of the endosperm. The degradation of the cell wall allows for enzymes to have access to cell contents.

The microbial form is the most common form of glucanase that is supplemented in diets. In this form, fungal and bacterial hosts are used in the production of microbial enzymes which can potentially lead to the contamination of the product[8]. Genetically modified corn can be used as an alternative to microbial enzymes. In the process of making genetically modified corn, high concentrations of recombinant enzymes are produced within transgenic corn grain to be used as feed additives at low inclusion rates[9-11]. The corn expressed glucanase (CEG) that was used in this experiment was AC1. AC1 expresses a recombinant carbohydrase, with endo-β-1,4-glucanase activity[12].

The objective of this study was to compare the efficacy of corn-expressed glucanase, (Grainzyme® Glucanase AC1) at different inclusion rates in broilers. It can be expected that the addition of corn expressed glucanase will improve AID and AME. Additionally, CEG can be used as a cheaper alternative since corn is already used in poultry diets.

MATERIALS AND METHODS

Animal husbandry, diet and experimental design: A total of 864 Cobb 500 male broiler chicks were used in this experiment. The birds were randomly assigned to one of seven dietary treatments (Table 1), including a positive control (PC, no enzyme added and typical commercial broiler diet), a negative control (NC, -100 kcal kg^{-1} ME reduction from PC diet) and NC diets with various inclusion rates of corn expressed glucanase (AC1, Agrivida, Woburn, MA, USA). The birds were fed a three-phase diet consisting of a starter (day 0-14, crumble), grower (day 14-28, pellet) and finisher (day 28-42, pellet). Both PC and NC diets were corn/soybean-based diets and were formulated based on the Cobb guide. Birds were allowed *ad libitum* access to feed and water. Titanium dioxide (TiO_2) was included at 0.5% in the grower diets as an indigestible marker and used for the determination of apparent ileal digestibility (AID) of P and Ca. Bird management was in accordance with guidelines outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching[13], all procedures were approved by Texas A&M University animal care and use committee (IACUC 2018-0181).

Table 1: Dietary treatment description and abbreviation for birds fed AC1

Treatments	Abbreviation	Glucanase U kg^{-1}
Positive control	PC	-
Negative control	NC	-
Negative control +0.18 kg t^{-1} of corn expressed glucanase (AC1)	0.18AC1	25
Negative control +0.35 kg t^{-1} of corn expressed glucanase (AC1)	0.35AC1	50
Negative control +0.50 kg t^{-1} of corn expressed glucanase (AC1)	0.50AC1	75
Negative control +0.75 kg t^{-1} of corn expressed glucanase (AC1)	0.75AC1	100
Negative control +2.00 kg t^{-1} of corn expressed glucanase (AC1)	2.00AC1	300

Digestibility and AME: On day 28, intestinal contents were collected from the ileum by gently finger-stripping the intestinal segment from 48 birds/treatment, frozen on a Labconco FreeZone 12+lyophilizer (Labconco, Kansas City, Missouri, USA) and subsequently analyzed for ileal digestibility (IDE). Calcium and total phosphorus in feed and ileal digesta samples were determined by Inductively Coupled Plasma-Optical Emission Spectrometry in accordance with method of AOAC, 2011.14 (AOAC, 2011) following microwave assisted acid digestion. Titanium concentration was determined via a protocol outlined by Short *et al.*[14]. This procedure consisted of a half gram of each dried sample being weighed and ashed. Following ashing, each sample was titrated with 10 mL of sulfuric acid (7.4 M) and then boiled at 200°C for 2 hrs until dissolved. Samples were then titrated with 20 mL of 30% hydrogen peroxide and brought to 100 mL total volume using distilled water. Samples were then analyzed for absorption using a Thermo Fisher Scientific Genesys 10S UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) at 410 nm. Apparent ileal digestibility of P and Ca were calculated based on the following formula[15], using titanium dioxide as the inert marker:

$$\text{Indigestibility factor (IF)} = \frac{\text{Marker concentration in feed}}{\text{Marker concentration in digesta}}$$

$$\text{AID} = 1\text{-(IF)}\times\text{(Ni/Nd)}$$

Ni is the nutrient (P or Ca) concentration in the ileal digesta and Nd is the nutrient concentration in the diet.

On day 42 intestinal contents were collected from the large intestine by gently finger-stripping the intestinal segment from 48 birds/treatment, frozen on a Labconco FreeZone 12+lyophilizer (Labconco, Kansas City, Missouri, USA) and subsequently analyzed for AME and AME corrected for nitrogen. AME and AMEn was calculated by markers (TiO_2)[16]:

$$\text{Indigestibility factor (IF)} = \frac{\text{Marker concentration in feed}}{\text{Marker concentration in digesta}}$$

$$\text{AME (kcal kg}^{-1}\text{)} = \text{GEdiet} - \text{(Gedigesta}\times\text{IF)}$$

$$\text{AMEn (kcal kg}^{-1}\text{)} = \text{GEdiet-(GEdigesta}\times\text{IF)}+8.22\times\text{[Ndiet-(Ndigesta}\times\text{IF)]}$$

For GE for both feed and digesta determination, samples were dried at 100°C for 24 hrs and gross energy of feed and digesta was determined using a Parr 6400 bomb calorimeter (Parr Instrument Company, Moline, IL). Nitrogen concentration of each dried sample was determined via combustion method, using an Elementar Rapid N Cube Analyzer (Elementar Americas, Inc., Mt. Laurel, NJ).

Statistical analysis: All data was analyzed using Minitab Software via One-Way ANOVA using the General Linear Model (GLM) procedure. Differences of $p<0.05$ were considered statistically significant. Treatment means that were determined to be significant were further separated using Fishers LSD Test.

RESULTS

Ileal digestibility: The means for Ileal digestibility are shown in Table 2. When evaluating ileal continents for calcium digestibility, NC and PC performed similarly ($p>0.05$). Birds that were fed 0.35 kg t^{-1} of AC1 and higher had increased calcium digestibility when compared to the NC ($p<0.05$). The 0.35AC1 treatment had higher ($p<0.05$) calcium digestibility than the PC, all other dietary treatments performed similarly to the PC ($p>0.05$).

When evaluating ileal contents for phosphorus digestibility, the PC outperformed the NC ($P < 0.05$). Birds that were fed AC1 at 0.35 kg t^{-1} and higher outperformed the NC and performed similarly to the PC ($p<0.05$).

Apparent metabolizable energy: Means for AME and AMEn are shown in Table 3. The PC birds had higher AME than the

Table 2: Apparent ileal digestibility of calcium and phosphorus at day 28 of broilers feed corn expressed glucanase

Treatments	Calcium digestibility (%)	Phosphorus digestibility (%)
PC	54.8^{bc}	63.8^{ab}
NC	48.0^{cd}	56.0^{c}
0.18AC1	42.7^{d}	61.3^{bc}
0.35AC1	63.2^{a}	68.2^{a}
0.50AC1	61.6^{ab}	66.6^{ab}
0.75AC1	58.4^{ab}	68.1^{a}
2.00AC1	55.7^{b}	62.3^{ab}
SEM	1.1	0.9
p-value	<0.0001	<0.0001

$^{a-d}$Means within columns with different superscripts differ significantly ($p<0.05$)

Table 3: Apparent metabolizable energy (AME, kcal kg^{-1}) and AME adjusted for nitrogen (AMEn, kcal kg^{-1}) at d 42 of broilers feed corn expressed glucanase

Treatments	AME	AMEn
PC	3764.0^{a}	3422.2^{a}
NC	3465.9^{f}	3111.5^{c}
0.18AC1	3537.8^{e}	3118.2^{c}
0.35AC1	3564.8^{cde}	3230.7^{b}
0.50AC1	3579.8^{cd}	3217.0^{b}
0.75AC1	3537.4^{de}	3242.3^{b}
2.00AC1	3689.9^{b}	3377.8^{a}
SEM	9.9	14.9
p-value	<0.0001	<0.0001

$^{a-f}$Means within columns with different superscripts differ significantly ($p<0.05$)

NC and all other dietary treatments ($p<0.05$). Birds that were fed AC1 had higher AME than the NC ($p<0.05$). When AME was corrected for Nitrogen, birds that were fed 2.0 kg t^{-1} AC1 and PC performed similarly and outperformed all other dietary treatments ($p<0.05$). All birds that were fed AC1 at 0.35 kg t^{-1} and higher had better AMEn than the NC ($p<0.05$).

DISCUSSION

Most poultry diets contain cereal grains which contain non-starch polysaccharides (NSPs). The presence of NSPs such as beta glucans in cereal grains can negatively affect nutrient utilization and reduce growth performance of broilers[17]. Glucanase enzymes are used in poultry diets due to their ability to degrade plant cell walls and release nutrients from grain endosperm[18]. It was expected that the use of corn expressed glucanase enzymes (AC1) would decrease intestinal viscosity and enhance nutrient digestibility; this in turn will improve utilization of fibrous feed ingredients as previously observed[19].

The current study observed that the addition of AC1 did improve calcium and phosphorus digestibility at inclusion rates above 0.35kg t^{-1} in diets with decreased ME (-100 kcal kg^{-1}). Similar results have been seen in studies using microbial produced phytase[20]. Additionally, a similar opinion was expressed by Kim *et al.*[21] who used microbial produced glucanase. In this current study, AME and AMEn were improved at all inclusion rates of AC1 at 0.35 kg t^{-1} and above when compared to the NC. The 2.00AC1 inclusion rate was even able to improve AMEn to the same level as the PC treatment. Similar results were observed in previous research utilizing glucanase[22-24]. Based on the composition it was observed that AC1 is capable of hydrolyzing and ameliorating some of the anti-nutritive components commonly found in broiler diets[22]. This resulted in improved digestion and absorption which lead to improved broiler performance and welfare.

CONCLUSION

This study showed that Ca and P reduced diet can improve AME and ileal digestibility. Further studies will need to be conducted to see if broiler performance and bone strength improve as a result of improved digestibility and AME. A larger reduction in energy should also be investigated to when the efficacy of AC1 is reached. If further studies show improved broiler performance and welfare, AC1 would be a feasible additive for poultry diets as it can be grown in the corn that is already being used in diets. It can be a cheaper alternative to other glucanase products since there is not any expensive microbial process related to its cost.

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