# Evaluation of the precision-fed rooster assay for detecting effects of supplemental enzymes on metabolizable energy

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**ABSTRACT** The precision-fed rooster assay has been used extensively to determine nitrogen-corrected true metabolizable energy (**$TME_n$**) of feed ingredients for poultry. However, this assay has not generally been used to evaluate effects of supplemental enzymes for this purpose. Therefore, 2 precision-fed rooster assays were conducted to evaluate several different carbohydrase enzymes on $TME_n$ for a corn/soybean meal diet, a pearled barley diet, and diets containing different inclusion levels of rye/corn. In both rooster assays, Single Comb White Leghorn roosters were fasted for 26 h and then crop intubated with either 25 or 30 g of the test diets, depending on the assay. Excreta were then collected quantitatively for 48 h after feeding. In the first rooster assay with 56 birds, 6 carbohydrase combinations and/or levels (xylanase/alpha-galactosidase were evaluated using a corn/soybean meal control diet. All carbohydrase additions either numerically or significantly ($P < 0.05$) increased $TME_n$ and the mean increase for the enzyme treatments was 66 kcal/kg DM compared with the corn/soybean meal control diet. The second assay consisted of twenty dietary treatments; 120 roosters were crop-intubated with 25 g of diets that were composed of 100% barley, 100% rye, 50% rye: 50% corn, or 25% rye: 75% corn. The diets were fed with and without inclusion of 2 different levels of either $\beta$-glucanase, xylanase, or a multi-carbohydrase combination. Both $\beta$-glucanase and the multi-carbohydrase significantly ($P < 0.05$) increased $TME_n$ of the 100% barley diet, with the multi-carbohydrase increasing it from 3,722 to 4,086 kcal/kg DM at the highest inclusion rate. The xylanase and multi-carbohydrase either numerically or significantly ($P < 0.05$) increased $TME_n$ of the 100% rye diet, with the multi-carbohydrase increasing it from 3,581 to 3,909 kcal/kg DM at the highest inclusion rate. The magnitude of enzyme response decreased as the level of rye in the diets decreased. Overall, results of this study indicated that the precision-fed rooster assay can detect effects of enzymes, primarily carbohydrase, on $TME_n$ of diets containing corn/soybean meal, pearled barley, and/or rye.

**Key words:** barley, rye, enzymes, xylanase, glucanase

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## INTRODUCTION

Inclusion of dietary exogenous enzymes in poultry diets has clearly been established as beneficial, especially as it pertains to diets with a high nonstarch polysaccharide (**NSP**) content such as barley and rye. Research in this area is highly developed and has been ongoing for decades. Less established is whether enzymes are consistently able to provide benefits in diets containing nonviscous cereal grains such as corn, which has huge potential since about 80% of global pig and poultry feed is based on corn (Barletta, 2010). Interest in the development of enzyme feed technology is not expected to diminish in the near future, as it is an essential part of the pursuit to achieve safe, affordable methods that make our animal-based food system more efficient to meet the growing world needs for food. In addition, there is a need to have the ability to use a broad range of nonconventional feed ingredients, thereby improving the sustainability of the animal protein industry.

Traditionally, a growth-type assay with chicks is used to evaluate prototypical enzyme products for potential benefits such as increased metabolizable energy. However, this method is laborious and usually requires significant time, money, birds, and resources including substantial amounts of the test enzyme itself. The precision-fed rooster assay may be an alternative to the growth assay by providing a faster and more economic option to evaluate current and new carbohydrase products for metabolizable energy. This balance assay also allows for the repeated use of adult birds instead of chicks, the flexibility



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to evaluate many samples at one time, and possibly the most compelling advantage, the ability to feed diets containing 100% of the target ingredient which increases the enzyme's target substrate. Only a few studies have been conducted to evaluate the precision-fed rooster assay for evaluating feed enzymes and the results have not been consistent. For example, studies by Meng et al. (2006) and Slominski et al. (2006) experienced success using the precision-fed rooster assay to detect the effects of a multi-carbohydrase combination containing xylanase, cellulase, amylase, protease, mannanase, invertase, and pectinase on $TME_n$ of full-fat canola seed and full-fat flaxseed, respectively, while Assadi et al. (2011) did not have success using the rooster assay for detecting effects of enzymes on full-fat canola seed. The objective of the current study was to more extensively evaluate the precision-fed rooster assay for determining the effects of carbohydrase enzymes on $TME_n$ of diets containing corn, soybean meal, barley, and rye.

## MATERIALS AND METHODS

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee (animal use protocol 20131).

### Experimental Design

Two experiments were conducted to measure $TME_n$ values of diets containing varying types and levels of carbohydrases using the precision-fed rooster assay. Both experiments followed the general guidelines of Sibbald (1986) with some modifications (Zhang et al., 1994). In Experiment 1, there were 7 dietary treatments and 8 replicates per treatment, with an individually caged bird serving as the experimental unit. Conventional roosters were fasted for 26 h to allow adequate clearing of the gastrointestinal tract and were housed in individual metabolism cages with wire flooring. After the feed withdrawal, each rooster was precision-fed 30 g of one treatment diet and returned to its respective cage, and excreta were then quantitatively collected for 48 h on plastic trays placed beneath the cages. Water was provided ad libitum and the roosters were housed in an environmentally controlled room with a daily 16 h light and 8 h dark photoperiod cycle. Basal endogenous energy losses were determined using roosters that had been fasted for 48 h.

In Experiment 2, there were only slight modifications. Roosters were precision-fed a lower amount of 25 g of diet due to concerns of lower diet bulk density and high viscosity effects on digesta passage rate from feeding high-NSP diets, and each treatment diet was replicated 6 times with an individually caged bird serving as the experimental unit.

### Diet Composition

In Experiment 1, six different combinations and/or levels of xylanase and α-galactosidase were evaluated for effects on $TME_n$ of a corn/soybean meal-based diet. A basal diet (Table 1) was mixed in one batch to reduce variation among treatments and served as Diet 1 for a negative control. Diets 2 to 7 contained different levels and/or combinations of the enzymes which were added at the expense of the basal diet and mixed in 1,000 g batches in a tabletop mixer to allow for accurate weighing and mixing of the exogenous enzymes at low inclusion levels. All diets were fed in mash form. Enzymes were in liquid form and added to the feed following instructions for product application. The two enzymes were supplied by a commercial company but they are not commercially available and the specific enzyme treatment combination quantities as well as the level or units of activity of the two enzymes are confidential.

**Table 1.** Composition of corn/soybean meal negative control diet (Diet 1) in Experiment 1.

| Item | Inclusion (%) |
|---|---|
| Corn | 58.81 |
| Soybean meal | 36.00 |
| Soy oil | 2.10 |
| NaCl | 0.10 |
| DL Met | 0.24 |
| L-Lys HCl | 0.15 |
| Limestone | 0.85 |
| Dicalcium phosphate | 1.40 |
| Vitamin mix[1] | 0.20 |
| Mineral mix[2] | 0.15 |
| Analyzed content: | |
| CP | 22.0 |
| Lys | 1.31 |
| Met + Cys | 0.92 |
| Ca | 0.78 |
| Total P | 0.60 |

[1]Provided per kilogram of complete diet: retinyl acetate, 4,400 IU; cholecalciferol, 25 μg; dl-α-tocopheryl acetate, 11 IU; vitamin $B_{12}$, 0.01 mg; riboflavin, 4.41 mg; d-Ca-pantothenate, 10 mg; niacin, 22 mg; and menadione sodium bisulfite complex, 2.33 mg.

[2]Provided per kilogram of complete diet: Mn, 75 mg from MnO; Fe, 75 mg from $FeSO_4 \bullet 7H_2O$; Zn, 75 mg from ZnO; Cu, 5 mg from $CuSO_4 \bullet 5H_2O$; I, 0.75 mg from ethylene diamine dihydroiodide; and Se, 0.1 mg from $Na_2SeO_3$.

In Experiment 2, there were 20 diets mixed in 1,000 g batches to allow for accurate weighing and mixing of exogenous xylanase, β-glucanase, or multi-carbohydrase combination at low inclusion levels. The enzymes were substituted in the diets at the expense of the entire diet. The primary grains used in the study were barley and rye and were designed to provide a high level of the targeted substrate (β-glucans in barley and arabinoxylans in rye) and thereby increase the response for the various test enzymes. The barley diets contained 100% barley. Because of concerns that excessive intestinal digesta viscosity could potentially interfere with nutrient digestibility and efficacy of the enzymes (Lazaro et al., 2003), the rye was fed alone (100%) and also at decreasing inclusion levels mixed with increasing levels of corn (50% rye: 50% corn and 25% rye: 75% corn). These rye: corn combinations or levels were chosen to more closely approximate the level of rye that might be fed in practical poultry diets. The enzymes evaluated were β-glucanase in the barley diets, xylanase in the rye and rye:corn diets and a multi-carbohydrase combination was

evaluated in both the barley and rye-containing diets and the enzymes were included at 2 inclusion levels. The lower level of enzyme inclusion was chosen to reflect the recommended commercial feeding level, while the higher level of each enzyme (10 or 20 times the commercial level, depending on the enzyme product) was the level recommended by the respective supplier in an attempt to magnify any potential enzyme response. The xylanase (endo-1, 4-beta-xylanase) and β-glucanase (endo-1, 3 (4)-β-glucanase) products were spray dried but stabilized with various sugar alcohols. Both enzymes were *Trichoderma reesei*-produced and were supplied by AB Vista, Marlborough, UK. The multi-carbohydrase contained xylanase, glucanase, cellulase, amylase, protease, mannanase, invertase, and pectinase and was supplied by Canadian Bio-Systems, Calgary, CA.

The xylanase (Econase XT P) and β-glucanase (Econase GT P) used in the second experiment were supplied and analyzed by AB Vista (Plantation, FL) for xylanase and β-glucanase activity. Based on the analyzed values, the xylanase provided 148,000 BXU/g of xylanase activity and 9,110 BU/g β-glucanase activity, and the β-glucanase provided 209,000 BXU/g xylanase activity and 2,560,870 BU/g β-glucanase activity. The unit BXU is defined as the amount of enzyme that produces 1 nmol of reducing sugar from birch xylan as xylose per second (at pH 5.3 and 50°C), while the unit BU represents the amount of enzyme producing 1 nmol of reducing sugar from barley β-glucan per second (at pH 4.8 and 50°C). Although each product contained activity levels of both enzymes, the xylanase in the Econase XT and the β-glucanase in the Econase GT are genetically modified to increase their thermo- and gastric stability, while the non-genetically modified activity of the ancillary enzyme in each product is not particularly stable. The occurrence of contaminant activities in enzymes on the market is not unusual. The multi-carbohydrase, Omegazyme, was reported by Canadian Bio-Systems (Calgary, CA) to contain 8,800 U/g xylanase, 4,000 U/g glucanase, 10,000 U/g cellulase, 15,000 U/g amylase, 6,000 U/g protease, 400 U/g mannanase, 3,000 U/g invertase, and 1,800 U/g pectinase. Based on these values, the 0.10% dietary level provided the activities listed above per kg of feed.

### Chemical Analysis

After the 48 h collection period, excreta from both experiments were frozen immediately after collection and subsequently freeze-dried, weighed, and ground in a coffee grinder for homogenization. Diet and excreta samples were analyzed for gross energy at the University of Illinois using an adiabatic bomb calorimeter (Model 1261, Parr Instruments, Moline, IL) that was standardized using benzoic acid. Diet samples were also analyzed for dry matter (Method 934.01; AOAC International, 2007). Analysis for total nitrogen via combustion (Method 990.03; AOAC International, 2007) for diet and excreta was performed at the Experiment Station Laboratories, University of Missouri-Columbia. Further analyses of the barley and rye were also conducted at the University of Missouri-Columbia (Methods 990.03, 934.01, 920.39, 978.10, 942.05, and 991.43 [AOAC International, 2007] for crude protein, moisture, crude fat, crude fiber, ash, and total, soluble, and insoluble dietary fiber, respectively).

### Statistics and Calculations

The $TME_n$ values were calculated using the following equation:

$$\begin{aligned} &TME_n \text{ of diets} = \\ &[FI \times GE_{diet} - ((\text{Excreta output from fed birds}) \times GE_{excreta}) \\ &\quad + (8.22 \times \text{Nitrogen retained by fed birds}) \\ &\quad + ((\text{Excreta output from fasted birds} \times GE_{excreta}) \\ &\quad + (8.22 \times \text{Nitrogen retained by fasted birds}))]/FI \end{aligned}$$

where FI = feed intake and GE = gross energy.

Data from Experiment 1 and 2 were analyzed using the one-way ANOVA procedure of SAS for completely randomized designs (SAS Institute Inc., 2010). Statistical significance of differences among treatment means were determined using Fisher's Least Significant Difference test. For Experiment 1, two single degree of freedom contrasts for a comparison of Diet 1 vs. Diets 2 to 7 and Diet 1 vs. Diets 2, 3, 4, 6, and 7 were also performed using the GLM procedure of SAS. All significant differences were assessed at $P < 0.05$.

## RESULTS AND DISCUSSION

The analyses for the barley and rye used in Experiment 2 were typical for both grains and composition of the two ingredients were generally similar (Table 2). The rye contained a higher level of crude, insoluble, and total fiber than the barley.

### Experiment 1

The $TME_n$ values for the 7 dietary treatments are presented in Table 3. The negative control diet was determined to have a $TME_n$ value of 3,560 kcal/kg DM. All enzyme treatments numerically increased $TME_n$ when compared with the negative control diet, and Diet 5 (3,702 kcal/kg DM) resulted in a significant increase of 142 kcal/kg DM. This increase was slightly larger than

**Table 2.** Proximate analysis (%) of the barley and rye used in Experiment 2 on an as-fed basis.

| Component | Barley | Rye |
|---|---|---|
| Crude protein | 10.72 | 10.85 |
| Moisture | 12.64 | 11.75 |
| Crude fat | 0.62 | 0.67 |
| Crude fiber | 0.72 | 1.55 |
| Insoluble dietary fiber | 8.2 | 12.0 |
| Soluble dietary fiber | 4.0 | 4.3 |
| Total dietary fiber | 12.3 | 16.3 |
| Ash | 1.34 | 1.70 |
| Gross energy (kcal/kg) | 3,828 | 3,862 |

**Table 3.** Nitrogen-corrected true metabolizable energy values for Experiment 1.

| Dietary treatment | $TME_n$ (kcal/kg DM)[1,2] |
|---|---|
| 1. Negative control (NC) | 3,560[b] |
| 2. NC + xylanase/α-galactosidase combination 1 | 3,586[b] |
| 3. NC + xylanase/α-galactosidase combination 2 | 3,617[b] |
| 4. NC + xylanase/α-galactosidase combination 3 | 3,604[b] |
| 5. NC + xylanase/α-galactosidase combination 4 | 3,702[a] |
| 6. NC + xylanase/α-galactosidase combination 5 | 3,624[b] |
| 7. NC + xylanase/α-galactosidase combination 6 | 3,625[b] |
| Pooled SEM | 23.5 |

[a-b]Means with no common superscript differ ($P < 0.05$).

[1]Values are means of 8 individually-caged conventional roosters.

[2]Single degree of freedom contrasts for Diet 1 vs. Diets 2-7 and Diet 1 vs. Diets 2, 3, 4, 6, and 7 were significant ($P < 0.05$).

**Table 4.** Nitrogen-corrected true metabolizable energy values for Experiment 2.

| Dietary treatment | $TME_n$ (kcal/kg DM)[1] |
|---|---|
| 1. 100% barley | 3,722[hi] |
| 2. As 1 + 0.01% β-glucanase[2] | 3,841[efgh] |
| 3. As 1 + 0.20% β-glucanase | 3,895[ef] |
| 4. 100% rye | 3,581[j] |
| 5. As 4 + 0.01% xylanase[3] | 3,680[ij] |
| 6. As 4 + 0.20% xylanase | 3,657[ij] |
| 7. 50% rye: 50% corn | 3,772[fghi] |
| 8. As 7 + 0.01% xylanase | 3,834[efgh] |
| 9. As 7 + 0.20% xylanase | 3,864[efg] |
| 10. 25% rye: 75% corn | 3,891[ef] |
| 11. As 10 + 0.01% xylanase | 3,949[bcde] |
| 12. As 10 + 0.20% xylanase | 3,917[cde] |
| 13. As 1 + 0.10% multi-carbohydrase[4] | 4,032[abcd] |
| 14. As 1 + 1.00% multi-carbohydrase | 4,086[a] |
| 15. As 4 + 0.10% multi-carbohydrase | 3,732[ghi] |
| 16. As 4 + 1.00% multi-carbohydrase | 3,909[de] |
| 17. As 7 + 0.10% multi-carbohydrase | 3,907[de] |
| 18. As 7 + 1.00% multi-carbohydrase | 3,968[abcde] |
| 19. As 10 + 0.10% multi-carbohydrase | 4,054[ab] |
| 20. As 10 + 1.00% multi-carbohydrase | 4,044[abc] |
| Pooled SEM | 47.9 |

[a-j]Means with no common superscript differ ($P < 0.05$).

[1]Values are means of 6 individually caged conventional roosters.

[2]0.01% β-glucanase (Econase GT, AB Vista, Marlborough, UK) provided 256,087 BU β-glucanase activity and 20,900 BXU xylanase activity per kg of feed.

[3]0.01% xylanase (Econase XT, AB Vista, Marlborough, UK) provided 14,800 BXU xylanase activity and 911 BU β-glucanase activity per kg of feed.

[4]0.10% multi-carbohydrase (Omegazyme, Canadian Bio-Systems, Calgary, CA) provided 8,800 U xylanase, 4,000 U glucanase, 10,000 U cellulase, 15,000 U amylase, 6,000 U protease, 400 U mannanase, 3,000 U invertase, and 1,800 U pectinase per kg of feed.

the 58 to 130 kcal/kg increase in ileal digestible energy obtained by Jasek et al. (2018) for a multi-carbohydrase containing xylanase and galactosidase. For another assessment of enzyme effect, 2 single degree of freedom contrasts were also used. The contrast for the negative control (Diet 1) vs. Diets 2 to 7 containing enzyme demonstrated a significant difference. To ensure that the significant effect of Diet 5 was not overly influencing the significance of the contrast, a second contrast was performed between the negative control (Diet 1) and Diets 2, 3, 4, 6, and 7, excluding Diet 5. This contrast was also significant. Thus, all enzyme treatments had some positive effect on $TME_n$ and the mean $TME_n$ increase for all enzyme treatments was 66 kcal/kg DM compared with the corn/soybean meal control diet. The results of this experiment showed that the precision-fed rooster assay was sensitive enough to detect positive effects of the supplemental xylanase and α-galactosidase enzymes on $TME_n$ of a corn/soybean meal diet.

## *Experiment 2*

The $TME_n$ values for the 20 dietary treatments are presented in Table 4. As expected, $TME_n$ of the 100% barley diet was significantly higher than the 100% rye diet (3,722 vs. 3,581 kcal/kg DM, respectively), and $TME_n$ of the diets containing rye increased as the level of corn increased and rye decreased. For the purpose of discussion, the treatments will be discussed in groups based on their basic diets composed of 100% barley, 100% rye, 50% rye: 50% corn, or 25% rye: 75% corn.

The $TME_n$ of the 100% barley diet was 3,722 kcal/kg DM, which is greater than the value reported by the NRC (1994) of 3,258 kcal/kg on a DM basis. However, the ME of barley depends on many factors such as barley cultivar, viscosity, and β-glucan content (Rotter et al., 1990). Additionally, the barley used in this study was pearled, which is dehulled barley that was steam processed to remove the bran (Jacob and Pescatore, 2012). Both β-glucanase and the multi-carbohydrase combination either numerically or significantly increased $TME_n$ of the 100% barley diet, with the multi-carbohydrase increasing $TME_n$ from 3,722 to 4,086 kcal/kg DM at the highest inclusion rate for a significant difference of 364 kcal/kg DM. Even though it was not significant, the commercial level (0.01%) of the β-glucanase product yielded an increase of over 100 kcal when it was added to the 100% barley diet, and the higher level (0.20%) of β-glucanase product significantly increased $TME_n$.

The $TME_n$ of the 100% rye diet was 3,581 kcal/kg DM, which is numerically greater than the value reported by the NRC (1994) of 3,331 kcal/kg on a DM basis. However, as with barley, energy values of rye can vary depending on many factors. Both xylanase and the multi-carbohydrase either numerically or significantly increased $TME_n$ of the 100% rye diet, with the multi-carbohydrase significantly increasing it from 3,581 to 3,909 kcal/kg DM at the highest inclusion rate for a difference of 328 kcal/kg DM.

As expected, the 50% rye: 50% corn treatment resulted in a $TME_n$ value that was significantly greater than the 100% rye diet, while the 25% rye: 75% corn yielded an additional numeric increase. Corn has a greater ME than rye (NRC, 1994), explaining why $TME_n$ increased with increasing level of corn. The xylanase numerically increased $TME_n$ of the 2 rye:corn blends and the multi-carbohydrase significantly increased the $TME_n$ of both rye:corn blends; at the most effective inclusion rates of 1.00 and 0.10%, the multi-carbohydrase increased $TME_n$ of the 50% rye: 50% corn and the 25% rye: 75% corn by 196 and 163 kcal/kg DM, respectively. The magnitude of the enzyme responses

generally decreased as the level of rye in the diets decreased, which was expected as the rye functioned as the primary target substrate for the xylanase. It follows that larger responses to enzymes were observed with treatments containing larger percentages of rye. For example, when the 100% rye and 25% rye: 75% corn diets were supplemented with 1.00% of the multi-carbohydrase, the magnitude of the improvement in $TME_n$ was 328 kcal/kg DM compared with 153 kcal/kg DM, respectively. The 25% rye: 75% corn treatment is a closer representation to what would more likely be seen in a commercial poultry diet.

Overall, results of this study indicated that the precision-fed rooster assay can detect effects of enzymes, primarily carbohydrase, on $TME_n$ of diets containing pearled barley and/or rye. The multi-carbohydrase combination consistently produced greater $TME_n$ values than with the enzyme products containing mainly either $\beta$-glucanase or xylanase; however, a direct comparison of the enzyme products would be difficult to make. Any ancillary activities of enzymes in a combination can be a large confounder of direct comparisons (Bedford, 2018). Cost of the enzyme supplement is also an important factor. Furthermore, enzyme ME responses in typical commercial diets are expected to be smaller than those in diets containing very high levels of target ingredients such as those often used herein, and will likely require a larger sample size of birds and increased replication in the precision-fed rooster assay to detect a statistically significant response. Using the standard errors from the experiments in the current study, an estimate can be made for the approximate bird numbers and replication needed to detect expected ME responses from exogenous enzymes in future experiments.

Although not directly comparable, the magnitude of energy differential detected from the use of the two precision-fed rooster assays in the current study are within the range of results of other experiments reported in the literature, albeit on the lower end of the spectrum. The responses to carbohydrases, however, have been highly variable in previous studies. In the two experiments of the current study, carbohydrases in corn/soybean meal diets produced an approximately 1 to 4% increase in $TME_n$, while carbohydrases in 100% barley diets produced a 3 to 10% increase, 100% rye diets a 2 to 9% increase, and 25% rye: 75% corn diets a 1 to 4% increase. Comparatively, for studies involving barley, Marquardt et al. (1994) reported that inclusion of a fungal enzyme preparation with high xylanase activity improved ($P < 0.05$) $AME_n$ of diets containing over 60% rye, hulless barley, or wheat for young chicks but yielded no significant effect in a 63% corn diet. In that study, the $AME_n$ of the wheat-based diet increased from 3,344 to 3,478 kcal/kg (4%), the barley-based diet increased from 3,179 to 3,564 kcal/kg (12%), and the rye-based diet increased from 3,009 to 3,317 kcal/kg (10%) by the high xylanase fungal enzyme. The positive response of xylanase on ME of barley may largely be due to the fact, as mentioned earlier, that the xylanase also contains glucanase activity. As this shows, much higher improvement in ME was obtained with diets containing rye and hulless barley than with diets containing wheat or corn. These results are in agreement with the results of the current study, where larger enzyme responses were obtained in 100% barley and rye diets than in a 75% corn diet.

Rotter et al. (1990) evaluated the $AME_n$ response in young chicks fed barley diets containing an enzyme supplement high in $\beta$-glucanase activity. With hulless Scout barley at 75% of the diet, there was an energy difference of 27% between unsupplemented and enzyme supplemented diets (2,773 vs. 3,521 kcal/kg). Subsequently, the same diets were fed to mature roosters and the energy difference between unsupplemented and enzyme supplemented diets was only 4% (3,578 vs. 3,719 kcal/kg) as compared with 27% for $AME_n$ in chicks. The authors suggested that the response differential was due to the age of the bird, and possibly differences in physiological condition of the gut or gut maturity/development, so that $\beta$-glucans do not have as much of an effect in adult roosters as young chicks; therefore, the energy values for barley derived from $AME_n$ studies are a better predictor of performance for young broilers. Lastly, the same authors evaluated the high $\beta$-glucanase enzyme preparation on several cultivars of barley in a 62.5% barley diet fed to chicks. The greatest $AME_n$ response was observed with Scout barley with a 25% increase, but no increase was observed with hulled Bedford barley. Tilman et al. (2006) found that $\beta$-glucanase supplementation to diets of broiler chickens fed various types of barley cultivars significantly increased AME, with improvements ranging from 5.4 to 21.9%. And lastly, Leeson and Proulx (1994) noted a 7.1% increase in ME from $\beta$-glucanase inclusion when feeding Rhode Island White roosters diets containing 40% high $\beta$-glucan barley; however, no increase was observed when low $\beta$-glucan barley was used. Differences in enzyme responses among studies may most likely be due differences in in the soluble $\beta$-glucan and associated intestinal viscosity content of the grains.

For studies involving diets containing large amounts of rye, Smulikowska et al. (2002) reported that xylanase added to 40% rye diets fed to broiler chicks improved $AME_n$ by approximately 9%, from 3,179 to 3,466 kcal/kg. Smulikowska and Mieczkowska (1996) noted that xylanase/$\beta$-glucanase added to 45% rye broiler diets significantly improved $AME_n$ by 11%, from 3,143 to 3,494 kcal/kg, and Pettersson and Åman (1989) observed an 8% increase in $AME_n$ value of a wheat/rye diet for broilers when supplemented with an enzyme. Dänicke et al. (1997) found that supplementation of a 61% rye diet with xylanase improved $AME_n$ in broiler chicks but that this effect was influenced by both age and dietary fat type, such that the benefit of xylanase on $AME_n$ tended to decrease with time, and $AME_n$ of diets containing beef tallow often showed greater improvements from the enzyme than diets containing soy oil, possibly due to the lower $AME_n$ and higher saturated fatty acid content of beef tallow. Improvement in $AME_n$

from the addition of xylanase in this latter study averaged approximately 9%.

For studies involving both barley and rye diets, Friesen et al. (1992) reported an increase in $AME_n$ for diets containing 70% Gazelle spring rye, hulled Bedford barley, or hulless Scout barley when supplemented with fungal enzyme and fed to young broiler chicks. Increases were by 14, 7, and 42%, respectively, when compared with their unsupplemented counterparts. Lastly, Lázaro et al. (2003) reported that a fungal $\beta$-glucanase/xylanase increased the $AME_n$ of 35% rye-containing diets and 50% barley-containing diets fed to laying hens by 1.9 and 5.7%, respectively, but not for 50% wheat diets. The variation in responses observed in the literature is not surprising, because results are dependent on many variables such as bird age and type, enzyme type and purity, and enzyme inclusion level, as well as grain characteristics, type, and inclusion level.

In summary, the results of the current study indicate that the precision-fed rooster assay is sensitive enough to detect positive effects of carbohydrase enzymes on ME of poultry diets. The.precision-fed rooster assay may particularly pose benefits for screening novel enzymes in the process of development. There is great potential for new products, as human capability to search nature and evolve new enzymes better suited to the role for which they are intended improves (Bedford and Partridge, 2010). However, this ability is met with a counterbalance of financial and regulatory hurdles for bringing new products to market. In the European Union, for example, the process for a new product from discovery to market may take 4 to 7 yr and upward of US$2 million, and the efficacy requirements (animal trials) account for approximately 75% of these costs and 1 to 2 yr of this process (Bedford and Partridge, 2010). In light of this, the precision-fed rooster assay can offer a relatively fast and less expensive method for evaluating novel enzymes for increases in $TME_n$. It is also advantageous that ingredients containing target substrates can be fed at 100% of the diet which is useful for evaluating specific individual feed ingredients whose utilization is likely to be improved by enzymes. In essence, an experiment may be designed to provide the "best case scenario" for an enzyme product, which could then serve as a reference point for further analysis, such as an $AME_n$ assay using broiler chicks. The $AME_n$ assays continue to be advantageous in the sense that they employ young chicks, which may generate a larger response to the enzyme than older birds that have more developed gastrointestinal tracts and a greater ability to tolerate higher digesta viscosity. Growth-type $AME_n$ assays can also provide additional data that may be highly valuable, such as feed efficiency. Also drawing significant attention is the development of thermostable carbohydrases that are able to withstand pelleting temperatures. After enzyme products are subjected to high feed processing temperatures, the rooster assay could be used to quickly evaluate any negative thermal consequences on enzyme activity, reflected as changes in $TME_n$.

## DISCLOSURES

There is no conflict of interest on manuscript.

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